Original Article

Association of Diabetes Duration and Diabetes Treatment With the Risk of Hepatocellular Carcinoma

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BACKGROUND: Despite the observed association between diabetes mellitus and hepatocellular carcinoma (HCC), little is known about the effect of diabetes duration before HCC diagnosis and whether some diabetes medications reduced the risk of HCC development. This objective of the current study was to determine the association between HCC risk and diabetes duration and type of diabetes treatment. **METHODS:** A total of 420 patients with HCC and 1104 healthy controls were enrolled in an ongoing hospital-based case-control study. Multivariate logistic regression models were used to adjust for HCC risk factors. **RESULTS:** The prevalence of diabetes mellitus was 33.3% in patients with HCC and 10.4% in the control group, yielding an adjusted odds ratio (AOR) of 4.2 (95% confidence interval [95% CI], 3.0-5.9). In 87% of cases, diabetes was present before the diagnosis of HCC, yielding an AOR of 4.4 (95% CI, 3.0-6.3). Compared with patients with a diabetes duration of 2 to 5 years, the estimated AORs for those with a diabetes duration of 6 to 10 years and those with a diabetes duration >10 years were 1.8 (95% CI, 0.8-4.1) and 2.2 (95% CI, 1.2-4.8), respectively. With respect to diabetes treatment, the AORs were 0.3 (95% CI, 0.2-0.6), 0.3 (95% CI, 0.1-0.7), 7.1 (95% CI, 2.9-16.9), 1.9 (95% CI, 0.8-4.6), and 7.8 (95% CI, 1.5-40.0) for those treated with biguanides, thiazolidinediones, sulfonylureas, insulin, and dietary control, respectively. **CONCLUSIONS:** Diabetes appears to increase the risk of HCC, and such risk is correlated with a long duration of diabetes. Relying on dietary control and treatment with sulfonylureas or insulin were found to confer the highest magnitude of HCC risk, whereas treatment with biguanides or thiazolidinediones was associated with a 70% HCC risk reduction among diabetics. ***Cancer*** **2010;116:1938–46.**

KEYWORDS: diabetes mellitus, metformin, sulfonylurea, cirrhosis, hepatocellular carcinoma.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and inadequate secretion of or receptor insensitivity to endogenous insulin,[1] and it is a major public health problem and the fifth leading cause of death in the United States.[2,3] This high death rate is partially due to the high incidence of renal and heart diseases among patients with diabetes mellitus.[3,4] In addition, diabetes is associated with increased risks of colon, kidney, and pancreatic cancers.[5]

Because the liver plays a crucial role in glucose metabolism, it is not surprising that diabetes mellitus is an epiphenomenon of many chronic liver diseases such as chronic hepatitis, fatty liver, liver failure, and cirrhosis. The association between diabetes mellitus and hepatocellular carcinoma (HCC) has been reported by cohort[6-9] and case-control studies.[10-12] Although such an association could be related to the underlying chronic liver diseases that preceded the development of HCC,[13-16] there are several lines of evidence suggesting that diabetes is in fact an independent risk factor for HCC development. This evidence includes 1) results from review and meta-analysis reports concluding that diabetes is a risk factor of HCC,[4,17-19] 2) findings that the positive association between diabetes and HCC is independent of underlying cirrhosis and chronic liver diseases,[11,16] 3) findings that the association is positively correlated with disease duration,[12,20,21] 4) demonstration of the synergistic interaction between diabetes and other HCC risk factors,[6,10,12] 5) findings of HCC recurrence after liver resection and transplantation among patients with diabetes,[22,23] 6) suggestion of a

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biological plausibility that underlies the association between diabetes and HCC,[18,19,24] and 7) the observation of risk of HCC development among patients with type 1 diabetes mellitus.[10]

Because diabetes mellitus is a complication of many chronic liver diseases and because transient hyperglycemia can be a symptom of metastatic tumors or side effect of chemotherapy intake,[25] detailed information regarding patients' duration of diabetes mellitus before HCC development may be crucial for properly studying the association between diabetes and HCC. Moreover, it is not known whether diabetes control reduces the risk of HCC or whether specific medication regimens for diabetes confer a high risk of developing HCC. Therefore, we embarked on a case-control study to address these questions after controlling for established HCC risk factors.

