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Correlations of Chronic Hepatitis B Virus Infection and Cigarette Smoking with Elevated Expression of neu Oncoprotein in the Development of Hepatocellular Carcinoma[1]

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ABSTRACT

To investigate the potential role of neu oncogene expression in hepatocarcinogenesis, a nested case-control study was conducted within a cohort of 9691 male adults in Taiwan. Blood samples of study subjects were collected during 1984–1986 and frozen at −30°C until subsequent analysis. The neu oncoprotein level in the stored serum was measured by an enzyme-linked immunosorbent assay for 27 cases of newly developed hepatocellular carcinoma (HCC), 12 liver cirrhosis cases, and 40 healthy controls. The mean level of neu oncoprotein was significantly higher in HCC and liver cirrhosis cases than in controls. The risk of HCC increased significantly with increasing serum level of neu oncoprotein (trend test, $P = 0.02$). The proportion of subjects having an elevated serum level of neu oncoprotein, defined as a level greater than the mean level of all controls, was significantly higher among asymptomatic HBsAg carriers than noncarriers ($P = 0.05$), showing a multivariate-adjusted odds ratio of 4.0. Among HCC cases, a strong association was observed between cigarette smoking and elevated prediagnostic serum level of neu oncoprotein. The association remained highly significant ($P = 0.017$) even when adjustment was made for potential confounders. The multivariate-adjusted odds ratio of having an elevated serum level of neu oncoprotein, defined as a level greater than the mean plus 1 SD of control levels, for HCC cases who smoked more than 10 cigarettes a day was as high as 386.5 compared with the cases who smoked less than 10 cigarettes a day or nonsmoking cases. The results suggest that both HBsAg carrier status and cigarette smoking are related to the increased expression of neu oncogene, and cigarette smoking seems to play a significant role in the latter stages of hepatocarcinogenesis. There was no association between alcohol drinking and serum neu oncoprotein level.

INTRODUCTION

Liver cancer, largely HCC,[3] is one of the most common fatal malignant neoplasms in the world. It ranks as the first leading cause of death due to cancer for men and the third for women in Taiwan (1). Epidemiological studies have provided strong evidence for the link between chronic HBV infection and the induction of HCC (2, 3). A variety of mechanisms by which the virus may contribute to the pathogenesis of HCC have also been proposed on the basis of molecular biological and animal studies (4–14). However, many uncertainties about the mechanism of HBV-related hepatocarcinogenesis in humans still exist (15).

The evolution of HCC is a pathogenic process involving multiple stages associated with exposures to multiple risk factors. In addition to HBV chronic carrier status, many possible etiological factors, including hepatitis C virus (16, 17), aflatoxin exposure (18, 19), alcohol consumption, cigarette smoking (2, 3, 20–23), and elevated serum level of endogenous testosterone (24), have also been implicated in the etiology of HCC. Among these environmental HCC risk factors other than HBV, cigarette smoking, and alcohol consumption are the most common in the general population. Their relationships to risk of HCC have been documented in Taiwan (2, 3) and other countries (20–23). Alcohol drinking has long been postulated as a HCC risk factor because of its relationship to the development of liver cirrhosis. Cigarette smoke contains many chemical carcinogens associated with tumor initiation and promotion. A wide range of human malignancies have been associated with cigarette smoking. However, the apparent carcinogenic effect of cigarette smoking differs substantially for various cancer sites. There is a strong association between cigarette smoking and lung cancer with a relative risk as high as 10 (25), while the relative HCC risk for cigarette smoking is only 2–3-fold (2, 3, 21–23). The role of cigarette smoking in hepatocarcinogenesis continues to challenge epidemiologists interested in the etiology of HCC.

Genome alterations which affect the expression or function of genes controlling cell growth and differentiation have been considered to be the main cause of cancer (26). Molecular research focusing on the identification of genes that are altered in various tumor types and the elucidation of their roles in tumorigenesis have contributed substantially to our understanding of the carcinogenic process. Among oncogenes known to be important in the development of human malignancies, the *neu* oncogene (also known as *HER-2* or *c-erbB-2*) has received much attention for its role in mammary carcinogenesis (27, 28). The *neu* gene encodes a M_r 185,000 transmembrane protein which is structurally and functionally similar to the epidermal growth factor receptor (29). Amplification and/or overexpression of the *neu* oncogene have been implicated in cell transformation and animal tumorigenesis (30, 31) and in a variety of human cancers, including carcinomas of the breast, ovary, stomach, colon, lung, and salivary gland (27, 28, 32–35). Clinical studies on breast cancer have suggested a role of *neu* oncogene in the neoplastic process (27, 28). Recently, overexpression of this gene in a significant proportion of HCC and liver cirrhosis patients has also been reported from a study of immunohistochemical staining of a limited number of liver specimens (36). This prompted us to further study the expression of *neu* oncogene during HCC development.

