

Risk Factors for Hepatocellular Carcinoma: Synergism of Alcohol With Viral Hepatitis and Diabetes Mellitus

Manal M. Hassan,¹ Lu-Yu Hwang,² Chiq J. Hatten,¹ Mark Swaim,³ Donghui Li,¹ James L. Abbruzzese,¹ Palmer Beasley,² and Yehuda Z. Patt¹

Risk factors associated with hepatocellular carcinoma (HCC) are well documented, but the synergisms between these risk factors are not well examined. We conducted a hospital-based, case-control study among 115 HCC patients and 230 non-liver cancer controls. Cases and controls were pathologically diagnosed at The University of Texas M. D. Anderson Cancer Center and were matched by 5-year age groups, sex, and year of diagnosis. Information on risk factors was collected by personal interview and medical records review. Blood samples were tested for the presence of antibodies to hepatitis C virus antigen (anti-HCV), hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core antigen (anti-HBc). Conditional logistic regression was used to determine odds ratios (ORs) by the maximum likelihood method. Multivariate ORs and 95% confidence intervals (CIs) were 15.3 (4.3-54.4), 12.6 (2.5-63.1), 4.5 (1.4-14.8), and 4.3 (1.9-9.9) for anti-HCV, HBsAg, heavy alcohol consumption (≥ 80 mL ethanol/d), and diabetes mellitus, respectively. Synergistic interactions on the additive model were observed between heavy alcohol consumption and chronic hepatitis virus infection (OR, 53.9; 95% CI, 7.0-415.7) and diabetes mellitus (OR, 9.9; 95% CI, 2.5-39.3). Independent of the effect of HCV, HBV, and diabetes mellitus, heavy alcohol consumption contributes to the majority of HCC cases (32%), whereas 22%, 16%, and 20% were explained by HCV, HBV, and diabetes mellitus, respectively. In conclusion, the significant synergy between heavy alcohol consumption, hepatitis virus infection, and diabetes mellitus may suggest a common pathway for hepatocarcinogenesis. Exploring the underlying mechanisms for such synergisms may indicate new HCC prevention strategies in high-risk individuals. (HEPATOLOGY 2002;36:1206-1213.)

See Editorial on Page 1046

Hepatocellular carcinoma (HCC), which is one of the most common cancers worldwide, is rarely detected early and is usually fatal within a few months of diagnosis. HCC also has been recognized as having a high incidence in sub-Saharan Africa, China,

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; anti-HCV, antibody to hepatitis C antigen; HBsAg, hepatitis B surface antigen; anti-HBc, antibody to hepatitis B core antigen; OR, odds ratio; CI, confidence interval; S, S Index; PAR%, population attributable risk percent.

From the ¹Department of Gastrointestinal Medical Oncology, and ³the Department of Gastrointestinal Medicine and Nutrition, The University of Texas M. D. Anderson Cancer Center, Houston, TX; and ²The University of Texas Health Science Center at Houston School of Public Health, Houston, TX.

Received March 19, 2002; accepted July 20, 2002.

Address reprint requests to: Manal M. Hassan, M.D., M.P.H., Ph.D., The University of Texas M. D. Anderson Cancer Center, Department of Gastrointestinal Medical Oncology, Box 426, 1515 Holcombe Blvd., Houston, TX 77030. E-mail: mhassan@mdanderson.org; fax: 713-745-1163.

Copyright © 2002 by the American Association for the Study of Liver Diseases.

0270-9139/02/3605-0024\$35.00/0

doi:10.1053/jhep.2002.36780

and the Far East but a low incidence in the United States and Europe.¹ However, recent reports showed a statistically significant increase in HCC incidence in the United States over the past 2 decades.² Specifically, the incidence rose from 1.4 per 100,000 during the period from 1979 to 1980 to 2.4 per 100,000 during the period from 1991 to 1995.

Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are established etiologic agents of HCC worldwide.³ There is a remarkable geographic correlation between the incidence of HCC and the prevalence of HBV infection, with both occurring most often in China and Southeast Asia. Moreover, in Egypt, which has the highest prevalence of HCV infection worldwide,⁴ more than 60% of all HCC cases are attributed to HCV infection alone.⁵ However, in the United States, it has been estimated that only 9% and 20% of HCC cases are attributed to HCV and HBV infection, respectively.⁶

Facing the increase in HCC incidence in the United States and low proportion of HCC cases attributed to viral hepatitis, we embarked on this case-control study to

provide a more precise assessment of the potential risks of alcohol consumption and diabetes mellitus, in addition to HCV and HBV infection, and evaluate possible synergisms between the risk factors examined.

