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Original Article

Diabetes mellitus and changes of peripheral blood cells in Chinese patients with hepatocellular carcinoma

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Abstract: Background: Diabetes mellitus (DM) has been identified as a new risk factor for hepatocellular carcinoma (HCC) that is also associated with changes of peripheral blood cells. However, no information was available for the relationship between DM and changes of peripheral blood cells. Our present study was designed to determine this relationship in our Chinese HCC patients. Methods: Patients who were diagnosed with HCC and hospitalized at our hospital in the period from January 2003 to April 2012 were included in this study. Their data, including demographic, metabolic, laboratory, biochemical, instrumental, radiological and pathological features, were analyzed. Results: A total of 375 HCC patients were included and 63 (16.8%) of which were diagnosed with diabetes. Compared with patients without DM, the HCC patients with DM were older, and had increased levels of fasting glucose and systolic blood pressure (SBP), lower values of hemoglobin and platelet count, and a lower percentage of patients with HBV infection. Multivariable analysis showed that hemoglobin (OR=0.982; 95% CI, 0.971-0.993; P=0.002) and SBP (OR=1.017; 95% CI, 1.001-1.034; P=0.039) were independently associated with HCC and DM. Considering the effect of cirrhosis and HBV infection for DM, sub-group analyses were performed and similar results were gained for hemoglobin (OR=0.984, 95% CI=0.970-0.998, P=0.030; OR=0.982, 95% CI=0.969-0.995, P=0.006; respectively). For treatment of diabetes, patients with biguanide use had increased levels of hemoglobin, compared with those without biguanide use. Conclusions: Hemoglobin concentrations may be affected by diabetes in our HCC patients and treatment with biguanide maybe has the potential to prevent this change.

Keywords: Hepatocellular carcinoma, diabetes mellitus, peripheral blood cell, hemoglobin, biguanide

Introduction

Hepatocellular carcinoma (HCC) accounts for more than 80% of all primary liver cancers and ranks globally as the third leading cause of cancer-related deaths [1, 2]. Annual mortality rates of HCC remain comparable to its yearly incidence, making it one of the most lethal varieties of solid-organ cancers [3]. Risk factors for HCC which have been identified include hepatitis B virus (HBV), HCV, cirrhosis, heavy alcoholic consumption, alfatoxin exposure, non-alcoholic steatohepatitis (NASH); however, no specific risk factor has been found for 15-50% of HCC patients [4-7].

Diabetes mellitus (DM) has been suggested as a potential risk factor for HCC [8-10]. In general, DM is associated with about two- to three-fold increased risk of HCC and may also increase the risk of death from HCC [11]. Moreover, the prognosis of HCC patients after curative therapy can be affected by diabetes, which is independent of the basic demographics, liver cirrhosis, and other comorbidities [6, 12, 13].

Tumor-host interactions extend beyond the local microenvironment and cancer development largely depends on the ability of malignant cells to hijack and exploit the normal physiological processes of the host [14, 15]. For example, growing evidences showed that gene expression profiling of peripheral blood cells is valuable to assess the gene signatures related to solid tumors [16, 17]. One population-based case-control study performed in Norway showed that gene expression of peripheral blood cells is markedly perturbed by the presence of breast cancer [15].

Changes of peripheral blood cells have also been associated with the HCC [18-20]. An ear-

lier study found that the number of lymphocyte, B cell, T cell and T-cell subsets of HCC patients was significantly decreased as compared with those of normal healthy individuals and asymptomatic hepatitis B virus surface antigen carriers [18]. In addition, more and more studies showed that the neutrophil to lymphocyte ratio (NLR) is a useful predictor of overall survival in HCC patients who undergo hepatectomy and in those HCC patients treated with hepatic arterial infusion chemotherapy [19-21].

However, no information was available for the effect of diabetes on the changes of peripheral blood cells in HCC patients. Our study was designed: (1) to determine this relationship between diabetes and the peripheral blood cells in Chinese HCC patients; (2) to perform the subgroup analysis when the study patients were restricted into those with cirrhosis and those with HBV infection, considering the effect of cirrhosis/HBV infection on the peripheral blood cells; and (3) to study the relation between count and amount of peripheral blood cells, fasting glucose, and duration/treatment of diabetes.

