[CANCER RESEARCH 51, 5621–5625, October, 15, 1991]

Association between Hepatitis C Virus Antibodies and Hepatocellular Carcinoma in Taiwan[1]

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ABSTRACT

In order to assess the association between antibodies to hepatitis C virus (anti-HCV) and hepatocellular carcinoma (HCC), as well as the interaction of anti-HCV with other HCC risk factors in Taiwan, a total of 127 pairs of newly diagnosed HCC patients and healthy community controls were studied. Case-control pairs were individually matched for age (±3 years), sex, residence, and ethnicity. Serum samples from study subjects were examined for anti-HCV by enzyme immunoassays as well as hepatitis B surface antigen (HBsAg) and e antigen (HBeAg) by radioimmunoassays using commercial kits. The habits of cigarette smoking, alcohol drinking, and peanut consumption were obtained through standardized interviews according to a structured questionnaire. Both the anti-HCV as well as the carrier status of HBsAg and HBeAg were significantly associated with HCC showing a multivariate-adjusted odds ratio of 24.8 for carriers of HBsAg alone, 33.5 for carriers of both HBsAg and HBeAg, and 23.7 for those who were positive for anti-HCV. The population-attributable risk percentage was estimated as 3% for anti-HCV alone, 69% for HBsAg carrier status alone, and 6% for both anti-HCV and HBsAg in Taiwan. There were also synergistic effects on HCC development for anti-HCV with HBsAg carrier status, cigarette smoking, and habitual alcohol drinking.

INTRODUCTION

Chronic viral hepatitis and cirrhosis have long been suggested to play an important role in the development of HCC.[3] In addition to HBV, HCV and HDV have also been documented to be associated with HCC. Although HBV is causally associated with HCC, there is a wide variation in the proportion of HCC attributable to HBV in different countries ranging from 10 to 90% (1). The attributable risk has been estimated as high as 79% in Taiwan where HBV is hyperendemic with an infection rate of 85–90% and a chronic carrier rate of 15–20% (2). A significantly elevated prevalence of anti-HDV IgG among HCC patients has been reported in some countries (3, 4). However, HDV seems to play a relatively minor role in the development of HCC in Taiwan (5). Non-A, non-B posttransfusional hepatitis has long been suggested to play a part in the pathogenesis of HCC based on clinical observations (6, 7), but this association could be investigated only recently after the HCV genome had been cloned and specific assays for circulating anti-HCV were developed (8, 9). Patients with advanced chronic liver disease and HCC were reported to have an increased proportion of anti-HCV positivity in Spain, Italy, the United States, and South Africa (10–13). A recent study also showed a higher prevalence of anti-HCV in HCC patients than volunteer blood donors in Taiwan (14).

In addition to persistent viral hepatitis infection, cigarette smoking, alcohol drinking, and aflatoxin exposure have also been observed to be associated with an increased risk of HCC in some countries including Taiwan (2, 15, 16). However, there has never been a study designed to elucidate interactive effects of anti-HCV with HBsAg carrier status, cigarette smoking, habitual alcohol drinking, and aflatoxin exposure. In this study, anti-HCV was found to be associated with an increased risk of HCC and have synergistic interactions with HBsAg carrier status, cigarette smoking, and alcohol drinking.

PATIENTS AND METHODS

Case Recruitment and Control Selection. Most HCC patients are referred to teaching hospitals for diagnosis and treatment in Taiwan where HBV is hyperendemic with an infection rate of 85–90% and an HBsAg carrier rate of 15–20%. Newly diagnosed HCC patients were recruited from two major teaching general hospitals in northern and southern Taiwan, the Chang-Gung Memorial Hospital and Kaohsiung Medical College Hospital, from August 1986 to July 1987. A total of 127 HCC patients diagnosed on the basis of either pathological examination or elevated α-fetoprotein level (400 ng/ml or above) combined with at least one positive image on angiography, sonography, liver scan, and/or computerized tomography scans were consecutively recruited. Among these 127 HCC cases, 80% were diagnosed pathologically and 20% by elevated α-fetoprotein and liver images compatible to HCC. The mean and SD of age of HCC patients were 50.4 and 11.8 years, respectively. There were 121 male and 6 female cases. They were Fukien Taiwanese (81%), Hakka Taiwanese (10%), and mainland Chinese (9%).