Patients and Methods

We conducted a hospital-based prospective case-control study in which a total of 345 subjects (115 histologically confirmed HCC cases and 230 non-liver cancer controls) were enrolled at The University of Texas M. D. Anderson Cancer Center.

The 115 patients having HCC received their diagnosis and underwent examination at M. D. Anderson Cancer Center from January 1994 to December 1995. We routinely obtained lists of newly diagnosed HCC cases from the Pathology Department and obtained permission from the attending physician to contact each of the patients. Two controls matched with each case according to sex, age (± 5 years), and year of diagnosis, were recruited within 15 days of case enrollment. All of the controls had histologically confirmed malignant neoplasms other than HCC, which included primary tumors of the gastrointestinal tract (44.3%), urogenital tract (18.7%), respiratory tract (17.8%), and skin (19.1%). Written informed consent to participate in the study was obtained from all of the subjects, and the study was approved by the Institutional Review Boards of The University of Texas-Houston and M. D. Anderson.

All of the subjects were interviewed using a questionnaire structured to yield basic demographic data and information about their history of liver disease, family history of liver cancer, history of smoking and drinking, and oral contraceptive use. Ten milliliters of blood was acquired from a peripheral vein in each participant and then separated. The samples were tested for the presence of antibodies to hepatitis C antigen (anti-HCV) using a second-generation enzyme-linked immunosorbent assay (ELISA; Abbott Laboratories, North Chicago, IL); positive results prompted confirmatory testing using a radioimmunoassay (RIBA; Chiron, Emoryville, CA). The samples were also tested for the presence of hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc) using the ELISA.

Smokers were defined as subjects who had smoked at least 100 cigarettes during their lifetime and subclassified into current smokers and ex-smokers.

The subjects were questioned about their lifetime history of consumption of beer, wine, and hard liquor. Alcohol drinkers were defined as subjects who consumed at least 100 servings of any of these alcoholic beverages during their lifetime. The alcohol drinkers were asked to estimate their prior frequency of alcohol consumption

and place it into 1 of 4 categories: (1) none or less than 100 servings ever, (2) infrequent use, (3) occasional use (at least one serving per week), and (4) regular use (at least one serving per day). The consumed-serving unit was defined as 12.0 oz for beer, 4.0 oz for wine, and 1.5 oz for hard liquor, which were all considered to be approximately equivalent to 12.0 mL of ethanol.⁷ Moreover, the subjects were classified according to the volume of total ethanol consumed in milliliters. This volume was obtained by multiplying the number of standardized servings by the number of milliliters of ethanol per drink and then adding the results for beer, wine, and hard liquor intake. In addition, all patients' medical records were reviewed to confirm alcohol consumption and to estimate the consumed amount. Heavy alcohol consumption was defined as consumption of ≥ 80 mL of ethanol per day.

Additionally, diagnosis of diabetes mellitus at least 1 year prior to that of cancer was confirmed by reviewing the medical records of both the HCC patients and controls. Information that confirmed the diagnosis at M. D. Anderson and the use of insulin treatment of diabetes mellitus was also extracted from the medical records. Only patients who received a diagnosis and treatment of diabetes mellitus were considered to be diabetic for this study.

All of the HCC patients were examined for the signs of cirrhosis during their clinical evaluation by a hepatobiliary oncologist and a hepatologist; these signs included manifestations related to portal hypertension, *e.g.*, ascites, bleeding from esophageal varices, and hepatic encephalopathy. Minor signs were also noted clinically, such as palmar erythema, spider angioma, and clubbing of the fingers. In addition, computed tomography scans of all of the HCC patients were reviewed for evidence of cirrhosis.⁸

The STATA software program (STATA Statistical Software, College Station, TX) was used for data management and statistical analysis. For univariate analysis, conditional logistic regression was used with maximum-likelihood estimate of parameter values for assessing the risk of HCC, while for multivariate analysis, the significant variables ($P < .05$) were modeled using conditional logistic regression. The adjusted odds ratio (OR) and 95% confidence interval (CI) for each variable were estimated using the logistic regression coefficient.