Recent studies have demonstrated that the extracellular domain of the neu oncoprotein is released and is measurable in the serum of patients with various types of cancers using enzyme-linked immunosorbent assay (37–40). It was also indicated that elevated serum levels of neu oncoprotein are related to the increased expression of this oncoprotein in tumor tissues (39, 41). This suggests that the neu oncoprotein level in the serum can be used as an indicator for assessing the expression of *neu* oncogene. We have previously reported an increased level of neu oncoprotein in the serum from the individuals who subsequently developed HCC before the clinical diagnosis of the disease was made (38). In our current investigation,

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[3] The abbreviations used are: HCC, hepatocellular carcinoma; CI, confidence interval; HBV, hepatitis B virus.

we applied the same approach to quantitate the neu oncoprotein in the serum samples from the individuals who subsequently developed HCC as well as from liver cirrhosis patients and healthy controls in a large scale cohort study. We also extended our study to the identification of putative HCC risk factors associated with the overexpression of *neu* oncogene. The role of *neu* oncogene overexpression in human hepatocarcinogenesis will be discussed in this paper.

SUBJECTS AND METHODS

This nested case-control study was conducted within a cohort of 9691 male adults whose blood samples were collected in 1984–1986 and stored at −30°C for subsequent assays (24). At the time of blood collection, each study subject was also personally interviewed according to a structured questionnaire, collecting information of demographic characteristics, long term habits of cigarette smoking and alcohol drinking, as well as personal and family history of various liver diseases and cancers diagnosed by physicians. A habit of cigarette smoking was defined as having smoked cigarettes more than 4 days a week for at least 6 months. Alcohol drinking was defined as having drunk alcohol more than 3 days a week for at least 6 months.

The participants of this study cohort were followed by telephone interviews and home visits annually until March 1990. The causes of death of study subjects were investigated through data linkage and matching with a computerized file of national death certification system in Taiwan. After an average follow-up period of 4.6 years, a total of 36 newly diagnosed liver cancer cases were identified. As the stored serum samples of 9 eligible liver cancer cases were not available for analysis of neu oncoprotein, a total of 27 liver cancer cases served as the case group in the present study. The 27 liver cancer cases included in this study and the other 9 eligible cases not included were comparable with respect to all the demographic characteristics and distributions of potential HCC risk factors. It seems reasonable to assume that the cases included in this study were representative of the eligible cases. The most accepted diagnostic criteria for HCC in Taiwan are either pathological examinations or elevated α-fetoprotein level (400 ng/ml or greater) combined with at least one positive image on angiography, sonography, liver scan, and/or computerized tomography scans. Among the 27 liver cancer cases with available sera in this study, 19 (70%) were double-checked with either hospital records or data files of the National Cancer Registry in Taiwan (about one-half were diagnosed pathologically and the other half by elevated α-fetoprotein and liver images), whereas 8 were identified only by death certificates.

The subjects of this study were selected from a previous nested case-control study on the role of elevated serum testosterone in the development of HCC (24). All the controls who were matched to the cases in the previous study and had sufficient serum samples for testing were included as controls in this study. A total of 40 controls were thus selected for the 27 liver cancer cases. The controls were individually matched to the cases on age (within 5 years), date of questionnaire interview and blood collection (within 3 months), and residential townships. They were originally selected from all the cohort members who were alive and free of cancer on the dates at the diagnosis of liver cancer cases to whom they were matched. Two controls were matched to each of the 13 liver cancer cases, and one to each of the other 14 cases. As 2 HBsAg-positive and 2 HBsAg-negative controls were selected for each liver cancer case in the previous study, approximately equal numbers of HBsAg-positive and HBsAg-negative controls were included in this study. This will make the comparison of serum levels of neu oncoprotein between these 2 groups more efficient. Since only one HBsAg-positive control had a past history of liver disease at the blood collection, most of the HBsAg-positive controls in this study were asymptomatic chronic HBV carriers.