In order to choose a representative and comparable control group, healthy community residents were selected from household registration offices where the complete demographical information of family members in each household is mandatorily registered and double-checked annually. Community controls were individually matched with cases for age (±3 years), sex, ethnicity, and residence. The mean and SD of age of community controls were 49.5 and 11.5 years, respectively. Controls were recruited during the same study period as cases.

Serum Collection and Serological Examination. An aliquot of 10 ml blood was also collected from HCC cases on the first day of hospitalization and from community controls at the time of personal interview at home. Serum samples separated on the same day as blood collection were kept at −30°C until examinations for serological markers of HBV and HCV infections. Anti-HCV was examined in duplicate by enzyme immunoassays according to the instructions of the manufacturer. Positive samples from the first test were retested. Only repeatedly positive samples were considered as anti-HCV positive. Among 254 serum samples examined, there were 14 definite positives and 2 borderline positives for anti-HCV identified from the first test. The retest of these 16 positive samples confirmed that all were positive. HBsAg and HBeAg were examined by radioimmunoassays. All laboratory examinations were performed blindly using commercial kits (Abbott Laboratories, Chicago, IL).

Structured Questionnaire and Standardized Interview. A structured questionnaire was used to obtain information concerning sociodemo-

Received 1/9/91; accepted 7/30/91.

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[1] This study was supported by research grants from the Department of Health, Executive Yuan, Republic of China. C-J. C. was a Fogarty International Research Fellow sponsored by the National Institutes of Health (1 F05 TW04161–01).

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[3] The abbreviations used are: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; anti-HCV, anti-hepatitis C virus antibody; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; 95% CI, 95% confidence interval.

graphic characteristics, habits of cigarette smoking, alcohol drinking, and consumption frequency of peanut products which were reported to be major aflatoxin-contaminated foodstuffs in Taiwan. The structured questionnaire was administered at the time of enrollment. All interviews were carried out by two experienced nurse interviewers who had been trained concerning study details and interview techniques. Almost equal numbers of cases and controls were assigned to each interviewer.

Statistical Methods. Matched crude and multivariate-adjusted relative risks and their 95% CI for various risk factors were estimated using conditional logistic models (17). Statistical significance of the risk estimates was examined by Z test. Unmatched odds ratio was also estimated to assess the HCC risk for various combinations of anti-HCV status with HBsAg carrier status, cigarette smoking, alcohol drinking, and peanut consumption. Mantel's χ^2 test for a trend was used to examine the dose-response relationship for the unmatched HCC risk estimates of various combinations of anti-HCV status and other risk factors. In the stratified data analysis, cases and controls were grouped as nonsmokers and smokers for the habit of cigarette smoking. Habitual alcohol drinking was defined as drinking alcohol >3 days/week for >15 years. Peanut consumption frequency was divided into two groups: "one or more meals a week" and "less than one meal a week."To compare the population-attributable risk for HCV and HBV, the prevalence of anti-HCV and HBsAg in the control group was used as the prevalence in the general population. Population-attributable risk for anti-HCV positivity and HBsAg carrier status was calculated from the matched odds ratios in the univariate analysis and the prevalence of two viral markers in the control group (18).

RESULTS

Anti-HCV and HBsAg. The anti-HCV status and carrier status of HBsAg and HBeAg among HCC cases and matched controls are shown in Table 1.