Patients and methods

Study patients

Our present study included all the patients who were diagnosed with HCC for the first time and hospitalized at our hospital (China-Japan Friendship Hospital, Beijing, China) in the period from January 2003 to April 2012. Diagnosis of HCC was based on the histological findings, typical radiological features and serum AFP levels. Those patients with serum HBsAg-positive for more than six months or at the diagnosis of HCC were regarded as chronic HBV infection. The Human Research Ethics Committee of China-Japan Friendship hospital approved our research project and it was implemented according to the principles of the *Declaration of Helsinki*.

Exclusion criteria included: (1) those patients who were less than 18 years old or more than 75 years or non-Chinese; (2) those patients who had been diagnosed with HCC for more than fifteen days or had been treated with any HCC-associated therapies at inclusion; (3) those patients who had been diagnosed with primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, hemachromatosis, Budd-Chiari syndrome, Wilson's disease, schistosomiasis, rheumatic diseases or allergic disorder; (4) those patients with a presence of other malignancies, including leukemia and lymphoma; and (5) those patients who had serious diseases, for example severe heart failure, acute exacerbations of chronic obstructive pulmonary disease, and uremia.

Determination of HCC and diabetes

HCC was diagnosed when patients fulfilled one of the following criteria: (1) the confirmed diagnosis of histological findings of needle biopsy or surgery; (2) the typical radiological features derived from at least two image examinations, including ultrasound, contrast-enhanced dynamic computed tomography, magnetic resonance imaging and hepatic angiography; and (3) a serum AFP level >400 ng/mL with typical radiological features by one image examination [4, 5, 22, 23].

The determination of diabetes was based on one of the following criteria: (1) fasting plasma glucose of 126 mg/dL or greater on at least two occasions; (2) plasma glucose of 200 mg/dL or greater at 2-hour oral glucose tolerance test (OGTT); and (3) the need for oral hypoglycemic drugs or insulin to control blood glucose [24].

Clinical and laboratory indicators

The demographic, biochemical, laboratory, metabolic, instrumental, radiological and pathological features of HCC patients were included in our analysis. All the data were obtained when those patients were diagnosed with HCC for the first time; however, those data would be excluded if they were examined more than 15 days before or after the confirmed diagnosis of HCC. Patients would also be excluded if they had any patchier data which could affect the statistical results. The Child-Turcotte-Pugh (CTP) score takes into account 5 indicators, including total bilirubin, albumin, international normalized ratio, ascites and hepatic encephalopathy. Systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg was diagnosed with hypertension. Mean artery pressure (MAP) was equal to 1/3 SBP plus 2/3 DBP. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of the height (in meters). According to the Asian

Table 1. Baseline characteristics of 375 hepatocellular carcinoma (HCC) patients and univariable analysis for patients with and without diabetes

