

## Increased international normalized ratio level in hepatocellular carcinoma patients with diabetes mellitus

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### Abstract

**AIM:** To determine the association of diabetes mellitus (DM) and international normalized ratio (INR) level in hepatocellular carcinoma (HCC) patients.

**METHODS:** Our present study included 375 HCC patients who were treated at the China-Japan Friendship Hospital, Ministry of Health (Beijing, China), in the period from January 2003 to April 2012, and with a hospital discharge diagnosis of HCC. The demographic, clinical, laboratory, metabolic and instrumental features were analyzed.  $\chi^2$  test, Student's *t* test and Mann-Whitney *U* test were used to compare the differences between HCC patients with and without DM. Unconditional multivariable logistic regression analysis was used to determine the association of DM and INR level in HCC patients. A sub-group analysis was performed to assess the effect of liver cirrhosis or hepatitis B virus

(HBV) infection on the results. The Pearson correlation test was used to determine the relationship between INR level and fasting glucose. In addition, association between diabetes duration, and diabetes treatment and INR level was determined considering the potentially different effects.

**RESULTS:** Of the total, 63 (16.8%) patients were diabetic (diabetic group) and 312 (83.2%) patients were diagnosed without diabetes (non-diabetic group). Their mean age was  $56.4 \pm 11.0$  years and 312 (83.2%) patients were male. Compared with patients without DM, the HCC patients with diabetes were older ( $59.5 \pm 10.3$  vs  $55.8 \pm 11.1$ ,  $P = 0.015$ ), had a lower incidence of HBV infection ( $79.4\%$  vs  $89.1\%$ ,  $P = 0.033$ ), had increased levels of systolic blood pressure (SBP) ( $133 \pm 17$  vs  $129 \pm 16$  mmHg,  $P = 0.048$ ) and INR ( $1.31 \pm 0.44$  vs  $1.18 \pm 0.21$ ,  $P = 0.001$ ), had lower values of hemoglobin ( $124.4 \pm 23.9$  vs  $134.2 \pm 23.4$ ,  $P = 0.003$ ) and had a platelet count (median/interquartile-range:  $113/64-157$  vs  $139/89-192$ ,  $P = 0.020$ ). There was no statistically significant difference in the percentages of males, overweight or obesity, drinking, smoking, cirrhosis and Child classification. After controlling for the confounding effects of age, systolic blood pressure, hemoglobin, platelet count and HBV infection by logistic analyses, INR was shown as an independent variable [odds ratio (OR) = 3.650; 95%CI: 1.372-9.714,  $P = 0.010$ ]. Considering the effect of liver cirrhosis on results, a sub-group analysis was performed and the study population was restricted to those patients with cirrhosis. Univariate analysis showed that diabetic patients had a higher INR than non-diabetic patients ( $1.43 \pm 0.51$  vs  $1.25 \pm 0.23$ ,  $P = 0.041$ ). After controlling for confounding effect of age, SBP, hemoglobin, platelet count and HBV infection by logistic analyses, INR level remained as the sole independent variable (OR = 5.161; 95%CI: 1.618-16.455,  $P = 0.006$ ). No significant difference in the relationship between INR level and fasting glucose was shown by Pearson test ( $r = 0.070$ ,  $P = 0.184$ ). Among the 63 diabetic patients, 35 (55.6%)

patients had been diagnosed with DM for more than 5 years, 23 (36.5%) received oral anti-diabetic regimens, 11 (17.5%) received insulin, and 30 (47.6%) reported relying on diet alone to control serum glucose levels. No significant differences were found for the association between DM duration/treatment and INR level, except for the age at diabetes diagnosis.

**CONCLUSION:** The INR level was increased in HCC patients with DM and these patients should be monitored for the coagulation function in clinical practice.

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**Key words:** International normalized ratio; Coagulation function; Diabetes mellitus; Hepatocellular carcinoma; Chinese patients

**Core tip:** This study showed that the international normalized ratio (INR) level was an independent variable associated with diabetes mellitus (DM) in hepatocellular carcinoma (HCC) patients compared with those HCC patients without DM, after controlling for the confounding effect of age, systolic blood pressure, hemoglobin, platelet and hepatitis B virus infection by logistic analyses. Considering the effect of liver cirrhosis on results, a sub-group analysis was performed and the study population was restricted to those HCC patients with cirrhosis. A similar result was obtained. These results indicated that INR level was increased in HCC patients with DM and this is independent of liver cirrhosis.