Anti-HCV was detected in 14 of 127 (11.0%) HCC patients and only 2 of 127 (1.6%) matched healthy controls. The association between anti-HCV status and HCC was significant with a matched odds ratio of 7.0 (95% CI 1.6–30.8). The HBsAg and HBeAg carrier rates were significantly higher among HCC cases (86.6 and 18.9%, respectively) than community controls (16.6 and 2.4%, respectively). Compared with noncarriers as the referent group, the matched odds ratio of developing HCC was 17.2 (95% CI 6.8–43.4) for carriers of HBsAg alone and 28.1 (95% CI 6.4–124.1) for carriers of both HBsAg and HBeAg. Based on a prevalence of 1.6% for anti-HCV and 16.6% for HBsAg carrier status in the control group as well as the relative risks associated with these two risk factors, the estimated population-attributable risk was 3% for anti-HCV positivity alone, 69% for HBsAg carrier status alone, and 6% for both anti-HCV positivity and HBsAg carrier status.

Cigarette Smoking, Alcohol Drinking, and Peanut Consumption. Table 2 shows the frequency distributions of cigarette smoking, alcohol drinking, and peanut consumption of HCC cases and matched community controls. There was a significant association between cigarette smoking and HCC with a matched odds ratio of 1.1 (95% CI 0.5–2.2), 1.8 (95% CI 1.0–3.4) and 1.7 (95% CI 0.7–4.5), respectively, for those who smoked 1–10, 11–20, and >20 cigarettes/day, as compared with nonsmokers. Habitual alcohol drinking was also significantly associated with HCC. The matched odds ratio for the habitual alcohol drinkers was 2.0 (95% CI 1.2–3.3) as compared with nondrinkers. No association was found between HCC and peanut consumption.

Multivariate Regression Analysis. Results of the conditional logistic regression analysis of multiple risk factors of HCC are shown in Table 3. After the effects of cigarette smoking, habitual alcohol drinking, and peanut consumption were adjusted for, the carrier status of HBsAg and HBeAg remained significantly related to HCC with a multivariate-adjusted odds ratio of 24.8 (95% CI 7.6–80.9) for carriers of HBsAg alone and 33.5 (95% CI 6.6–170.9) for carriers of both HBsAg and HBeAg. Anti-HCV status also remained significantly associated with HCC. The multivariate-adjusted odds ratio was as high as 23.7 (95% CI 2.2–253.8) for those who were positive for anti-HCV as compared with those who were negative. However, cigarette smoking, habitual alcohol drinking, and peanut consumption were not found to be significantly associated with HCC.

Interaction of Anti-HCV with Other Risk Factors. The independent and interactive effects of anti-HCV and HBsAg carrier status on the development of HCC are shown in Table 4. Anti-HCV was detected in 29.4% (5 of 17) of HBsAg-negative HCC patients, 8.2% (9 of 110) of HBsAg-positive HCC patients, 1.9% (2 of 106) of HBsAg-negative matched controls, and 0% (0 of 21) of HBsAg-positive matched controls. Compared with those who were negative for both anti-HCV and HBsAg as the referent, the matched odds ratio and unmatched odds ratio of developing HCC for HBsAg noncarriers positive for anti-HCV were 15.6 (95% CI 1.8–134.8) and 20.6 (95% CI 3.0–238.6). The corresponding figures for HBsAg carriers negative for anti-HCV were 22.1 (95% CI 8.1–60.8) and 40.6 (95% CI 18.5–96.7). Although no controls were positive for both HBsAg and anti-HCV and thus no finite estimate for the relative risk or upper 95% CI could be calculated, the lower 95% CI of the unmatched odds ratio for HBsAg carriers who were positive for anti-HCV was as high as 14.3. Based on the Mantel χ^2 test for a trend, the relative risk of developing HCC was found to increase with the presence of anti-HCV alone, HBsAg alone, and both anti-HCV and HBsAg. The mean ± SD of HCC onset age was 57.8 ± 10.0 for patients who were negative for both HBsAg and anti-HCV, 60.4 ± 13.1 for those positive for anti-HCV alone, 49.0 ± 10.2 for those positive for HBsAg alone, and 49.0 ± 14.1 for those positive for both anti-HCV and HBsAg. Although HBsAg-positive patients had a younger onset age than HBsAg-negative patients, the onset age was similar for patients who were positive or negative on anti-HCV.