Variable	HCC patients, n=375*	HCC patients with diabetes (n=63)*	HCC patients without diabetes (n=312)*	*P* Value
Male sex, no. (%)	312 (83.2)	51 (81.0)	261 (83.7)	0.601
Mean age, years	56.4±11.0	59.5±10.3	55.8±11.1	0.015
BMI[a], kg/m^2	23.77±3.39	23.69±3.51	23.68±3.71	0.991
Overweight or obesity[a], no. (%)	159 (56.6)	27 (57.4)	132 (56.4)	0.896
Smoking, no. (%)	154 (41.4)	20 (31.7)	134 (42.9)	0.099
Alcohol intake, no. (%)	104 (27.7)	14 (22.2)	90 (28.8)	0.284
History of hypertension, no. (%)	88 (23.5)	17 (27.0)	71 (22.8)	0.470
SBP, mmHg	130±17	133±17	129±16	0.048
DBP, mmHg	79±10	78±10	79±10	0.455
Liver cirrhosis, no. (%)	199 (53.1)	38 (60.3)	161 (51.6)	0.206
HBV infection, no. (%)	328 (87.5)	50 (79.4)	278 (89.1)	0.033
HCV infection, no. (%)	22 (5.9)	3 (4.8)	19 (6.1)	0.908
Neutrophil, ×^{9}/L	4.13±2.49	3.88±2.52	4.18±2.49	0.389
Hemoglobin, g/L	132.5±23.8	124.4±23.9	134.2±23.4	0.003
Platelet count†, ×^{9}/L	130 (85-189)	113 (64-157)	139 (89-192)	0.020
Fasting blood glucose, mmol/L	5.82±2.09	8.83±3.12	5.21±1.07	<0.001
AFP>400 ng/mL[b], no. (%)	167 (45.8)	27 (43.5)	140 (46.2)	0.702
ALT†, U/L	45 (29-81)	44 (27-91)	45 (30-79)	0.943
GGT†, U/L	106 (55-233)	96 (51-190)	109 (57-239)	0.406
Albumin, g/L	37.4±6.0	36.4±5.9	37.6±6.0	0.148
Total cholesterol, mmol/L	4.26±1.34	4.18±1.12	4.28±1.39	0.662
Child-Turcotte-Pugh classification[c]				
Child A, no. (%)	243 (65.5)	35 (56.5)	208 (67.3)	0.101
Child B, no. (%)	92 (24.8)	17 (27.4)	75 (24.3)	0.600
Child C, no. (%)	36 (9.7)	10 (16.1)	26 (8.4)	0.061

BMI, mean body mass index; DBP, diastolic blood pressure; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SBP, systolic blood pressure. *Plus-minus value indicates mean ± standard deviation. †Median (interquartile range, Q1-Q3). Data were available in [a]281 (47+234), [b]365 (62+303) and [c]371 (62+309) patients. The numbers before the brackets indicate the total available cases in the two groups.

and Chinese criteria, BMI≥23 kg/m^2 was diagnosed as overweight and BMI≥25 kg/m^2 was as obesity [5, 6].

In the morning (after an overnight fasting of 8 hours), venous blood samples were taken and standard methods were used to measure for those indicators, including peripheral blood cells. Neutrophil, hemoglobin and platelet count were selected to be as the presentative indicators for the changes of peripheral blood cells. For the clinical classification and tumor-node-metastasis stage, results of physical examinations, radiological and pathological features of HCC patients would be re-assessed carefully by two authors independently, including US, CT, MRI and hepatic angiography. The 7th TNM staging system recommended by International Union against Cancer was used. For the clinical classification, HCC with a diameter of ≥5 cm was diagnosed as massive-type, HCC with diameters of <5 cm was diagnosed as nodular-type and HCC with a diameter of <3 cm for single or two nodules was as small cancer-type.

Statistic analysis

For univariable analysis, Chi-square test, Student's t-test and Mann-Whitney *U* test were used to compare the differences between HCC patients with and without DM. For multivariable analysis, based on the results of univariable analysis, unconditional multivariable logistic regression analysis was used to determine the

Table 2. Comparison of clinical classification and tumor-node-metastasis stage of HCC patients with diabetes (n=63) and patients without diabetes (n=312)

Variable	Total patients (n=375)	HCC patients with diabetes (n=63)	HCC patients without diabetes (n=312)	*P* Value
Clinical classification				
Massive, no. (%)	213 (56.8)	29 (46.0)	184 (59.0)	0.059
Nodular, no. (%)	117 (31.2)	22 (34.9)	95 (30.4)	0.485
Small-cancer, no. (%)	28 (7.5)	8 (12.7)	20 (6.4)	0.142
Diffuse, no. (%)	17 (4.5)	4 (6.3)	13 (4.2)	0.503
T stage				
Stage T1, no. (%)	82 (21.9)	15 (23.8)	67 (21.5)	0.683
Stage T2, no. (%)	90 (24.0)	19 (30.2)	71 (22.8)	0.210
Stage T3, no. (%)	194 (51.7)	28 (44.4)	166 (52.3)	0.204
Stage T4, no. (%)	9 (2.4)	1 (1.6)	8 (2.6)	0.991
N stage				
Stage N0, no. (%)	334 (89.1)	57 (90.5)	277 (88.8)	0.694
Stage N1, no. (%)	41 (10.9)	6 (9.5)	35 (11.2)	---
M stage				
Stage M0, no. (%)	269 (71.7)	49 (77.8)	220 (70.5)	0.243
Stage M1, no. (%)	106 (28.3)	14 (22.2)	92 (29.5)	---