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## INTRODUCTION

Hepatocellular carcinoma (HCC), with a mounting annual incidence of 4.9 per 100000 persons, is the third most common cause of cancer death worldwide<sup>[1-3]</sup>. In China, there is a particularly high incidence of 40 per 100000 persons per year<sup>[4,5]</sup>. Although many advances in treatment have been made, the prognosis of HCC is very poor and the total 5-year survival rate is as low as 10%, even in those developed countries like the United States<sup>[6]</sup>. Risk factors of HCC that were identified include hepatitis B virus (HBV), hepatitis C virus (HCV), cirrhosis, heavy alcohol consumption, non-alcoholic steatohepatitis (NASH), aflatoxin exposure, increasing age, male sex, and positive family history<sup>[7,8]</sup>; however, in 15%-50% of HCC patients no specific risk factor has been found<sup>[4,5,9]</sup>.

Recently, emerging evidence suggest that diabetes mellitus (DM) is a potential risk factor for HCC<sup>[10-13]</sup>, which has been strengthened by several meta-analyses<sup>[13,14-17]</sup>.

Generally, DM is associated with about two to three fold increased risk of HCC and diabetes may also increase the risk of death from HCC, which has been observed in a large cohort study conducted in Europe<sup>[18]</sup>. In addition, DM can affect the prognosis of HCC after curative therapy and this prognostic impact is independent of the basic demographics, liver cirrhosis, and other comorbidities of HCC patients<sup>[19-21]</sup>.

Blood coagulation disorders are common findings in cancer patients<sup>[22,23]</sup>. Cancer cells can activate blood coagulation through the expression of procoagulant molecules such as tissue factors and cancer procoagulant which consequently activate serine proteases factor VIIa, factor Xa and thrombin<sup>[24,25]</sup>. However, HCC patients with impaired liver function have a more complex hemostatic disturbance, especially those with liver cirrhosis<sup>[26,27]</sup>. Moreover, some preclinical and clinical studies have suggested that diabetes is associated with coagulation disorders, which are responsible for an increased thrombotic tendency and risk of cardiovascular disease<sup>[28-30]</sup>. Therefore, the mechanisms for these coagulation alterations may be complex; however, no information was available for the association between DM and coagulation disorders in HCC patients, to our best knowledge.

Prothrombin time (PT), which is used to measure the coagulation factors of the “extrinsic pathway”, is the most frequently used coagulation test in routine laboratories. International normalized ratio (INR), which was introduced to overcome the problem of marked variation in PT results among laboratories, has been used to standardize PT value in liver diseases and been included in some prognostic models of HCC and liver cirrhosis, such as Child-Turcotte-Pugh (CTP) score and the model for end stage liver disease (MELD)<sup>[31]</sup>. Considering that no information was available for the effect of DM on the INR level in HCC patients, our study was designed to determine the association of DM and INR in our Chinese HCC patients.

## MATERIALS AND METHODS

### Study population

A cohort of patients who were treated at our hospital in the period from January 2003 to April 2012, and with a hospital discharge diagnosis of HCC, were included in our present study. Chronic HBV infection was defined as serum HBsAg-positive for at least 6 mo or at diagnosis of HCC. Patients who followed these criteria would be excluded: (1) those who had been treated by any method at inclusion or with confirmed diagnosis of HCC for more than 15 d; (2) those who had other malignancies, including leukemia and lymphoma; (3) those who were non-Chinese; (4) those who had autoimmune hepatitis, schistosomiasis, primary biliary cirrhosis, Budd-Chiari syndrome, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, rheumatic diseases or allergic disorder; and (5) those who had serious diseases of other organs or systems, such as severe heart failure, uremia, or acute exacerbations of chronic obstructive pulmonary disease. The study was approved by the Human Research

Ethics Committee of the China-Japan Friendship hospital and it was in accordance with the principles of the Declaration of Helsinki.

### Subject determinations

HCC diagnosis was based on the histological findings of needle biopsy/surgery or typical radiological features shown by at least two image examinations including ultrasound (US), contrast-enhanced dynamic computed tomography (CT) and magnetic resonance imaging (MRI) and hepatic angiography or by a single positive imaging with a serum alpha fetoprotein level  $> 400$  ng/mL<sup>[32]</sup>. DM was characterized by fasting plasma glucose of 126 mg/dL or greater on at least two occasions, plasma glucose of 200 mg/dL or greater at 2-h oral glucose tolerance test, or the need for an oral hypoglycemic drug or insulin to control glucose<sup>[33]</sup>.