The synergisms between risk factors were evaluated by considering the additive model, since it is more appropriate to address biological interactions and public health concerns. Multiple logistic regression models were used to evaluate departure from additivity. By crossing alcohol consumption and diabetes mellitus and hepatitis viruses, dummy variables of 4 categories were obtained; 2 for the

Table 1. Characteristics of Study Subjects

Study Variables	HCC Patients (n = 115)		Controls (n = 230)		Total (n = 345)	
	n	%	n	%	n	%
Matched variables						
Sex						
Male	87	75.7	174	75.7	261	75.7
Female	28	24.3	56	24.3	84	24.3
Age (mean ± SD)	59.5 ± 10.7		59.1 ± 10.9		59.2 ± 10.9	
Unmatched variables						
Ethnicity						
White	77	66.9	183	79.6	260	75.4
Black	11	9.6	18	7.8	29	8.4
Hispanic	18	15.7	25	10.9	43	12.5
Asian	9	7.8	4	1.7	13	3.7
Education level						
≤High school	73	63.5	146	63.5	219	63.5
>High school	42	36.5	84	36.5	126	36.5

presence of each risk factor in the absence of other, 1 indicating the presence of joint risk factors, and 1 for unexposed to either risk factor. The latter was used as the reference category in the regression models. To assess deviation from the additive model of no interaction the Synergism index (S) and its 95% CI, proposed by Rothman was calculated²; $S = [OR_{11} - 1] / \{ [OR_{01} + OR_{10}] - 2 \}$. A value of S equal to unity was interpreted as indicative of additivity, whereas a value greater than unity was indicative of superadditivity and synergism.

Finally, the population attributable risk percentage (PAR%) for each of the statistical significant risk factors was calculated using the OR of that factor and its prevalence in the control group.

Results

Patient Characteristics

Table 1 shows the distribution of the HCC patients and controls according to sex, age, race, and level of education. Most of the subjects were white (HCC patients, 66.9%; controls, 79.6%); the rest were Hispanic (15.7%; 10.9%), black (9.6%; 7.8%), or Asian (7.8%; 1.7%). Also, Texas was the state of residence for 70.1% of the subjects. HCC patients and controls have a similar pattern of place of residence in the United States: most of the subjects were residents of Texas (HCC patients, 73%; controls, 70%).

Risk Factors for HCC Development

Univariate Analysis. A large statistically significant difference in the prevalence of anti-HCV positivity was observed between HCC patients and controls (22.6% and 2.2%, respectively) (Table 2). The univariate OR for the association of anti-HCV positivity with HCC was 16.4 (95% CI, 4.9-54.2). A large statistically significant difference in the prevalence of HBsAg positivity was also found between HCC patients (14.8%) and controls (0.9%). The univariate OR for the association between

Table 2. Risk Factors for HCC: Univariate (crude) and Multivariate (adjusted) OR and 95% CI

Risk Factor	HCC Patients (n = 115)		Controls (n = 230)		Crude OR (95% CI)	Adjusted OR (95% CI)*
	n	%	n	%		
HCV						
Anti-HCV-	89	77.4	225	97.8	1	-
Anti-HCV+	26	22.6	5	2.2	16.4 (4.9-54.2)	14.1 (4.0-49.7)
HBV						
HBsAg-/anti-HBc-	87	75.7	212	92.2	1	-
HBsAg-/anti-HBc+	11	9.6	16	6.9	0.6 (0.2-1.4)	-
HBsAg+/anti-HBc+	17	14.7	2	0.9	17.0 (3.9-73.6)	23.8 (3.9-141.6)
Alcohol consumption						
No	40	34.8	136	59.1	1	-
Yes	75	65.2	94	40.9	2.9 (1.8-4.9)	2.4 (1.3-4.4)
<80 mL ethanol per day	33	28.7	63	27.4	1.9 (1.3-3.4)	1.7 (0.9-3.7)
≥80 mL ethanol per day	42	36.5	31	13.5	8.3 (3.2-21.4)	4.5 (1.4-14.8)
Diabetes mellitus						
No	90	78.3	212	92.2	1	-
Yes	25	21.7	18	7.8	3.5 (1.7-7.1)	4.3 (1.9-9.9)
Insulin-dependent	10	8.7	9	3.9	2.9 (1.1-7.6)	4.4 (1.4-13.6)
Non-insulin-dependent	15	13	9	3.9	4.1 (1.7-9.8)	4.2 (1.5-11.9)
Cigarette smoking						
No	37	32.2	113	49.1	1	-
Yes	78	67.8	117	50.9	2.2 (1.3-3.7)	1.2 (0.6-2.4)

*Multivariate analysis using conditional logistic regression was applied to adjust for the significant risk factors including hepatitis virus infection (HBsAg+ and anti-HCV+), alcohol consumption, cigarette smoking, and diabetes mellitus.