effect of diabetes on the changes of peripheral blood cells in Chinese HCC patients. Five variables were entered in the multivariable analysis, including age, SBP, HBV infection, hemoglobin and platelet count. Stepwise multiple regression analysis (Backward: Wald; Entry: 0.05, Removal: 0.10) was used. Considering that cirrhosis and HBV infection affect the count and amount of peripheral blood cells, and most patients had HBV infection and/or cirrhosis, sub-group analysis was performed when the study patients were restricted into those with cirrhosis and those with HBV infection.

Based on the results of above-mentioned analyses, the amount of hemoglobin was found to be the sole independent indicator. Therefore, we would study the relationship between blood glucose, duration/treatment of diabetes and the amount of hemoglobin. Pearson correlation test was used for the relationship between hemoglobin and fasting glucose. Chi-square test was used to determine the relationship between amount of hemoglobin and duration/treatment of diabetes. SPSS for Windows, version 19.0 (SPSS, Chicago, IL, USA) was used for statistic analysis. We expressed results as odds ratios (ORs) and their 95% confidence intervals (CIs). For all tests, $P<0.05$ was considered statistically significant and all *P* values quoted are two-sided.

Results

Baseline characteristics of study population of 375 HCC patients

Three hundred and seventy-five HCC patients were included in our study according to the defined diagnostic, inclusion and exclusion criteria. Their demographic, clinical, laboratory, metabolic and instrumental features were shown in **Table 1**. **Table 2** showed their clinical classification and tumor-node-metastasis stage. Among these HCC patients, 63 (16.8%) patients were diagnosed with diabetes mellitus and regarded as the case group. The left 312 (83.2%) patients were non-diabetic and regarded as the control group. For these 63 diabetic patients, 74.6% (47 patients) had been diagnosed with diabetes for more than one year before they were diagnosed with hepatocellular carcinoma. For the total study population (375 patients), the mean age was 56.4±11.0 years and 312 (83.2%) patients were male. Three hundred and twenty-eight (87.5%) patients had HBV infection, 199 (53.1%) patients had liver cirrhosis, 104 (27.7%) patients were drinking, and 154 (41.4%) patients were smoking. For the clinical classification, 88.0% (330 patients) of them were found to be massive- or nodular-type HCC. Most of them were diagnosed with N0 and M0.

Table 3. Multivariable analysis: effect of diabetes mellitus on the changes of peripheral blood cells in HCC patients

Variable	AOR	95% CI	*P* Value
Mean age, years	1.015	0.987-1.045	0.292
SBP, mmHg	1.017	1.001-1.034	**0.039**
HBV infection, no. (%)	0.552	0.267-1.140	0.108
Hemoglobin, g/L	**0.982**	**0.971-0.993**	**0.002**
Platelet count, $\times^9$/L	0.997	0.994-1.001	0.149

AOR: adjusted odds ratio; CI: confidence interval. Multivariable logistic regression analysis was performed to determine the effect of diabetes mellitus on the changes of peripheral blood cells in HCC patients. Five variables were entered, including age, SBP, HBV infection, hemoglobin and platelet count. Stepwise multiple regression analysis (Backward: Wald; Entry: 0.05, Removal: 0.10) was used. Bold type was used for those variables with statistical differences.