### Clinical and laboratory parameters

The demographic, metabolic, biochemical, radiological, and pathological features of the patients with HCC were recorded. The data were obtained at the diagnosis of HCC, but excluded those obtained 15 d before or after the diagnosis. Patients who had missing values which may affect statistical results were excluded. Body mass index (BMI) was computed as body weight (in kilograms) divided by the square of the height (in meters). Overweight was defined as  $BMI \geq 23$  kg/m<sup>2</sup> and obesity  $BMI \geq 25$  kg/m<sup>2</sup>, according to the Asian and Chinese criteria<sup>[4,5]</sup>. The diagnosis of hypertension was defined as systolic blood pressure (SBP)  $\geq 140$  mmHg and/or diastolic blood pressure (DBP)  $\geq 90$  mmHg, and mean arterial pressure (MAP) was computed as  $1/3$  SBP plus  $2/3$  DBP. Blood pressure was measured in a quiet room at a comfortable temperature. Physical activity, smoking, coffee drinking, or having eaten within the past 30 min were to be avoided. Five associated parameters, including total bilirubin, albumin, INR, ascites and hepatic encephalopathy, were used to calculate the CTP score.

Venous blood samples were taken in the morning after a 12 h overnight fast. Standard methods were used to measure the laboratory parameters, including INR. Chronic HCV infection was defined as anti-HCV seropositivity and/or having detectable HCV RNA. Based on the recorded results, the findings of physical examinations and imaging techniques including US, CT, MRI and hepatic angiography were re-assessed carefully by at least two authors independently for tumor-node-metastasis (TNM) stage and clinical classification. We classified tumor stages according to the 7<sup>th</sup> TNM staging system recommended by International Union Against Cancer. In clinical classification, massive HCC was defined as a diameter of  $\geq 5$  cm, nodular HCC as a diameter of  $< 5$  cm, and small HCC as a diameter of  $< 3$  cm for single or two nodules.

### Statistical analysis

The statistical analysis was performed using SPSS for Windows, version 17.0 (SPSS, Chicago, IL, United

States). For the categorical variables, the numbers and proportions were described, and Pearson  $\chi^2$  test, continuity correction  $\chi^2$  tests or Fisher's exact test were used. For the continuous variables, mean  $\pm$  SD deviation was described and Independent-Samples *t* test was used. If the continuous variable had a skewed distribution, it would be described using the median value and inter-quartile range and analyzed by Mann-Whitney non-parametric *U* test. Unconditional multivariable logistic regression analysis was used to determine the association of DM and INR level in HCC patients. According to the results of univariate analysis, six variables were included, including age, SBP, HBV infection, hemoglobin, platelet count and INR level. To assess the effect of liver cirrhosis on our results, a sub-group analysis was performed and the study population was restricted to those HCC patients with and without cirrhosis. The Pearson correlation test was used to determine the relationship between INR level and fasting glucose. Stepwise multiple regression analysis (Backward: Wald; Entry: 0.05, Removal: 0.10) was used. We expressed results as odds ratio (OR) and their 95%CI. For all tests,  $P < 0.05$  was considered statistically significant and all *P* values quoted are two-sided.

## RESULTS

### Baseline characteristics of the study population

Our present study included 375 HCC patients based on the diagnostic, inclusion and exclusion criteria. Of the total, 63 (16.8%) patients were diabetic (diabetic group) and 312 (83.2%) patients were diagnosed without diabetes (non-diabetic group). Their baseline characteristics were shown in Table 1. Their mean age was  $56.4 \pm 11.0$  years and 312 (83.2%) patients were male. Of these patients, 328 (87.5%) had HBV infection and 22 (5.9%) patients had HCV infection. One hundred and ninety-nine (53.1%) patients were diagnosed with liver cirrhosis, 88 (23.5%) patients had a past history of hypertension, 159 (159/281, 56.6%) patients were overweight or obese, 104 (27.7%) patients were alcohol drinkers, and 154 (41.4%) patients were smokers. TNM stage and clinical classification of our study population were shown in Table 2. For the clinical classification, massive-type HCC (213, 56.8%) and nodular-type HCC (117, 31.2%) were the two major types, which accounted for nearly 90% of the total patients. For M stage, 269 (71.7%) were diagnosed with M0.

### Univariable analysis: Comparison of HCC patients with and without DM

Of the 63 diabetic patients, the mean age was  $59.5 \pm 10.3$  years, 51 (81.0%) were male, the mean BMI was  $23.69 \pm 3.51$  kg/m<sup>2</sup>, and 17 (27.0%) patients had a past history of hypertension. Twenty (31.7%) patients were smokers, and 14 (22.2%) were drinkers (Table 1). The duration and treatment of diabetes is shown in a later section.