MATERIALS AND METHODS

Study Design and Population

The current investigation is part of an ongoing hospital-based case-control study that was approved by the institutional review board at The University of Texas M. D. Anderson Cancer Center (MDACC). Written informed consent for participation was obtained from each study participant. Detailed description of cases and controls were previously reported.[26-28] Case patients were recruited from the population of patients with newly diagnosed HCC who were evaluated and treated at the gastrointestinal medical oncology and surgical oncology outpatient clinics at MDACC. The inclusion criteria were as follows: pathologically confirmed diagnosis of HCC, US residency, and the ability to communicate in English. The exclusion criteria were the presence of other types of primary liver cancer (such as cholangiocarcinoma or fibrolamellar hepatocarcinoma), unknown primary tumors, and a concurrent or past history of cancer at another organ site.

From January 2000 through July 2008, 652 patients with suspected HCC were identified, 518 of whom were eligible for this study. We enrolled 420 eligible patients with HCC; 98 eligible patients (18.9%) were not recruited because of patient refusal, patient sickness, or inadequate time to complete the interview. Statistical analyses indicated that the eligible patients who were not recruited did not differ from the recruited patients in terms of demographic, epidemiologic, or clinical factors (retrieved from patients' medical records).

The control subjects were healthy and genetically unrelated family members (ie, spouses and in-laws) of patients at MDACC who had cancers other than liver, gastrointestinal, lung, or head and neck cancer. The reason for excluding family members and spouses of patients with these cancers as controls was to prevent the introduction of selection bias connected with shared environmental and genetic factors that are highly associated with HCC (eg, alcohol consumption, diabetes mellitus, smoking, family history of cancer, and hepatitis virus infection).

The eligibility criteria for controls were the same as those for patients, except for having a cancer diagnosis. Control subjects were recruited from various diagnostic radiology clinics of MDACC, where cancer patients and their companions are sent to receive the initial cancer diagnosis or treatment follow-up examination. A short structured questionnaire was used to screen for potential controls on the basis of the eligibility criteria. Analysis of the answers received on the short questionnaire indicated that 83.6% of those questioned agreed to participate in clinical research. A comparison of those recruited as controls and those who refused to participate in the research revealed no significant differences in age, sex, race/ethnicity, educational level, personal history of cancer, or the accompanied patient's type of cancer.

We sought to confirm the control subjects' reasons for coming to the hospital with cancer patients and whether these reasons could have been related to the risk factors for HCC. We found that the underlying causes for the controls' companionship were care and altruism. Moreover, all spouses of patients with other cancers who served as control subjects reported that they would have chosen to be referred to MDACC if they had been diagnosed with cancer during the same time period because they tended to share the same family physician, had the same health insurance coverage, and lived in the same geographic location. All of the above mentioned results indicated that the patients and controls had the same catchments, which further supported the idea that the control subjects were representative of the MDACC population from which HCC patients were selected.[29-31] A total of 1286 eligible control subjects were ascertained in the current study. However, 172 control subjects were excluded due to limited blood samples for testing hepatitis B virus (HBV) and hepatitis C virus (HCV) markers. An additional 10 control subjects were excluded for living outside the United States, leaving a total of 1104 control subjects to be analyzed in this study.

HCC patients and controls were recruited simultaneously and were personally interviewed for approximately 25 to 30 minutes. No proxy interviews were conducted. The interviewers used a structured and validated questionnaire[32] to collect information regarding demographic features and HCC risk factors, such as personal smoking history, alcohol consumption, medical history, occupational history, and family history of cancer. The definitions used for smokers, alcohol drinkers, and individuals with a family history of cancer were previously reported.[26-28]

Diabetes Mellitus

Each participant was questioned about his or her prior history of diabetes mellitus, the type of diabetes (insulin-treated or non–insulin-treated), the age at diagnosis, and the duration of each type of diabetes. Subjects with a history of diabetes were questioned about medications used for diabetes control and the duration of treatment. Oral antidiabetic agents used were classified as biguanides (eg, metformin), sulfonylureas (eg, glyburide, glipizide), and thiazolidinediones (eg, rosiglitazone).[33]

Hepatitis Virus Infection

Blood samples from cases and controls were tested for HBV and hepatitis C virus HCV. HCV antibodies, hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core (HBc) antigen were detected by use of a third-generation enzyme-linked immunoadsorbent assay (ELISA) (Abbott Laboratories, North Chicago, Ill). Positive results prompted repeated confirmatory ELISA testing.