Table 4. Sub-group analysis: consideration of the effect of liver cirrhosis and HBV infection for the changes of peripheral blood cells

Variable	AOR	95% CI	*P* Value
HCC patients with liver cirrhosis			
SBP, mmHg	1.021	0.997-1.046	0.083
Hemoglobin, g/L	0.984	0.970-0.998	0.030
HCC patients with HBV infection			
Mean age, years	1.028	0.998-1.059	0.070
Hemoglobin, g/L	0.982	0.969-0.995	0.006

Subgroup analysis was performed considering the effect of liver cirrhosis and HBV infection. All the confounding factors shown in **Table 3** were entered and only those variables with statistical differences were given in this Table.

Changes of peripheral blood cells in HCC patients with and without DM

Neutrophil, hemoglobin and platelet count were selected to be as the presentative indicators for the changes of peripheral blood cells. The values were determined when the patients were diagnosed with HCC for the first time. For the total 375 patients, the mean (± standard deviation) or median (interquartile range) values were 4.13±2.49 $\times^9$/L, 132.5±23.8 g/L and 130 (85-189) $\times^9$/L, for neutrophil, hemoglobin and platelet count, respectively (**Table 1**). For the 63 diabetic patients, the values were 3.88±2.52 $\times^9$/L, 124.4±23.9 g/L and 113 (64-157) $\times^9$/L, respectively (**Table 1**).

Univariable analysis: comparison of HCC patients with and without DM

We used the Chi-square test, Student's t-test and Mann-Whitney *U* test to compare the differences between HCC patients with and without diabetes. Compared with patients without DM (**Table 1**), the HCC patients with diabetes had an older age (59.5±10.3 *vs*. 55.8±11.1, P=0.015), increased levels of fasting glucose (8.83±3.12 *vs*. 5.21±1.07, P<0.001) and SBP (133±17 *vs*. 129±16, P= 0.048), lower values of hemoglobin (124.4±23.9 *vs*. 134.2±23.4, P=0.003) and platelet count (median/interquartile-range 113/64-157 *vs*. 139/89-192, P=0.020), and a lower percentage of patients with HBV infection (79.4% *vs*. 89.1%, P=0.033). As shown in **Table 2**, no significant differences were found for their clinical classification and tumor-node-metastasis stage.

Multivariable analysis: effect of DM on changes of peripheral blood cells in HCC

Unconditional multivariable logistic regression analysis was used to determine the effect of diabetes on the changes of peripheral blood cells in Chinese patients with HCC. Based on the results of univariate analysis, five variables were included in the multivariable analysis (**Table 3**), including age, SBP, HBV infection, hemoglobin and platelet count. Fast blood glucose was excluded because of the dependent relationship with diabetes. As shown in **Table 3**, statistically significant differences were found for only two variables, including hemoglobin value (OR=0.982; 95% CI, 0.971-0.993; P= 0.002) and systolic blood pressure (OR=1.017; 95% CI, 1.001-1.034; P=0.039). However, no significant differences were found for the neutrophil and platelet count.

Sub-group analysis: consideration of the effect of cirrhosis/HBV infection on DM

We have known that liver cirrhosis and HBV infection affect the count and amount of peripheral blood cells according to the previously published literatures. In addition, four of the five variables with statistical differences shown in **Table 1** were associated with liver cirrhosis, including age, HBV infection, hemoglobin and platelet count. Considering that 328 (87.5%) patients had HBV infection and 199 (53.1%)