HBsAg positivity and HCC was 17.0 (95% CI, 3.9-73.6), but the presence of anti-HBc positivity with HBsAg negativity did not translate into a higher risk of HCC than in those not having HBV markers (OR, 0.6; 95% CI, 0.2-1.4).

A history of lifetime consumption of more than 100 servings of alcohol was statistically significant (HCC patients, 65.2%; controls, 40.9%), resulting in an estimated univariate OR of 2.9 (95% CI, 1.8-4.9). HCC risk gradient (OR) increased in proportion to the increase in alcohol consumption. A total of 42 HCC patients and 31 controls were classified as daily drinkers who consumed ≥ 80 mL of ethanol per day; the univariate OR was 8.3 (95% CI, 3.2-21.4) (Table 2), whereas, for those who consumed less than 80 mL of ethanol per day, the univariate OR was 1.9 (95% CI, 1.3-3.4). Moreover, the risk of HCC in relation to preference for different types of alcoholic beverages and to the total amount of alcohol consumed was also assessed. A significant HCC risk association was shown in those who consumed ≥ 80 mL of ethanol per day having a preference for hard liquor (HCC patients, 13%; controls, 6.1% [OR, 5.2; 95% CI, 1.4-18.9]) or beer (HCC patients, 28.7%; controls 10.1% [OR, 5.8; 95% CI, 2.2-15.5]) compared with that in nondrinkers.

The presence of diabetes mellitus (insulin or non-insulin dependent) was also statistically associated with development of HCC. Table 2 shows that patients having diabetes mellitus had a risk of HCC 4 times greater than that of nondiabetic patients.

In addition, a history of ever smoking was reported by 67.8% of HCC patients and 50.9% of controls, yielding a crude OR of 2.2 (95% CI, 1.3-3.7). Current smokers were subdivided according to the number of packs smoked (>1 or ≤ 1 pack/d) and the duration of their habit (>20 or ≤ 20 years smoking). No consistent dose-dependent responses were found in current smokers who smoked more than one pack per day (OR, 0.8; 95% CI, 0.3-2.7) or smoked for more than 20 years (OR, 1.7; 95% CI, 0.7-4.6) when compared with nonsmokers.

Multivariate Analysis. The relationship observed in multivariate analysis did not meaningfully differ from those observed in univariate analysis, as the OR and 95% CI were 15.3 (4.3-54.4), 12.6 (2.5-63.1), and 2.4 (1.3-4.4) for anti-HCV positivity, HBsAg positivity, and alcohol consumption, respectively. Daily consumption of ≥ 80 mL of ethanol also resulted in a greater risk of HCC as shown in Table 2, while the adjusted OR for daily consumption of less than 80 mL of ethanol was not statistically significant. Additionally, the multivariate adjusted OR for the presence of diabetes mellitus was 4.3 (95% CI, 1.9-9.9); both insulin- and non-insulin-depen-

Table 3. Interaction of Heavy Alcohol Consumption (≥ 80 mL ethanol/d) With Chronic Hepatitis Virus Infection (HBV or HCV) and Diabetes Mellitus: Logistic Regression Analysis With Adjusted OR

Interaction Variables		β Coefficient (\pm SE)	P	OR (95% CI)	S (95% CI)*	
Virus	Alcohol					
	Negative	Negative		1		
	Positive	Negative	2.9 (0.79)	.0001	19.1 (4.1-89.1)	
	Negative	Positive	0.87 (0.32)	.006	2.4 (1.3-4.4)	
Diabetes	Alcohol					
	Negative	Negative		1		
	Positive	Negative	0.87 (0.33)	.008	2.4 (1.3-4.5)	
	Negative	Positive	0.95 (0.34)	.004	2.6 (1.4-4.9)	
Positive	Positive	2.3 (0.69)	.001	9.9 (2.5-39.3)	2.9 (1.3-4.6)	

*S = Synergy Index described by Rothman⁹ = $(OR_{11} - 1)/(OR_{01} + OR_{10} - 2)$, where OR_{11} = odds ratio of the joint effect of 2 risk factors; OR_{01} and OR_{10} = OR of each risk factor in the absence of the other.

dent diabetes mellitus subjects had a statistically significant risk when taking into consideration the effect of other significant risk factors. However, the adjusted OR for smoking dropped to a nonsignificant OR ($P > .05$) (Table 2).