HCC usually occurs in cirrhotic liver, and liver cirrhosis has long been regarded as the premalignant lesion of HCC. Evaluation of the *neu* oncogene expression in liver cirrhosis cases will help understand the chronological changes of this gene expression in the pathogenesis of HCC. A total of 12 study subjects who died from liver cirrhosis during the follow-up period were also included in the analyses. They were all HBsAg-positive. As these cirrhotic patients died within 5 years after recruitment, with only 3 reporting a history of physician-diagnosed liver cirrhosis in the baseline interview, these patients probably already had subclinical liver cirrhosis at the time of blood collection.

All participants in the study cohort were tested for their HBsAg carrier status by reverse passive hemagglutination assay at the initial recruitment examination. Liver cancer cases and matched controls who were HBsAg-negative in the initial reverse passive hemagglutination assay were retested by radioimmunoassay using commercial kits (Abbott Laboratories, North Chicago, IL). Serum levels of the neu oncoprotein were assayed in a manner blinded with respect to disease status by a sandwich enzyme-linked immunosorbent assay (Oncogene Science, Inc., Uniondale, NY) using 2 different mouse monoclonal antibodies that react with independent epitopes on the extracellular domain of the p185 neu oncopeptide, as described in detail previously (38, 39). All serum samples were measured for the neu oncoprotein levels on the same day.

Since the distribution of serum neu oncoprotein levels showed no substantial deviation from normal distribution judging from histogram and coefficients of skewedness and kurtosis, a t test was applied to compare the mean serum levels of neu oncoprotein between groups. Categorical data analysis was also performed to assess the dose-response relationship between the serum level of neu oncoprotein and risk of HCC, and the association between elevated serum level of this protein and HCC risk factors. In the stratified data analysis, the serum level of neu oncoprotein was dichotomized or trichotomized based on the distribution for controls. Mantel's χ^2 test for a trend was used to examine the dose-response relationship. Correlation of the quantity of cigarettes smoked per day with serum neu oncoprotein levels was further examined by Spearman's rank correlation coefficient. Multivariate-adjusted odds ratios and their 95% CI were estimated by modeling the data through unconditional logistic regression. All statistical tests were based on 2-tailed probability.

RESULTS

There were 16 HBsAg-positive and 11 HBsAg-negative liver cancer cases. The age of liver cancer patients at initial recruitment examination ranged from 40 to 74 years. The mean ages ± SD at recruitment were 58.6 ± 9.7 and 58.2 ± 9.0 years for liver cancer cases and matched controls, respectively. The age of patients who died from liver cirrhosis ranged from 38 to 66 years, with a mean ± SD of 52.8 ± 10.6 years. They were younger than the liver cancer cases and controls.

Elevated Serum Level of neu Oncoprotein and Risk of HCC. The means ± SD of neu oncoprotein levels in serum were 1324.4 ± 590.8, 1482.8 ± 656.5, and 985.0 ± 516.7 human neu units/ml, respectively, for liver cancer cases, liver cirrhosis cases, and controls. The differences in mean levels of neu oncoprotein were statistically significant between liver cancer cases and controls ($P = 0.02$), and between liver cirrhosis cases and controls ($P = 0.008$). There was no significant difference in mean levels of neu oncoprotein between liver cancer and liver cirrhosis cases.

Table 1 shows a clear dose-response relationship between the serum levels of neu oncoprotein and risk of HCC (trend test, $P = 0.02$). As compared with the individuals who had a neu oncoprotein level below the mean level of all controls, the odds ratio of developing HCC was 2.0 (95% CI = 0.5–7.3) for those who had a neu oncoprotein level greater than the mean but less than the mean plus 1 SD of control levels. The odds ratio increased up to 6.4 (95%

Table 1 *Comparisons of the serum levels of neu oncoprotein among 27 liver cancer cases, 12 liver cirrhosis cases, and 40 controls*

Serum neu oncoprotein levels (HNU[a]/ml)	Control	Liver cancer		Liver cirrhosis	
	(*n*)	*n*	OR (95% CI)	*n*	OR (95% CI)
≤985.0[b]	20	7	1.0[c]	2	1.0[c]
>985.0 and ≤1501.7	16	11	2.0 (0.5–7.3)	4	2.5 (0.3–23.0)
>1501.7	4	9	6.4 (1.2–36.8)	6	15.0 (1.7–174.6)

[a] HNU, human neu unit; OR, odds ratio.
[b] The serum levels of neu oncoprotein were trichotomized according to the mean and the mean plus 1 SD of the levels of all controls.
[c] Mantel's χ^2 test for a trend was significant.