Table 5. Relationship between duration/treatment of diabetes and hemoglobin

Variable	Diabetic patients, n=63	Hemoglobin <120 g/L		Hemoglobin ≥120 g/L		P
		N=26	%	N=37	%	
Duration of diabetes, yr						
<1	16	5	19.2	11	29.7	0.346
≥1	47	21	80.8	26	70.3	---
Age at diabetes diagnosis, yr						
<60	49	18	69.2	31	83.8	0.171
≥60	14	8	30.8	6	16.2	---
Diabetes treatment						
Diet only						
Non-users	33	14	53.8	19	51.4	0.845
Users	30	12	46.2	18	48.6	---
Oral drug treatment						
Non-users	40	19	73.1	21	56.8	0.185
Users	23	7	26.9	16	43.2	---
Insulin treatment						
Non-users	52	20	76.9	32	86.5	0.517
Users	11	6	23.1	5	13.5	---
Type of oral drug treatment						
α-glucosidase inhibitor						
Non-users	54	23	88.5	31	83.8	0.875
Users	9	3	11.5	6	16.2	---
Biguanide						
Non-users	54	26	100.0	28	75.7	0.008
Users	9	0	0.0	9	24.3	---
Sulfonylureas						
Non-users	52	22	84.6	30	81.1	0.979
Users	11	4	15.4	7	18.9	---

patients had liver cirrhosis, sub-group analysis was performed when the study population was restricted into those patients with liver cirrhosis and those with HBV infection.

For those 199 HCC patients with cirrhosis, univariate analysis showed that diabetic patients had a lower amount of hemoglobin than non-diabetic patients (120.4±27.3 *vs.* 129.4±24.2, P=0.046). After the same confounding factors shown in **Table 3** were controlled by logistic analysis (**Table 4**), hemoglobin was found to be an independent variable (OR=0.984; 95% CI= 0.970-0.998; P=0.030). When the study population was restricted to those patients with HBV infection, the similar results with statistical differences were found in univariate analysis (124.4±25.6 *vs.* 135.2±22.5, P=0.002) and multivariable analysis (OR=0.982; 95% CI= 0.969-0.995; P=0.006).

Relationship between fasting blood glucose and hemoglobin in HCC

Based on the results of univariate, multivariable and sub-group analyses, the amount of hemoglobin was the sole independent variable for the changes of peripheral blood cells induced by diabetes in the HCC patients. Then, we would study the relationship between blood glucose, duration/treatment of diabetes and the amount of hemoglobin. Pearson correlation test was used to determine the relationship between hemoglobin and fasting blood glucose. Compared with patients without DM, the diabetic HCC patients had an increased level of fasting glucose (8.83±3.12 *vs.* 5.21±1.07, P<0.001); however, no significant correlation was found by Pearson test (r=-0.031, P=0.546). The same result was obtained when the analysis were restricted to those diabetics.

Relationship between duration/treatment of DM and hemoglobin in HCC

Chi-square test, continuity correction chi-square tests or Fisher's exact test were used to determine the relationship. Among the 63 diabetic patients (**Table 5**), 47 (74.6%) patients had been diagnosed with DM for more than one year, 49 (77.8%) were less than 60 years old at diabetes diagnosis, 23 (36.5%) received oral anti-diabetic regimens, 11 (17.5%) received insulin use treatment, and 30 (47.6%) reported relying on diet alone to control serum glucose level. The cutoff value of 120 g/L was selected according to the diagnostic criteria of anemia. As shown in **Table 5**, a significant difference was found only for the treatment with biguanide (P=0.008). All the nine patients treated with biguanide had no anemia (hemoglobin ≥120 g/L).

Discussion

Our study was designed to determine the effect of DM on the changes of peripheral blood cells in our Chinese patients with HCC, considering the tumor-host interactions and association of peripheral blood cells with carcinomas. We found that, after the confounding factors were controlled, including age, SBP, HBV infection and platelet count, the amount of hemoglobin remained as an independent indicator associated with DM in HCC patients. Taking into account the effect of liver cirrhosis and HBV infection, sub-group analysis was performed and the similar results were obtained. Moreover, we found that treatment with biguanide was associated with the increased level of hemoglobin and it maybe has the potential to prevent the anemia in HCC.

For the effect of diabetes on changes of peripheral blood cells, the major concern was the population-based case-control study, which could not provide definite evidences to clarify the causal association. However, in our study, all the HCC patients were diagnosed for the first time and hospitalized in our hospital. To avoid the effect of HCC on peripheral blood cells as far as possible, those data would be excluded if they were examined more than 15 days before or after the confirmed diagnosis of HCC. Moreover, 74.6% of the 63 diabetic patients had been diagnosed for more than one year before the HCC diagnosis and more than fifty percent of them had the need for hypoglycemic drugs or insulin to control blood glucose. Therefore, to a certain extent, we provided some evidences to clarify the causal association of DM with blood cells.