Statistical Analysis

Stata software (Stata Corp, College Station, Tex) was used for statistical analysis. Univariate analysis was performed using the chi-square or Fisher exact test for categorical variables and the Kruskal-Wallis test for continuous variables. To test for the association between diabetes and HCC, we performed multivariable unconditional logistic regression analyses using all variables significant at $P < .05$ in the univariable analyses and have a confounding effect on the association between diabetes and HCC. To determine the association between HCC development and diabetes duration and diabetes treatment, we performed restricted analysis among diabetic cases and controls. For each factor, we calculated the adjusted odds ratio (AOR) and 95% confidence interval (95% CI), using maximum likelihood estimation. All odds ratios (ORs) were adjusted for age, sex, race, education level, cigarette smoking, alcohol consumption, diabetes mellitus, family history of cancer, and HBV/HCV infection. The final model was chosen on the basis of biological plausibility and the lowest −2 log likelihood function.

RESULTS

The baseline demographic characteristics of patients and controls are summarized in Table 1. Most study subjects were non-Hispanic white men; the men-to-women ratio was 2.5 to 1 for HCC patients. Case patients were slightly older than control subjects, with a mean difference of 3 years (95% CI 2-5; the mean [± standard error (SE)] ages were 63 ± .6 years for HCC patients and 60 ± .3 years for controls.

Table 2 shows that the prevalences of hepatitis virus infection (detected by anti-HCV, HBsAg, or anti-HBc), cigarette smoking, alcohol consumption, and family history of cancer were significantly higher for cases than for controls. Our previous reports from the same population indicated that each factor is an independent risk factor for HCC development.[26-28]

A total of 140 HCC patients (33.3%) and 115 controls (10.4%) recalled a prior history of diabetes mellitus that conferred a 4-fold increase in HCC risk when compared with nondiabetic individuals ($P = .001$; AOR, 4.2 [95% CI, 3.0-5.9]) (Fig. 1). The prevalence of diabetes mellitus stratified by demographic characteristics (Table 1) and HCC risk factors (Table 2) was significantly higher in cases than in controls.

The significant risk of HCC development among patients with diabetes mellitus was observed for both men (AOR, 5.2; 95% CI, 3.3-8.3 [$P < .001$]) and women (AOR, 3.2; 95% CI, 1.6-6 [$P = .001$]).

To ensure that diabetes was not induced by the cancer, analysis of the association between diabetes and HCC risk was restricted to those who were diagnosed with diabetes more than 1 year before HCC diagnosis or before control recruitment (122 cases and 86 controls) (Fig. 1); the AORs were 4.4 (95% CI, 3.0-6.3) for all subjects, 5.2 (95% CI, 3.3-8.3) for men, and 3.5 (95% CI, 1.7-7.1) for women.

Table 3 presented results of restricted analyses among diabetic cases and controls. The estimated AORs of developing HCC were 1.8 (95% CI, 0.8-4.1) for patients diagnosed with diabetes 6 to 10 years before HCC diagnosis and 2.2 (95% CI, 1.2-4.8) for those with a duration of diabetes >10 years.

Table 1. Prevalence of Diabetes Mellitus by Participant Characteristics

Demographic Variables	Total Population				*P*	Prevalence of Diabetes				*P*[a]
	Cases		Controls			Cases		Controls		
	N=420	%	N=1104	%		N=140	%	N=115	%	
Sex					.001					
Male	299	71.2	636	57.6		112	37.5	78	12.3	<.0001
Female	121	28.8	468	42.4		28	23.1	37	7.9	<.0001
Age, y					.001					
≤40	15	3.6	50	4.5		1	6.7	1	2.0	.4
41-50	46	10.9	181	16.4		5	10.9	14	7.7	.3
51-59	119	28.3	336	30.4		33	27.7	34	10.1	<.0001
60-69	117	27.9	358	32.4		55	47.0	39	10.9	<.0001
≥70	123	29.3	179	16.2		46	37.4	27	15.1	<.0001
Ethnicity					.001					
Non-Hispanic white	294	70.0	973	88.1		92	31.3	92	9.5	<.0001
Hispanic	56	13.3	84	7.6		32	57.1	14	16.7	<.0001
African American	40	9.5	39	3.5		11	27.5	6	15.4	.1
Asians	30	7.1	8	0.7		5	16.7	3	37.5	.2
Educational level					.001					
≤High school	198	47.1	316	28.6		67	33.8	39	12.3	<.0001
Some college	94	22.4	287	26.0		32	34.0	26	9.1	<.0001
≥College degree	128	30.5	501	45.4		41	32.0	50	10.0	<.0001
State of residency					.5					
TX, LA, AK, NM, OK	308	73.3	809	73.3		106	34.4	90	11.1	<.0001
Other states	112	26.7	295	26.7		34	30.4	25	8.5	<.0001