Interaction Between HCC Risk Factors

Table 3 shows the independent and joint effects of heavy alcohol consumption (≥ 80 mL of ethanol per day), chronic hepatitis virus infection (HBV or HCV), and presence of diabetes mellitus. In particular, there was a synergism between hepatitis virus infection and daily consumption of ≥ 80 mL of ethanol adjusted for the effect of diabetes mellitus as well as between daily consumption of ≥ 80 mL of ethanol and diabetes mellitus adjusted for the effect of chronic hepatitis virus infection. Both of these interactions fit the assumption of additive scales. Using the OR as an estimate for the relative risk of disease development, the relative excess risk for patients having daily consumption of ≥ 80 mL of ethanol plus hepatitis virus infection or diabetes mellitus exceeded the sum of the relative excess risks for each risk factor alone: $53.9 - 1.0 > (19.1 - 1.0) + (2.4 - 1.0)$ and $9.9 - 1.0 > (2.6 - 1.0) + (2.4 - 1.0)$. The estimated synergism index (S) and its 95% CI was significantly greater than 1, indicating a departure from additivity in the joint effect of heavy alcohol consumption and hepatitis viruses ($S = 2.7$; 95% CI, 1.1-5.2) and in the joint effect of heavy alcohol consumption and diabetes mellitus ($S = 2.9$; 95% CI, 1.3-4.6). This indicated that heavy alcohol consumption, in addition to its own direct effects, may exacerbate the effect of diabetes mellitus and chronic hepatitis virus infection on chronic liver diseases and HCC.

Furthermore, the joint effect of HBV and HCV cannot be estimated after adjustment for the effect of alcohol

consumption and diabetes mellitus because of the small number of cases and lack of controls who had infection of both viruses in the absence of other risk factors.

Excluding Asian subjects from the data set and restricting the analysis to the non-Asians did not change the results of the multivariate analysis or the synergism of heavy alcohol consumption with hepatitis viruses or with diabetes mellitus.

In this study population we estimated that the PAR% explained by anti-HCV positivity, HBsAg positivity, daily alcohol consumption, and the presence of diabetes mellitus was 22%, 16%, 32%, and 20%, respectively.

Discussion

This is the largest case-control study, investigating the potential risk factors of HCC and interaction between these factors published to date in the United States. It shows that chronic HCV or HBV infection, alcohol consumption, and diabetes mellitus alone are significant risk factors for HCC development. Moreover, synergy between alcohol consumption and the other two risk factors exists in this study population. Specifically, chronic HCV and HBV infection increase the risk of HCC approximately 15-fold and 13-fold, respectively. Additionally, the presence of cirrhosis seems to play a central role in both viruses, because generally 60% to 80% of HCC cases are associated with the disease.¹⁰ In our study 69% of HCV-infected HCC patients and 35% of HBV-infected HCC patients had an established diagnosis of cirrhosis. Even though HCC developed in some HBV- or HCV-infected patients in the absence of cirrhosis, a direct carcinogenic role of HBV and HCV has been suggested. Integration of the HBV genome into cellular DNA has been found in the majority of HBV-induced HCC patients,¹¹ and the HCV core protein has been implicated to have a direct carcinogenic role in HCC development.^{12,13} Direct effects of both viruses may further contribute to histopathologic changes in the liver that induce HCC.

This study also shows that, like chronic hepatitis virus infection, alcohol consumption is an independent risk factor for HCC. Although the positive association between alcohol consumption and HCC was observed in the ever-drinking group in our study, we believe that the true risk of alcohol consumption is related to the heavy consumption category, defined as daily consumption of ≥ 80 mL of ethanol. After adjustment for all cofounders, subjects in this category continued to demonstrate this risk. The observation that HCC development is correlated with the magnitude of alcohol consumption is consistent with data from previous case-control studies^{14,15} and supported by the estimation by Pequignot et al.¹⁶ that the risk of alcohol-related liver diseases is increased 5

times with an increase in daily alcohol consumption of 80 to 160 mL/d and 25 times if daily alcohol consumption exceeds 160 mL. We do not believe, though, that the observed risk of HCC development can be attributed to recall bias in the liver cancer patients, because the link between alcohol consumption and cirrhosis and liver cancer is more specific than that with other cancers. However, both HCC patients and controls were interviewed by the same interviewer, and the information collected about alcohol consumption was confirmed using their medical records, as we observed no discrepancies between the two sources. Most importantly, the prevalence of heavy drinking in the control group was consistent with the prevalence in the US general population reported by Caetano et al.¹⁷