$\chi^2$  test, Student's *t* test and Mann-Whitney *U* test were used to compare the differences between HCC patients with and without diabetes. As shown in Table 1, compared

**Table 1** Baseline characteristics of the study population and univariate analysis of comparison of hepatocellular carcinoma patients with and without diabetes *n* (%)

Variable	Total patients ( <i>n</i> = 375)	HCC patients with diabetes ( <i>n</i> = 63)	HCC patients without diabetes ( <i>n</i> = 312)	<i>P</i> value
Male sex	312 (83.2)	51 (81.0)	261 (83.7)	0.601
Mean age, yr (mean ± SD)	56.4 ± 11.0	59.5 ± 10.3	55.8 ± 11.1	0.015
Body weight <sup>1</sup> , kg (mean ± SD)	68.1 ± 10.9	69.3 ± 13.0	67.8 ± 10.5	0.346
Body height <sup>2</sup> , cm (mean ± SD)	168.8 ± 7.0	168.6 ± 8.0	168.9 ± 6.8	0.810
BMI <sup>3</sup> , kg/m <sup>2</sup> (mean ± SD)	23.77 ± 3.39	23.69 ± 3.51	23.68 ± 3.71	0.991
Overweight or obesity <sup>3</sup>	159 (56.6)	27 (57.4)	132 (56.4)	0.896
History of hypertension	88 (23.5)	17 (27.0)	71 (22.8)	0.470
SBP, mmHg (mean ± SD)	130 ± 17	133 ± 17	129 ± 16	0.048
DBP, mmHg (mean ± SD)	79 ± 10	78 ± 10	79 ± 10	0.455
Smoking	154 (41.4)	20 (31.7)	134 (42.9)	0.099
Alcohol intake	104 (27.7)	14 (22.2)	90 (28.8)	0.284
HBV infection	328 (87.5)	50 (79.4)	278 (89.1)	0.033
HCV infection	22 (5.9)	3 (4.8)	19 (6.1)	0.908
Liver cirrhosis	199 (53.1)	38 (60.3)	161 (51.6)	0.206
Fatty liver	11 (2.9)	2 (3.2)	9 (2.9)	1.000
Child-Turcotte-Pugh classification <sup>4</sup>				
Child A	243 (65.5)	35 (56.5)	208 (67.3)	0.101
Child B	92 (24.8)	17 (27.4)	75 (24.3)	0.600
Child C	36 (9.7)	10 (16.1)	26 (8.4)	0.061
AFP > 400 ng/mL <sup>5</sup>	167 (45.8)	27 (43.5)	140 (46.2)	0.702
Neutrophil, × 10 <sup>9</sup> /L (mean ± SD)	4.13 ± 2.49	3.88 ± 2.52	4.18 ± 2.49	0.389
Hemoglobin, g/L (mean ± SD)	132.5 ± 23.8	124.4 ± 23.9	134.2 ± 23.4	0.003
Platelet count, × 10 <sup>9</sup> /L	130 (85-189)	113 (64-157)	139 (89-192)	0.020
ALT, U/L	45 (29-81)	44 (27-91)	45 (30-79)	0.943
AST, U/L	62 (38-117)	52 (34-97)	66 (39-119)	0.124
ALP, U/L	111 (82-176)	111 (84-171)	111 (81-177)	0.907
GGT, U/L	106 (55-233)	96 (51-190)	109 (57-239)	0.406
INR <sup>6</sup> (mean ± SD)	1.20 ± 0.27	1.31 ± 0.44	1.18 ± 0.21	0.001
Total bilirubin, mg/L	16 (10-27)	17 (10-31)	16 (10-25)	0.561
Albumin, g/L (mean ± SD)	37.4 ± 6.0	36.4 ± 5.9	37.6 ± 6.0	0.148
Total cholesterol, mmol/L (mean ± SD)	4.26 ± 1.34	4.18 ± 1.12	4.28 ± 1.39	0.662
BUN, mmol/L	5.14 (4.00-6.36)	5.12 (3.92-6.46)	5.18 (4.04-6.34)	0.738
Creatinine, μmol/L	80 (71-88)	80 (70-97)	80 (71-88)	0.860

Data were available in <sup>1</sup>366 (60 + 306), <sup>2</sup>286 (49 + 237), <sup>3</sup>281 (47 + 234), <sup>4</sup>371 (62 + 309), <sup>5</sup>365 (62 + 303) and <sup>6</sup>367 (62 + 305) patients. The numbers before the brackets indicate the total available cases in the two groups. BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; INR: International normalized ratio; SBP: Systolic blood pressure; ALT: Aminolucine transferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Galactosylhydroxylslyl glucosyltransferase.