Further analyses were carried out to examine interactive effects of anti-HCV with cigarette smoking, habitual alcohol drinking, and peanut consumption frequency as shown in Table 5. There were also significantly synergistic effects of anti-HCV with cigarette smoking, habitual alcohol drinking, and peanut consumption frequency. As compared with nonsmokers who were negative for anti-HCV as the referent, the unmatched odds ratio of HCC development was 1.5 for smokers who were negative for anti-HCV, 5.6 for nonsmokers who were positive for anti-HCV, and 13.9 for smokers who were positive for anti-HCV. The Mantel χ^2 test for a trend indicated that the relative risk of developing HCC increased in a dose-response pattern.

Table 1 *Prevalence of anti-HCV and HBsAg/HBeAg in HCC cases and matched controls*

Variable	Cases No.	Cases %	Controls No.	Controls %	Matched odds ratio (95% CI)
Anti-HCV					
Negative	113	89.0	125	98.4	1.0
Positive	14	11.0	2	1.6	7.0[a] (1.6–30.8)
HBsAg/HBeAg					
Negative/negative	17	13.4	106	83.5	1.0
Positive/negative	86	67.7	18	14.2	17.2[a] (6.8–43.4)
Positive/positive	24	18.9	3	2.4	28.1[a] (6.4–124.1)

[a] $P < 0.01$.

Table 2 *Frequency distributions of cigarette smoking, alcohol drinking, and peanut consumption in matched HCC cases and community controls*

Variable	Cases No.	Cases %	Controls No.	Controls %	Matched odds ratio (95% CI)
Cigarette smoking (cigarettes/day)					
None	37	29.1	48	37.8	1.0
1–10	19	15.0	25	19.7	1.1 (0.5–2.2)
11–20	57	44.9	42	33.1	1.8[a] (1.0–3.4)
>20	14	11.0	12	9.4	1.7 (0.7–4.5)
Habitual alcohol drinking[b]					
No	61	48.0	83	65.4	1.0
Yes	66	52.0	44	34.6	2.0[c] (1.2–3.3)
Peanut consumption frequency (meals/week)					
<1	60	47.2	60	47.2	1.0
1+	67	52.8	67	52.8	1.0 (0.6–1.7)

[a] $0.05 < P < 0.06$.
[b] Habitual alcohol drinking defined as drinking three or more times per week for more than 15 years.
[c] $P < 0.01$.

Table 3 *Conditional regression analysis of multiple risk factors of HCC based on 127 matched pairs of HCC cases and healthy community controls in Taiwan*

Variable and group	Multivariate-adjusted odds ratio	95% CI
HBsAg/HBeAg		
Negative/negative	1.0	
Positive/negative	24.8[a]	7.6–80.9
Positive/positive	33.5[a]	6.6–170.9
Anti-HCV		
Negative	1.0	
Positive	23.7[a]	2.2–253.8
Cigarette smoking (cigarettes/day)		
None	1.0	
1–10	0.4	0.1–1.9
11–20	1.3	0.4–4.2
>20	2.1	0.3–13.5
Habitual alcohol drinking		
No	1.0	
Yes	1.4	0.5–4.4
Peanut consumption (meal/week)		
<1	1.0	
1+	0.6	0.2–1.5

[a] $P < 0.01$.