CI = 1.2–36.8) for those who had a neu oncoprotein level greater than the mean plus 1 SD of control levels. A strong association was also observed between liver cirrhosis and elevated serum level of neu oncoprotein. The percentage of liver cirrhosis cases who had serum neu oncoprotein levels greater than the mean but less than the mean plus 1 SD of control levels was 33.3% (4 of 12) compared with 40% (16 of 40) for the controls, the percentage of the liver cirrhosis cases who had serum neu oncoprotein levels greater than the mean plus 1 SD of control levels was 50% (6 of 12) compared with 10% (4 of 40) for the controls. When an analysis was performed for the association between liver cirrhosis and serum neu oncoprotein level, a highly significant trend for the odds ratios was noted ($P = 0.006$).

There were 13 liver cancer cases diagnosed within 2 years and 14 cases diagnosed more than 2 years after blood collection. The distribution of serum neu oncoprotein level in the early onset cases was similar to that of the late onset cases.

Serum Level of neu Oncoprotein and HCC Risk Factors. In this study, the serum samples of the liver cancer cases were collected up to 5 years prior to the occurrence of clinical disease. It seems reasonable to speculate that the liver cancer cases had preneoplastic lesions or small tumors at the time of blood collection, but they were asymptomatic. Since chronic HBV infection, cigarette smoking, and alcohol drinking may have a different effect on the expression of *neu* oncogene at different stages of neoplastic process, the associations of these factors with serum neu oncoprotein levels were evaluated in controls and liver cancer cases, separately.

Table 2 shows the association with elevated serum level of neu oncoprotein for HBsAg carrier status, alcohol drinking, and cigarette smoking among controls. More HBsAg-positive controls (63.6%) than HBsAg-negative controls (33.3%) had an elevated serum level of neu oncoprotein, defined as a level greater than the mean level of all controls, giving an odds ratio of 3.5 (95% CI = 0.9–13.0, $P = 0.056$). The association between HBsAg carrier status and elevated serum level of neu oncoprotein was statistically significant after adjustment for age, cigarette smoking, and alcohol drinking ($P = 0.05$). The multivariate-adjusted odds ratio of having an elevated serum level of neu oncoprotein for HBsAg carrier status was 4.0 (95% CI = 1.0–16.0). Alcohol drinking and cigarette smoking were not significantly associated with the elevated neu oncoprotein level among controls.

The associations of elevated prediagnostic serum level of neu oncoprotein with HBsAg carrier status, alcohol drinking, and cigarette smoking among liver cancer cases are depicted in Table 3. The percentage of liver cancer cases who had a serum neu oncoprotein level greater than the mean plus 1 SD of control levels was 43.8% for HBsAg-positive cases and 18.2% for HBsAg-negative cases, showing an odds ratio of 3.5 (95% CI = 0.6–21.7). But the association between HBsAg carrier status and elevated serum level of neu oncoprotein was not statistically significant. No association with the elevated serum neu oncoprotein level was observed for alcohol drinking.

Table 2 *Associations between putative HCC risk factors and elevated serum level (human neu unit/ml) of neu oncoprotein in 40 controls*

Variable	Group	Total no.	% with elevated neu oncoprotein[a]	Odds ratio	P value[b]
HBsAg	Negative	18	33.3	1.0	
	Positive	22	63.6	3.5	0.056
Alcohol drinking habit	No	33	48.5	1.0	
	Yes	7	57.1	1.4	0.50
Cigarette smoking (cigarettes/day)	≤20	36	47.2	1.0	
	>20	4	75.0	3.4	0.30

[a] Elevated serum level of neu oncoprotein was defined as a level greater than the mean level of all controls.
[b] Statistical analyses were performed by Fisher's exact test.

Table 3 *Associations between putative HCC risk factors and elevated prediagnostic serum level of neu oncoprotein (human neu unit/ml) in 27 liver cancer cases*

Variable	Group	Total no.	% with elevated neu oncoprotein[a]	Odds ratio	P value[b]
HBsAg	Negative	11	18.2	1.0	
	Positive	16	43.8	3.5	0.17
Alcohol drinking habit	No	20	30.0	1.0	
	Yes	7	42.9	1.8	0.43
Cigarette smoking (cigarettes/day)	≤10	15	6.7	1.0	
	>10	12	66.7	28.0	0.001

[a] Elevated serum level of neu oncoprotein was defined as a level greater than the mean plus 1 SD of control levels.
[b] Statistical analyses were performed by Fisher's exact test.