Considering the underlying mechanisms of alcohol-induced HCC, previous studies have suggested that heavy alcohol consumption could induce direct hepatic cellular injury and toxicity leading to the development of liver fibrosis and cirrhosis.¹⁸ Moreover, oxidation and metabolism of ethanol in the liver by microsomal enzymes may contribute to increased conversion of procarcinogens to carcinogens.¹⁹ Generation of acetaldehyde and oxygen-free radicals during ethanol metabolism is highly associated with the development of alcohol-related liver diseases through oxidative stress, which may directly induce liver-cell damage by initiating peroxidation of membrane lipids.²⁰ Indeed, one might speculate that the intensity of any of these mechanisms would increase with an increase in alcohol consumption and that the development of alcohol-related liver diseases is correlated with the magnitude of alcohol consumption.

Both simultaneously and consistent with previous reports²¹⁻²³ our data shows that diabetes mellitus is significantly related to the risk of HCC development after adjustment for the confounding variables, chronic virus infection and alcohol consumption. It is unlikely that the observed relationship between diabetes mellitus and HCC was due to information bias between HCC patients and controls. Indeed, both groups of subjects in the current hospital-based setting were equally evaluated for a past history of diabetes mellitus, type of diabetes, duration of disease, treatment intervention, and confirmed diagnosis at M. D. Anderson. It is also doubtful that such a chronic disease would be misreported in cancer patients. Moreover, the prevalence of diabetes mellitus in our study control group is consistent with the reported estimates in the American population generated from the National Health and Nutrition Examination Survey, Epidemiologic Follow-up Study from 1971-1991.²⁴ Possible biological mechanisms of the association between diabetes mellitus and HCC have been elucidated, and it has been

suggested that patients having non–insulin-dependent diabetes mellitus experience hyperinsulinemia and an elevated level of insulin-like growth factor I, which may stimulate liver-cell proliferation and increase susceptibility to cancer development.²⁵ However, in the study, both insulin- and non–insulin-dependent diabetes mellitus were significantly associated with HCC. Furthermore, the estimated ORs for both types of the disease have a similar magnitude, suggesting that other mechanisms may be relevant. For example, the metabolic effect of diabetes may increase the risk of HCC through nonalcoholic steatohepatitis and cryptogenic cirrhosis.²⁶ In fact, the majority of our HCC patients having diabetes mellitus had evidence of cirrhosis in the absence of viral hepatitis (67%) and alcohol consumption (62%).

The most important finding of this study is the synergy (excess over additivity) between heavy alcohol consumption and chronic hepatitis virus infection as well as diabetes mellitus independent of each other's effects, in the etiology of HCC, which would have both biologic and public health implications.^{27–29}

With respect to the interaction between heavy alcohol consumption and chronic hepatitis virus infection, both risk factors are significantly associated with cirrhosis. One common mechanism through which both infection and alcohol consumption may contribute to cirrhosis as well as hepatocarcinogenesis is oxidative stress.^{30,31} As with HCV, it has been suggested that the increased free radical levels induced by chronic alcohol consumption may promote HBV gene expression and favor the establishment of a chronic HBV carrier state following acute infection or reactivation of virus replication in a previously established infection.³² An increased HBV or HCV load in liver cells may further intensify liver disease chronicity induced by heavy alcohol consumption toward advanced cirrhosis and/or liver cancer.

The mechanisms through which heavy alcohol consumption and diabetes mellitus promote the development of HCC are unknown. However, many researchers have supported a role for oxidative stress in the pathogenesis and complications of diabetes mellitus as a result of hyperglycemia.^{33–37} In diabetes mellitus patients an increased blood glucose level may stimulate glycosylation of proteins, including hemoglobin, leading to an increase in the release of iron from hemoglobin and further production of free radicals causing oxidative stress.^{34,35} A recent study in the United States found a high concentration of serum ferritin in patients having diabetes mellitus, suggesting that it may reflect increased body iron stores.³⁵ The fact that iron is a powerful pro-oxidant and that oxidative stress is increased in impaired glucose tolerance states suggests a possible role for oxidative stress in patho-

genesis of diabetes mellitus and its complications, such as cirrhosis. It is also possible that alcohol-induced oxidative stress may increase the susceptibility of patients having diabetes mellitus to cirrhosis, DNA damage, and HCC development. Meanwhile, alcohol-induced oxidative stress may increase the susceptibility of diabetes mellitus development, which may explain the initial observation in the American study²¹ followed by the Italian one,²² that early onset of diabetes is related to HCC development. The role of oxidative stress in the interaction between alcohol consumption and other risk factors is currently under investigation at M. D. Anderson.