As compared with nondrinkers who were negative for anti-HCV as the referent, the unmatched odds ratio of developing HCC was 2.1 for drinkers who were negative for anti-HCV and 6.1 for nondrinkers who were positive for anti-HCV. A higher HCC risk was found for drinkers who were positive for anti-HCV. The Mantel's χ^2 test for a trend also indicated that the relative risk of developing HCC increased in a dose-response pattern. Compared with those who consumed peanuts less often than one meal/week and were negative for anti-HCV as the referent, the unmatched odds ratio of developing HCC was 0.9 for those who consumed peanuts during one or more meals/week and were negative for anti-HCV, 5.3 for those who consumed peanuts less often than one meal/week and were positive for anti-HCV, and 9.5 for those who consumed peanuts during one or more meals/week and were positive for anti-HCV. The Mantel's χ^2 test for a trend indicated that the relative risk of developing HCC increased in a dose-response pattern. All matched odds ratios estimated for various combinations of anti-HCV status and risk factors shown in this table were very similar to the unmatched odds ratios with a more narrow 95% CI.

DISCUSSION

Increasing attention has recently been paid to the role of HCV in the pathogenesis of HCC. Recent studies suggest that HCV may be a more important cause of HCC than HBV in Europe (10, 11), but HBV is the more important etiological agent in South Africa (13). In this study, we also observed a significant association with HCC for anti-HCV and HBsAg. Previous study in Taiwan indicated an anti-HCV positivity rate in the general population of 0.95% with a 95% CI between 0.02 and 1.88%. In our study, the prevalence of anti-HCV in the control group was within that range (1.6%). Although storage of frozen serum samples may cause false-positive anti-HCV results, the duration of serum storage prior to testing was the same for cases and controls.

It might not bias the relative risk estimates associated with the anti-HCV status. Even though the frozen storaged serum samples and commercial kits we used might give false-positive results, the odds ratio may thus be underestimated. In other words, the odds ratio was obtained under a conservative circumstance. Based on the prevalence of HBsAg carrier status and anti-HCV in the control group, we found that 69% of HCC cases in Taiwan can be attributable to HBsAg alone, 3% to anti-HCV alone, and 6% to both HBsAg and anti-HCV. The results of this study strengthen the close association between HCV and HCC but indicates the relatively minor role of HCV in the determination of HCC development in an area hyperendemic for HBV.

Our data also revealed a considerably higher prevalence of anti-HCV in HBsAg-negative (29.4%) than in HBsAg-positive (8.2%) HCC patients. The discrepancy is greater in Taiwan than in Italy (70 *versus* 54%) (11) and South Africa (34 *versus* 26%) (13). Although our sample size was not large, a significant synergistic effect on HCC risk was observed when both HBsAg and anti-HCV were present. Similar results have been reported in Italy, the United States, and South Africa (11–13). HCV infection was found to be low in the general population and high in hemophiliacs, parenteral drug abusers, and prostitutes in Taiwan (14), while the HBV infection rate of persons 20 years of age is approximately 90% and most of the HBsAg carriers are infected in early childhood in Taiwan (19). The co-occurrence of HBsAg and anti-HCV among HCC patients may less likely be due to common transmission routes. It seems more reasonable to speculate that HCV superinfection in HBsAg carriers may synergistically increase the risk of devel-

Table 4 *Independent and interactive effects of anti-HCV positivity and HBsAg carrier status on the risk of HCC*

Anti-HCV/HBsAg	Cases No.	Cases %	Controls No.	Controls %	Matched odds ratio (95% CI)	Unmatched odds ratio[a] (95% CI)
Negative/negative	12	9.5	104	81.9	1.0	1.0[b]
Positive/negative	5	3.9	2	1.6	15.6[c] (1.8–134.8)	20.6[d] (3.0–238.6)
Negative/positive	101	79.5	21	16.5	22.1[d] (8.1–60.8)	40.6[d] (18.5–96.7)
Positive/positive	9	7.1	0	0.0	∞	∞[d] (14.3–∞)

[a] Tests of significance and point estimates and confidence intervals of odds ratios are based on exact conditional distribution.
[b] Mantel's χ^2 test for a trend was significant; $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.01$.