A significantly higher proportion of liver cancer cases who smoked more than 10 cigarettes a day had elevated serum levels of neu oncoprotein (66.7%) than those who smoked less than 10 cigarettes a day or nonsmokers (6.7%), giving an odds ratio of 28.0 (95% CI = 2.7–295.7, $P = 0.001$). The association of cigarette smoking with elevated serum levels of neu oncoprotein was highly significant after adjustment for potential confounders including age, onset time of liver cancer, HBsAg carrier status, and alcohol drinking ($P = 0.017$). The multivariate-adjusted odds ratio for those who smoked more than 10 cigarettes a day was as high as 386.5 (95% CI = 3.0–50,531.3) compared with those who smoked less than 10 cigarettes a day or nonsmokers. There was no association between elevated serum level of neu oncoprotein and age, onset time of liver cancer, HBsAg carrier status, and alcohol drinking among liver cancer cases.

The correlation between the quantity of cigarettes consumed per day and serum neu oncoprotein level was further examined using Spearman's rank correlation coefficient. This analysis showed a significant correlation between cigarettes smoked per day and prediagnostic serum levels of neu oncoprotein among liver cancer cases ($r = 0.58$, $P = 0.0015$). There was essentially no correlation between cigarettes smoked per day and the serum neu oncoprotein levels among controls ($r = -0.14$, $P = 0.4$).

DISCUSSION

Although various oncogenes in hepatocarcinogenesis have been investigated in rat liver cancers and rat or human liver cancer cell lines (42–45), only a few studies have been done on oncogenes in human HCC (36, 46, 47). This nested case-control study suggests an association between elevated prediagnostic serum levels of neu oncoprotein and the development of HCC. However, the exact role for overexpression of neu oncoprotein in hepatocarcinogenesis is unknown. Because the neu oncoprotein is presumed to be a growth factor receptor, the elevated expression of neu oncoprotein may simply reflect the increased cell proliferation in preneoplastic liver. Alternatively, the increased expression may result from exposure to carcinogens in the course of hepatocarcinogenesis. The close association between elevated serum level of neu oncoprotein and some putative HCC risk factors observed in this study is compatible with the latter hypothesis and suggests a role for *neu* oncogene in the induction of HCC in humans.

HBsAg carrier status has been well-documented as the most important environmental risk factor of HCC, with a relative risk 10 to 20 times higher for HBsAg carriers as compared with noncarriers (2, 3). Based on the strong epidemiologic association between HBV and HCC, the role of HBV in hepatocarcinogenesis has been extensively explored in molecular and animal studies (4–14). However, many uncertainties about the mechanism of HBV-related human hepatocarcinogenesis remain (15). In this study, a significantly higher pro-

portion of asymptomatic HBsAg carriers were found to have elevated serum levels of neu oncoprotein than noncarriers. Whether the increased neu expression in chronic HBV carriers plays a relatively early role in HBV-modulated hepatocarcinogenesis needs to be established.

The mechanism by which chronic HBV infection causes an increased serum level of neu oncoprotein remains to be elucidated. In view of observations from previous experimental work, it is likely that multiple independent or interdependent mechanisms are involved. HBV has been proposed to act as an insertional mutagen that may alter cellular gene structures and/or functions (4–9). HBV X-gene product has been demonstrated to be a transcriptional activator able to stimulate a variety of cellular promoters which do not share a common *cis*-regulatory element (10–12). As the X protein has the ability to affect signal transduction (12), it may also perturb the flow of information in the cell and thus affect the expression of many genes. Persistent infection with HBV is also associated with chronic phasic necroinflammation and regenerative hyperplasia of the liver (48). The continuous hepatocyte regeneration caused by prolonged hepatocellular injury has been shown to initiate a cascade of events associated with the pathogenesis of HCC including transcriptional deregulation and chromosomal aberrations in HBV transgenic mouse model (14). In this study, we also observed a close relationship between liver cirrhosis and elevated serum level of neu oncoprotein. This result provides evidence supporting the theory that prolonged hepatocellular injury caused by chronic HBV infection results in overexpression of neu oncoprotein. Many oncogenes may be expected to be overexpressed in regenerating livers (42). Significantly higher levels of c-*myc* gene expression have also been observed in liver cirrhotic tissues (47). As liver cirrhosis has long been regarded as the premalignant lesion of HCC, the elucidation of oncogenes associated with liver cirrhosis may help us to understand the multistep hepatocarcinogenesis at the molecular level in humans.