HCC indicates hepatocellular carcinoma.
[a]*P* value for the difference in diabetes prevalence between cases and controls in each demographic characteristic.

Table 2. Prevalence of Diabetes Mellitus by HCC Risk Factors

Risk Factors	Total Population				*P*	Prevalence of Diabetes				*P*[a]
	Cases		Controls			Cases		Controls		
	N=420	%	N=1104	%		N=140	%	N=115	%	
Hepatitis virus					<.0001					
None	232	55.2	1066	96.6		94	40.5	109	10.2	<.0001
Anti-HCV+	94	22.4	6	0.5		22	23.4	2	33.3	.6
HBsAg+/Anti-HBc+	30	7.1	4	0.4		4	13.3	1	25.0	.5
HBsAg−/Anti-HBc+	24	5.7	25	2.3		10	41.7	2	8	.006
Both HCV and HBV	40	9.5	3	0.3		10	25.0	1	33.3	.7
Cigarette smoking					<.0001					
No	126	30	582	52.7		42	33.3	57	9.8	<.0001
Yes[b]	294	70	522	47.3		98	33.3	58	11.1	<.0001
Smoking quantity					.007					
≤20 pack-y	115	27.4	258	23.4		35	30.4	23	8.9	<.0001
>20 pack-y	176	41.9	264	23.9		62	35.2	35	13.3	<.0001
Alcohol consumption					<.0001					
No	137	32.6	485	43.9		52	38.0	55	11.3	<.0001
Yes[c]	283	67.4	619	56.1		88	31.1	60	9.7	<.0001
Alcohol quantity					<.001					
<60 mL ethanol/d	192	45.7	551	49.9		60	31.3	53	9.6	<.0001
≥60 mL ethanol/d	89	21.2	65	5.9		27	30.3	7	10.8	.003
Family history of cancer					<.0001					
No	132	31.4	355	32.2		48	36.4	44	12.4	<.0001
Yes[d]	264	62.9	740	67		82	31.1	71	9.6	<.0001
Liver cancer (first-degree)	24	5.7	9	0.8		10	41.7	0	0	<.0001

HCC indicates hepatocellular carcinoma; HCV, hepatitis C virus; +, positive; HBsAg, hepatitis B surface antigen; HBc, hepatitis B core; −, negative; HBV, hepatitis B virus.
[a]*P* value is shown for the difference in diabetes prevalence between cases and controls by risk factors.
[b]Duration of smoking was missing for 3 HCC cases.
[c]Duration of drinking was missing for 2 HCC cases and 3 controls.
[d]Any cancer in first-degree and second-degree relatives.

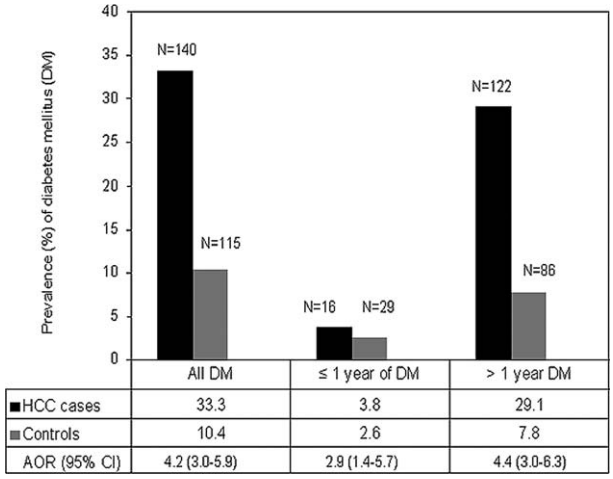

Figure 1. The prevalence of diabetes mellitus (DM) in cases (n = 420) and controls (n = 1104) and the adjusted odds ratio (AOR) for the association between the development of hepatocellular carcinoma (HCC) and diabetes according to the duration of diabetes (all, ≤1 year, and >1 year) are shown. Odds ratios were adjusted for the confounding effect of age, sex, race, educational level, cigarette smoking, alcohol drinking, hepatitis C virus, hepatitis B virus, and family history of cancer using unconditional multivariable logistic regression analyses. Duration of diabetes was missing for 2 patients with HCC. 95% CI indicates 95% confidence interval.