Table 5 *Interactive effects of anti-HCV positivity with cigarette smoking, habitual alcohol drinking, and peanut consumption*

Variable	Cases No.	Cases %	Controls No.	Controls %	Matched odds ratio (95% CI)	Unmatched odds ratio[a] (95% CI)
Cigarette smoking/anti-HCV						
Nonsmoker/negative	33	26.0	47	37.0	1.0	1.0[b]
Smoker/negative	80	63.0	78	61.4	1.6 (0.9–3.0)	1.5 (0.8–2.6)
Nonsmoker/positive	4	3.1	1	0.8	6.0 (0.6–57.3)	5.6 (0.5–286.4)
Smoker/positive	10	7.9	1	0.8	14.6[c] (1.8–121.0)	13.9[d] (1.8–630.9)
Alcohol drinking/anti-HCV						
Nondrinker/negative	53	41.7	81	63.8	1.0	1.0[b]
Drinker/negative	60	47.3	44	34.6	2.1[d] (1.2–3.7)	2.1[d] (1.2–3.6)
Nondrinker/positive	8	6.3	2	1.6	6.1[c] (1.2–30.1)	6.0[c] (1.1–60.6)
Drinker/positive	6	4.7	0	0.0	∞	∞[d] (1.7–∞)
Peanut consumption (meals/week)/anti-HCV						
<1/negative	55	43.3	59	46.4	1.0	1.0[b]
1+/negative	58	45.7	66	52.0	0.9 (0.6–1.6)	0.9 (0.6–1.6)
<1/positive	5	3.9	1	0.8	4.9 (0.6–42.6)	5.3 (0.6–257.9)
1+/positive	9	7.1	1	0.8	8.8[c] (1.1–70.9)	9.5[c] (1.3–430.2)

[a] Tests of significance and point estimates and confidence intervals of odds ratios are based on exact conditional distribution.
[b] Mantel's χ^2 test for a trend was significant.
[c] $P < 0.05$.
[d] $P < 0.01$.

oping HCC, although the mechanism remains to be elucidated. Non-A, non-B hepatitis-associated HCC in Japan usually occurs in old age (20). In this study, we also observed that HCC patients who were anti-HCV-positive and HBsAg-negative had a higher mean onset age (60 ± 13 years) than those who were anti-HCV-negative and HBsAg-positive (49 ± 10 years). The difference may reflect a relatively earlier infection of HBV than HCV and/or a shorter induction period of HCC for HBV than HCV.

Considerable evidence indicates that HCC is multifactoral in origin. In addition to HBV and HCV, aflatoxins have long been thought to be the major hepatocarcinogens (21). A moderate excess risk of HCC associated with alcohol drinking (2, 22) and cigarette smoking (2, 23, 24) has also been well documented, although the relative importance of these two factors varies from one population to another. Our previous study also demonstrated an additively synergistic effect on HCC risk between HBsAg carrier status and habits of alcohol drinking and cigarette smoking (2). This study showed a strikingly increased HCC risk when anti-HCV was present among cigarette smokers and habitual alcohol drinkers. Cigarette smoking and alcohol drinking are common habits among males in Taiwan; the questions were not sensitive enough for people to intentionally underreport them. It was also believed that study subjects had no difficulty recalling their long-term habits of cigarette smoking and alcohol drinking. Furthermore, our well-trained interviewers made an equal effort to obtain the information from cases and controls. The possibility of reporting bias of these habits from study subjects was rather low.

Although the mechanism of such synergistic phenomena is unclear, interventions aimed at reducing alcohol drinking and cigarette smoking are also important for the prevention of HCC, especially for those who are positive for anti-HCV and/or HBsAg.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Kung-Yee Liang, Department of Biostatistics, School of Hygiene and Public Health, Johns Hopkins University, for his helpful suggestions.

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Cancer Res 1991;51:5621-5625.