In respect to the synergism between HCV and HBV, the small sample size of the study precludes a meaningful examination of joint infections with or without diabetes mellitus on the risk of HCC.

The present study carries some weaknesses. Because it is a hospital-based and not a population-based study, potential sources of bias caused by errors in determination of the study exposures or in ascertainment of study subjects may exist. This may explain our results of no significant effect of cigarette smoking in HCC etiology. On the other hand, as we stated before, HBV and HCV markers were tested at one laboratory for HCC patients and controls, whereas information of other risk factors was collected from subjects as well as from their medical records. Most importantly, the prevalence of the significant risk factors in the control subjects was comparable to that of the general population. Additionally, we do not believe that we have introduced an ascertainment bias due to misdiagnosis, differential referral patterns, or selection bias. Both HCC patients and controls were pathologically and newly diagnosed patients, who were prospectively ascertained from the outpatient clinics at M. D. Anderson. Moreover, controls were selected from a wide variety of admission diagnoses, all of which are believed to be neither caused by nor prevented by any of the study risk factors. Although, it is possible that some cancer types of the controls are related to alcohol; however, this may underestimate the apparent values of the current study ORs. Furthermore, to overcome possible bias caused by the referral pattern, we choose to use hospital controls and not population-based controls who were age and sex matched to the HCC patients.³⁸ Conditional logistic regression was performed to keep the matching during the statistical analysis.

To the extent that the proportions of the significant risk factors reflect the same pattern of the United States general population, the present study gives an accurate estimate of the independent effect of HBV and HCV infection, heavy alcohol consumption, and a history of diabetes mellitus on HCC development in the United States. However, the absence of the smoking-HCC asso-

ciation is more likely related to the use of hospital controls; we believe that the use of a population-based design is more appropriate to comment on the smoking effect.²¹

Despite the overwhelming evidence showing that chronic HBV and HCV infection are important causes of HCC, neither of these viruses explains the majority of HCC cases in the American patients in this study. However, heavy alcohol consumption contributed to almost a third of our HCC cases, whereas 10% of the cases were not explained by any of our study risk factors. It is possible that genetic metabolic disorders, particularly alpha anti-trypsin deficiency and hereditary hemochromatosis, were undetected risk factors for HCC in this study. Further studies are needed to determine the independent role of these disorders and their interaction with hepatitis viruses, alcohol, and diabetes mellitus. Moreover, public health considerations should prompt the study of HCC prevention strategies among high-risk individuals. It would seem very prudent for patients having chronic HBV or HCV infection, or diabetes mellitus to consider abstaining from alcohol consumption to reduce the effect modification of alcohol when combined with these risk factors. Finally, if indeed oxidative stress mediates the interaction between some of the risk factors, large studies of antioxidant supplement use may be needed to test their potential preventive role in high-risk populations.