Among patients who had diabetes for more than a year, most subjects were considered to have type 2 diabetes mellitus and were receiving an oral antidiabetic regimen, yielding an inverse association with HCC for all subjects (AOR, 0.3; $P = .009$). A total of 16 HCC case patients and 2 control subjects with diabetes reported relying on diet alone to control diabetes, yielding a significantly higher risk of HCC development (AOR, 7.8; 95% CI, 1.5-40). The majority of diabetic patients receiving oral antidiabetic regimens received agents in the biguanide and sulfonylurea classes. The AORs for HCC association with biguanide use were 0.3 (95% CI, 0.2-0.6) for all subjects, 0.3 (95% CI, 0.1-0.7) for men, and 0.2 (95% CI, 0.1-0.9) for women. Only 6 HCC patients and 16 controls received thiazolidinedione-class agents, which demonstrated a 70% risk reduction in HCC development (Table 3). Use of the sulfonylurea class of oral antidiabetics had a much higher association with HCC development: the AORs were 7.1 (95% CI, 2.9-16.9) for all subjects, 5.3 (95% CI, 1.9-14.2) for men, and 12.3 (95% CI, 1.6-96.9) for women. Moreover, insulin use was associated with risk for HCC development compared with the use of oral modalities, however, the association was not statistical significant ($P = .1$).

We found no significant association between early onset of diabetes diagnosis (age <50 years) and risk of HCC development. Moreover, we found no correlations between duration of diabetes and patients' age or types of treatment in this study population.

DISCUSSION

Results from the current study suggests that the magnitude of association between diabetes and HCC increased as the duration of diabetes increased and with specific antidiabetic treatment. A notable finding is that the use of sulfonylurea drugs (such as glyburide) among diabetics revealed a 7-fold increase in HCC risk compared with nonusers. Moreover, diabetic patients who were treated with exogenous insulin were at a higher risk for HCC development as compared with noninsulin treatment group; however, such elevated risk was not statistically significant.

Insulin sensitizing agents such as biguanides (including metformin) and thiazolidinediones are alternative options for treating obese patients with diabetes mellitus or patients with underlying nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In the current study, the use of metformin or thiazolidinediones was associated with a 70% risk reduction of HCC compared with the use of insulin or sulfonylureas.

The above findings of the elevated risk associated with the use of insulin or sulfonylureas and the reduced risk associated with the use of biguanide (metformin) are in agreement with newly published study by Donadon et al among Italian patients with cirrhosis and HCC,[34] which reported a significantly increased risk of HCC among diabetic patients treated with insulin and sulfonylureas (OR, 2.99; 95% CI, 1.34-6.65) and reduced HCC risk among diabetic patients treated with metformin (OR, 0.33; 95% CI, 0.1-0.7). Moreover, Bowker et al[35] reported that patients with type 2 diabetes exposed to sulfonylureas and exogenous insulin had a significant risk of cancer-related mortality compared with patients exposed to metformin. Both studies are in agreement with an earlier report by Evans et al[36] who observed lower incidence of cancer among diabetic patients treated with metformin compared with other diabetes treatments. Interestingly, such risk reduction was associated with duration and dosage of metformin treatment.