Changes of peripheral blood cells have been associated with HCC and diabetes. One earlier study which was published in the year of 1991 and included 175 HCC patients found that, compared with normal healthy individuals and asymptomatic hepatitis B virus surface antigen carriers, the HCC patients had the significantly decreased number of lymphocyte, B cell, T cell and T-cell subsets [18]. Another study showed that white blood cell count and NLR, not platelet count, were independent predictors of survival for the 419 HCC patients with extrahepatic metastasis, after the Child-Pugh classification and primary tumor stage were controlled [25]. Perioperative change in white blood cell count, not hemoglobin and platelet, was also found on multivariate analysis to be the only independent indicator which was positively associated with the recurrence and worse survival for HCC patients after hepatic resection [26].

Considering that no information was available for the effect of DM on the changes of peripheral blood cells in HCC patients, for the first time our study was designed to determine this relationship. We found that hemoglobin was the independent indicator associated with DM in HCC patients. Taking into account the possible effect of liver cirrhosis and HBV infection on the results, we performed the sub-group analysis and obtained the similar results. It is not surprised that hemoglobin is associated with DM because glycosylated hemoglobin has been regarded as one of the diagnostic criteria.

Diabetes is a complex and chronic metabolic disease characterized by hyperglycemia resulting from defects in the secretion and action of insulin [27]. One cross-sectional audit which was designed to investigate the prevalence and determinants of anemia in diabetic patients found that anemia was highly prevalent, affecting 59% of them. They also found that older age and longer duration of diabetes were the main predictors of anemia, whereas the presence of chronic kidney disease was a mediator rather than a direct cause [27]. The etiology of anemia in diabetes is multi-factorial. Chronic hypergly-

cemia may lead to hypoxia in the renal interstitium, resulting in tubulointerstitial damage and impaired production of erythropoietin by the peritubular fibroblasts [28]. Inappropriately low EPO levels in diabetes may lead to earlier development of anemia. Other factors include chronic inflammation, elevated levels of advanced glycosylation end products, diabetic neuropathy and inadequate iron stores [28].

Our study also found that treatment with biguanide was associated with the increased level of hemoglobin and it maybe has the potential to prevent anemia in HCC patients. All the nine patients treated with biguanide had no anemia and their hemoglobin exceeded the value of 120 g/L. Our previous study showed that metformin treatment was associated with reduced risk of HCC in diabetic patients [29]. For the first time metformin treatment was associated with the potential role to prevent the anemia in HCC. The possible mechanism may be deduced to be with the expression of PC-1, which is expressed in many tissues and inhibits insulin signaling either at the level of the insulin receptor or downstream at a post-receptor site. An elevated PC-1 content in insulin target tissues may play an important role in the development of insulin resistance in obesity and type 2 DM [30]. Insulin resistance is a characteristic feature of diabetes and anemia has been associated with DM. Metformin has been shown to decrease enzymatic activity of over-expressed PC-1 molecules in diabetics [30].

Some limitations should be acknowledged. The first is that most of the HCC patients were diagnosed clinically rather than by biopsy, and the diagnosis of most diabetics was dependent on their self-reported history or fasting serum glucose. The role of DM may be underestimated. However, we followed the diagnostic criteria recommended by the authorized institutes and used them widely in clinical practice. We believe that our results are more likely applicable in clinical practice. The second was due to the nature of our case-control study that some data could not be obtained and some possible factors could not be adjusted, for example NAFLD and NASH. Actually, biopsy was unnecessary and not recommended for these confirmed HCC patients.

In conclusion, our study showed that levels of hemoglobin were affected by diabetes in HCC patients and treatment with biguanide maybe has the potential to prevent this change. More studies, especially well-designed prospective studies, are required for a better understanding of this change and causal relationship.

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Disclosure of conflict of interest

None.

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