References

- McGlynn KA, Tsao L, Hsing AW, Devesa SS, Fraumeni JF Jr. International trends and patterns of primary liver cancer. *Int J Cancer* 2000;94:290-296.
- El Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N Engl J Med* 1999;340:745-750.
- International Agency for Research on Cancer (IARC). *Hepatitis Viruses*. Vol 59. Lyon, France: IARC Monogr Eval Carcinog Risks Hum, 1994.
- Habib M, Mohamed MK, Abdel-Aziz F, Magder LS, Abdel-Hamid M, Gamil F, Madkour S, et al. Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. *HEPATOLOGY* 2001;33:248-253.
- Hassan MM, Zaghoul AS, El Serag HB, Soliman O, Patt YZ, Chappell CL, Beasley RP, et al. The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. *J Clin Gastroenterol* 2001;33:123-126.
- Yu MC, Tong MJ, Coursaget P, Ross RK, Govindarajan S, Henderson BE. Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. *J Natl Cancer Inst* 1990;82:1038-1041.
- International Agency for Research on Cancer (IARC). *Alcohol Drinking*. Vol 44. Lyon, France: IARC Monogr Eval Carcinog Risks Hum, 1988.
- Brown JJ, Naylor MJ, Yagan N. Imaging of hepatic cirrhosis. *Radiology* 1997;202:1-16.
- Rothman KJ. The estimation of synergy or antagonism. *Am J Epidemiol* 1976;103:506-511.
- Bosch FX, Munoz N. Hepatocellular Carcinoma in the World: epidemiologic questions. In: Tabor E, Di Bisceglie AM, Purcell RH, eds. *Etiology, Pathology, and Treatment of Hepatocellular Carcinoma in North America*. Houston, Texas: Gulf Publishing Company, 1991;35-54.
- Kew MC. Hepatitis B and C viruses and hepatocellular carcinoma. *Clin Lab Med* 1996;16:395-406.
- Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 1998;4:1065-1067.
- Ray RB, Meyer K, Ray R. Suppression of apoptotic cell death by hepatitis C virus core protein. *Virology* 1996;226:176-182.
- Austin H, Delzell E, Grufferman S, Levine R, Morrison AS, Stolley PD, Cole P. A case-control study of hepatocellular carcinoma and the hepatitis B virus, cigarette smoking, and alcohol consumption. *Cancer Res* 1986;46:962-966.
- Tagger A, Donato F, Ribero ML, Chiesa R, Portera G, Gelatti U, Albertini A, et al. Case-control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC Study. *Int J Cancer* 1999;81:695-699.
- Peguignot G, Chabert C, Eydoux H, Corcowl MA. Increased risk of liver cirrhosis with intake of alcohol. *Rev Alcoholism* 1974;20:191-202.
- Caetano R, Clark CL. Trends in alcohol consumption patterns among whites, blacks and Hispanics: 1984 and 1995. *J Stud Alcohol* 1998;59:659-668.
- Batey RG, Burns T, Benson RJ, Byrh K. Alcohol consumption and the risk of cirrhosis. *Med J Aust* 1992;156:413-416.
- Lieber CS, Seitz HK, Garro AJ, Worner TM. Alcohol-related diseases and carcinogenesis. *Cancer Res* 1979;39:2863-2886.
- Lieber CS. Mechanism of ethanol induced hepatic injury. *Pharmacol Ther* 1990;46:1-41.
- Yu MC, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. *J Natl Cancer Inst* 1991;83:1820-1826.
- La Vecchia C, Negri E, Decarli A, Franceschi S. Diabetes mellitus and the risk of primary liver cancer. *Int J Cancer* 1997;73:204-207.
- Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. *J Natl Cancer Inst* 2000;92:1096-1099.
- Resnick HE, Valsania P, Halter JB, Lin X. Differential effects of BMI on diabetes risk among black and white Americans. *Diabetes Care* 1998;21:1828-1835.
- Moore MA, Park CB, Tsuda H. Implications of the hyperinsulinaemia-diabetes-cancer link for preventive efforts. *Eur J Cancer Prev* 1998;7:89-107.
- Silverman JF, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, Norris HT, et al. Liver pathology in morbidly obese patients with and without diabetes. *Am J Gastroenterol* 1990;85:1349-1355.
- Breslow NE, Day NE. The analysis of case-control studies. In: *International Agency for Research on Cancer. Statistical methods in cancer research*. Vol 1. Lyon, France: IARC, 1980:42-81.
- Rothman KJ. Interactions between causes. In: *Modern Epidemiology*. Boston: Little Brown and Company, 1986:311-326.
- Kleinbaum DG, Kupper LL, Morgenstern H. Interactions. Effect modification and synergism. In: *Epidemiologic Research*. New York: Van Nostrand Reinhold, 1982:407-417.
- Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Barton S, Lemoch H, Sudhop T, et al. Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection. *Eur J Clin Nutr* 1997;51:266-272.
- Zhang W, Cox AG, Taylor EW. Hepatitis C virus encodes a selenium-dependent glutathione peroxidase gene. Implications for oxidative stress as a risk factor in progression to hepatocellular carcinoma. *Med Klin* 1999;94(Suppl 3):2-6.
- Brecht C, Nalpas B, Feitelson MA. Interactions between alcohol and hepatitis viruses in the liver. *Clin Lab Med* 1996;16:273-287.
- Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes Metab Res Rev* 2001;17:189-212.

34. Kar M, Chakraborti AS. Release of iron from haemoglobin—a possible source of free radicals in diabetes mellitus. *Indian J Exp Biol* 1999;37:190-192.
35. Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 1999;22:1978-1983.
36. Oberley LW. Free radicals and diabetes. *Free Radic Biol Med* 1988;5:113-124.
37. Gillery P, Monboisse JC, Maquart FX, Borel JP. Does oxygen free radical increased formation explain long term complications of diabetes mellitus? *Med Hypotheses* 1989;29:47-50.
38. Schlesselman JJ, Stolley PD. Sources of bias. In: Schlesselman JJ, ed. *Case-Control Studies Design, Conduct, Analysis*. New York: Oxford, 1982: 124-143.