The results of the current study are consistent with the notion that the biological mechanism for liver-cell damage induced by type 2 diabetes mellitus involves insulin resistance and hyperinsulinemia.[4,37] HCC development related to hyperinsulinemia can be mediated

Table 3. Association Between Diabetes Duration/Treatment and HCC Risk

Diabetes Variables	HCC Patients		Controls		AOR (95% CI)[a]	*P*
	N=122	%	N=86	%		
Duration of diabetes, y						
2-5	30	24.6	33	38.4	1 (reference)	
6-10	38	31.1	23	26.7	1.8 (0.8-4.1)	.2
>10	54	44.3	30	34.9	2.2 (1.2-4.8)	.04
Age at diabetes diagnosis, y						
≥50	83	68.0	57	66.3	1 (reference)	
<50	39	32.0	29	33.7	1.5 (0.7-3.4)	.3
Diabetes treatment						
Oral treatment						.009
Nonusers	32	26.2	11	12.8	1 (reference)	
Users	90	73.8	75	87.2	0.3 (0.1-0.7)	
Insulin treatment						.1
Nonusers	95	77.9	73	84.9	1 (reference)	
Users	27	22.1	13	15.1	1.9 (0.8-4.6)	
Diet only						.01
Nonusers	106	86.9	84	97.7	1 (reference)	
Users	16	13.1	2	2.3	7.8 (1.5-40.0)	
Type of oral treatment						
Biguanide						
Nonusers	78	63.9	32	37.2	1 (reference)	
Users	44	36.1	54	62.8	0.3 (0.2-0.6)	<.001
Sulfonylureas						
Nonusers	75	61.5	58	67.4	1 (reference)	
Users	47	38.5	10	11.6	7.1 (2.9-16.9)	<.001
Thiazolidinediones						
Nonusers	116	95.1	70	81.4	1 (reference)	
Users	6	4.9	16	18.6	0.3 (0.1-0.7)	.01

HCC indicates hepatocellular carcinoma; AOR, adjusted odds ratio; 95% CI, 95% confidence interval.
[a]AOR for the confounding effect of age, sex, race, educational level, cigarette smoking, alcohol drinking, hepatitis C virus, hepatitis B virus, and family history of cancer using unconditional multivariable logistic regression analyses.

through inflammation, cellular proliferation, inhibition of apoptosis, and mutation of tumor suppressor genes.[4] Increased insulin levels lead to reduced liver synthesis and blood levels of insulin growth factor-binding protein-1, which may contribute to increased bioavailability of insulin-like growth factor-1 (IGF-1), the promotion of cellular proliferation, and the inhibition of apoptosis.[38] Insulin also binds to the insulin receptor and activates its intrinsic tyrosine kinase, leading to phosphorylation of insulin receptor substrate-1 (IRS-1).[39] Both IGF-1 and IRS-1 have been overexpressed in tumor cells.[40] Overexpression of IRS-1 has been associated with the prevention of apoptosis mediated by transforming growth factor-β.[41] In addition, insulin is associated with lipid peroxidation and increased oxidative stress and the generation of reactive oxygen species, which may contribute to DNA mutation. In fact, lipid peroxidation has been implicated in the up-regulation of peroxidation of proinflammatory cytokines, which has been involved in p53 tumor suppressor gene mutations.[42]

Metformin can reduce blood glucose in diabetic patients, predominantly through reduction of hepatic gluconeogenesis and glycogenolysis.[33,43,44] It also increases the insulin-stimulated glucose uptake in the skeletal muscles, suppresses oxidation of fatty acids, and reduces triglyceride levels in patients with hypertriglyceridemia. All of these effects may contribute to reducing hyperinsulinemia, improving hepatic insulin resistance, reducing steatosis, improving liver enzymes, and reducing body weight.

Although to our knowledge the molecular mechanisms of metformin's antidiabetic activity have yet to be fully identified, experimental studies in ob/ob mice indicated that the key role of metformin may be related to

decreased hepatic expression of tumor necrosis factor-α, a cytokine that promotes insulin resistance.[45] The beneficial effect of metformin treatment among patients with NAFLD was assessed by small-scale trials; improvement in liver enzymes, steatosis, and fibrosis was noted.[46-48] However, a recently reported study by Haukeland et al[49] indicated that metformin treatment for 6 months was not better than placebo in terms of improving liver histology in patients with NAFLD, even though body weight and metabolic profile improved significantly.

Unlike metformin, the use of sulfonylureas is associated with weight gain, hyperinsulinemia, and hepatotoxicity.[50] Therefore, it may not be the appropriate diabetes treatment for patients with underlying chronic liver diseases, obesity, or insulin resistance because of possible exacerbation of the underlying NAFLD or NASH observed in these patients and possible acceleration of HCC development.[51]

Although the mechanism for the antineoplastic activity of metformin is not fully understood, there is substantial evidence suggesting that metformin suppresses cellular proliferation and protein synthesis with AMP-independent protein kinase activation in both malignant and nonmalignant cells.[52,53] A recent review by Cazzaniga et al[54] reported that such AMP-activated protein kinase actions may be mediated by multiple pathways, including up-regulation of the p53 and reduction of cyclin D1 levels, which may eventually lead to antiproliferative effect.