A number of case-control studies have investigated the association between cigarette smoking and HCC. Most of the studies reported a relative risk of 2–3 for cigarette smoking (3, 21, 23). The association between cigarette smoking and HCC has also been documented in 2 large-scale cohort studies (2, 22). An unsolved issue is the mechanism of action of cigarette smoking. The most marked finding of this study was the strong association between elevated prediagnostic serum levels of neu oncoprotein and cigarette smoking among HCC patients. Although a recent smoking habit may be changed due to intercurrent illness and thus may result in a misclassification of exposure to smoking, the misclassification may be nondifferential. In other words, it may not be associated with the serum levels of neu oncoprotein. Even if cigarette-smoking liver cancer patients with high neu oncoprotein levels may be more likely to quit smoking than smoking patients with low neu oncoprotein levels due to unidentified symptoms, this may bias the effect of smoking toward the null only. This means that the association between cigarettes smoked per day and serum neu oncoprotein in this study was estimated under a conservative circumstance. In contrast, there was no significant association between cigarette smoking and elevated serum level of neu oncoprotein among controls. These results suggest that cigarette smoking may play a role in the late promotion and/or progression stages of HCC development. A significant etiological role of cigarette smoking in the development of HCC from liver cirrhosis was recently reported by a prospective study of Japanese patients with chronic liver disease (17). In this study, the relative risk of developing HCC from liver cirrhosis was much higher for current smokers than for exsmokers. This result is compatible with our hypothesis that cigarette smoking may act as a strong promoter or progressor in the late stages of hepatocarcinogenesis. It will be of interest to determine whether liver cirrhosis patients who smoked cigarettes had a higher serum level of neu oncoprotein than nonsmoking patients. However, we were not able to test the hypothesis in this study because of the small number of liver cirrhosis cases. Further study on this hypothesis may help clarify the role of cigarette smoking in the development of HCC from liver cirrhosis.

It has been reported that the overexpression of *neu* oncogene can be caused by gene amplification and gene deregulation through epigenetic mechanisms (49). Activation of the transforming potential of *neu* protooncogene caused by point mutation have also been shown *in vitro* and in chemically induced rat tumors (31, 50, 51). Cigarette smoke is a mixture of over 3800 chemical substances containing at least 43 known carcinogens. Many substances in cigarette smoke can be metabolized to form reactive intermediate products which can bind to DNA (52). The precise mechanism for cigarette smoking-associated overexpression of *neu* oncogene to induce HCC requires further examination.

There was no significant association between alcohol drinking and elevated serum level of neu oncoprotein. The average quantity of alcohol consumed by people in Taiwan is not large. In this study, habitual alcohol drinking was defined as having consumed alcohol more than 3 days a week for at least 6 months. Ethanol itself is not carcinogenic. There is good evidence suggesting that alcohol consumption may be a HCC risk factor only because it is involved in the development of liver cirrhosis (17, 53). Liver cirrhosis was shown to be significantly associated with an increase in serum level of neu oncoprotein in this study, but no significant association between alcohol drinking and elevated neu oncoprotein level was observed. This discrepancy may be due to the small quantity of alcohol consumed by habitual alcohol drinkers in this study, an amount possibly insufficient to induce liver cirrhosis. However, only a small fraction of cases and controls were habitual alcohol drinkers in our study. The sample size is too small to draw a definite conclusion on the association between alcohol drinking and serum level of neu oncoprotein.

To date, observations on the role of oncogenes in hepatocarcinogenesis and their relationship to risk factors of HCC are still sparse. This nested case-control study of HCC provided the first link between increased expression of *neu* oncogene and human hepatocarcinogenesis. However, since this study was based on a small sample size, further prospective studies on a larger number of cases are needed to validate this finding. On the other hand, only a fraction of the HCC patients in this study had increased prediagnostic serum levels of neu oncoprotein. There probably exists a variety of pathways for hepatocarcinogenesis. The use of serum oncoprotein levels as a biomarker offers a way to search for the genetic alterations involved in the development of HCC. Whether there are other oncogenes critical for the pathogenesis of HCC remains to be determined.

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