However, although the intake of thiazolidinediones was found to be significantly associated with reduced risk of HCC, only 6 HCC patients with diabetes recalled using this medication, which may not be enough to conclude the protective effect of thiazolidinediones treatment on HCC development.

In this study, cases were pathologically confirmed HCC patients who were newly diagnosed and prospectively enrolled in the study in which both cases and controls were personally and simultaneously interviewed, using a structured, validated questionnaire. Control subjects were selected to represent the study population from which cases were selected. To ensure the accuracy of our data, subjects with a history of diabetes were asked about the duration of their disorder, their age at diagnosis, and their treatment exposure. Questions of prior history of diabetes mellitus along with other chronic medical conditions were part of a long list of questions in which study subjects were blinded for the current study hypothesis and its specific aims. It is reasonable to assume that subjects who had received a definite diagnosis and had been treated could accurately report their prior history of medical conditions and recalled the condition duration. Upon reviewing the medical records of HCC patients, we found no discrepancy between interview information and patients' records. In fact, there is strong evidence supporting the reliability and validity of self-reported diabetes mellitus when agreement between self-reported disease diagnosis and medical conditions was observed.[55-57] It is partially attributable to patients' awareness of diabetes complications and the importance to monitor blood sugar during treatment. Therefore, it is not surprising that patients with diabetes mellitus tend to remember the name of exposed medications with and without therapeutic response during their lifetime.

The current study did have some limitations. Overweight and obesity may have a confounding effect on the observed association between diabetes and HCC and might modulate the antidiabetic treatment selection. Nevertheless, we have collected information regarding subjects' weight before HCC diagnosis or before control ascertainment. Such data were initiated in 2004 and are available for 184 HCC patients and 648 controls. Results indicated that the mean body mass index (BMI) at early age (between 20 and 40 years of age) (±SE) was significantly larger in HCC patients (24.06 ± 0.3) than in controls (23.04 ± 0.1) ($P = .001$). However, adjustment for the effect of prior BMI did not meaningfully change the observed significant association between diabetes and HCC; the estimated OR was 3.8 (95% CI, 2.3-6.1).

Although obesity is a risk factor for diabetes mellitus and HCC, obesity is not necessarily present in patients with NAFLD; a significant portion of patients with NAFLD have a normal body weight.[58] Therefore, it is not surprising that the association between diabetes and HCC is not confounded by obesity in the current study and other studies.[6,7]

We also noted that most patients with diabetes were treated with oral antidiabetic drugs, implicating type 2 diabetes. Although we do not know why some patients with type 2 diabetes received insulin treatment, it is possible that insulin was given to some patients for whom safety and efficacy considerations favor its use as the drug of choice, for example, patients with severe hepatic or renal impairment. It may also indicate that the diabetes was severe or that some patients required insulin therapy, either as monotherapy or in conjunction with oral antidiabetic therapy, to maintain long-term glycemic control. However, we lacked information regarding fasting blood glucose, diabetes complications, and glycosylated

hemoglobin to identify the average plasma glucose concentration over prolonged periods of time. This information is crucial to explain whether severity of diabetes is correlated with duration and type of treatment and why some diabetic patients with dietary control are at high risk for HCC development. Future large cohort studies among diabetic patients with detailed information concerning family history of diabetes, type of diabetes, diabetes treatment, response to diabetes therapy, diabetes-related complications, and clinicopathologic changes in liver tissues may reveal the explanation for the relationship between diabetes and HCC development reported by case-control studies.

The preliminary findings of the current study may indicate that choosing an appropriate and safe treatment for diabetes mellitus is critical in patients with underlying liver diseases. The need for developing specific guidelines for treating diabetic patients with underlying liver diseases—with consideration of subjects' BMI and whether they have NAFLD or NASH—is warranted. Such guidelines should outline appropriate and safe treatment for these patients, with the ultimate goal of preventing progressive liver disease and HCC development. Future studies should be aimed at investigating the preventive role of metformin on HCC development.

CONFLICT OF INTEREST DISCLOSURES

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