with patients without DM, the HCC patients with diabetes had: an older age (59.5 ± 10.3 *vs* 55.8 ± 11.1, *P* = 0.015); a lower incidence of HBV infection (79.4% *vs* 89.1%, *P* = 0.033); increased levels of SBP (133 ± 17 *vs* 129 ± 16, *P* = 0.048) and INR (1.31 ± 0.44 *vs* 1.18 ± 0.21, *P* = 0.001); 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). There was no statistically significant difference in the percentages of males, overweight or obesity, drinking, smoking, cirrhosis and Child classification. Results of univariable analysis for the TNM stage and clinical classification were shown in Table 2. No significant differences were demonstrated for T stage, N stage, M stage and the clinical classification.

### Multivariable analysis: Increased INR levels in HCC patients with DM

Unconditional multivariable logistic regression analysis was used to determine the association of DM and INR level in HCC patients. According to the results of univar-

iate analysis, six variables were included (Table 3), including age, SBP, HBV infection, hemoglobin, platelet count and INR level. Statistical differences were shown for three variables (Table 3): INR level (OR = 3.650; 95%CI: 1.372-9.714; *P* = 0.010), SBP (OR = 1.019; 95%CI: 1.002-1.036; *P* = 0.029), and hemoglobin value (OR = 0.987; 95%CI: 0.975-0.999; *P* = 0.038).

Considering that some factors may play possible roles in HCC based on the published literature and our current knowledge, for example male gender, alcohol drinking, HCV infection, liver cirrhosis, and CTP classification. To control the effect of these variables, they were included in the multivariable analysis, although no statistical significance were shown by univariate analysis (Table 1). For this purpose, more potentially confounding factors were controlled and logistic regression was repeated, including age, sex, HBV infection, HCV infection, alcohol drinking, liver cirrhosis, CTP classification and INR (Table 3). Results showed that INR level remained statistically significant (OR = 4.487; 95%CI: 1.713-11.754; *P* = 0.002).

**Table 2 Tumor-node-metastasis stage and clinical type of the study population *n* (%)**

Variable	Total patients ( <i>n</i> = 375)	HCC patients with diabetes ( <i>n</i> = 63)	HCC patients without diabetes ( <i>n</i> = 312)	<i>P</i> value
T stage				
T1	82 (21.9)	15 (23.8)	67 (21.5)	0.683
T2	90 (24.0)	19 (30.2)	71 (22.8)	0.210
T3a	157 (41.9)	22 (34.9)	135 (43.3)	0.221
T3b	37 (9.9)	6 (9.5)	31 (9.9)	0.920
T4	9 (2.4)	1 (1.6)	8 (2.6)	0.991
N stage				
N0	334 (89.1)	57 (90.5)	277 (88.8)	0.694
N1	41 (10.9)	6 (9.5)	35 (11.2)	-
M stage				
M0	269 (71.7)	49 (77.8)	220 (70.5)	0.243
M1	106 (28.3)	14 (22.2)	92 (29.5)	-
Clinical classification				
Massive	213 (56.8)	29 (46.0)	184 (59.0)	0.059
Nodular	117 (31.2)	22 (34.9)	95 (30.4)	0.485
Small-cancer	28 (7.5)	8 (12.7)	20 (6.4)	0.142
Diffuse	17 (4.5)	4 (6.3)	13 (4.2)	0.503

HCC: Hepatocellular carcinoma; T: Tumor; N: Node; M: Metastasis.

**Table 3 Multivariable analysis: Increased international normalized ratio level in hepatocellular carcinoma patients with diabetes mellitus**

Variable	AOR	95%CI	<i>P</i> value
Model 1			
INR	3.650	1.372-9.714	0.010
SBP	1.019	1.002-1.036	0.029
Hemoglobin	0.987	0.975-0.999	0.038
Model 2			
INR	4.487	1.713-11.754	0.002
Age	1.032	1.005-1.059	0.020

Model 1: Based on the results of univariate analysis, unconditional multivariable logistic regression analysis was performed. Six variables were included, including age, systolic blood pressure (SBP), hepatitis B virus (HBV) infection, hemoglobin, platelet count and international normalized ratio (INR). Statistical differences were shown for 3 variables (shown in Table), and other four variables were omitted because no significant differences were found; Model 2: According to the published literatures and our current knowledge, more potential confounding factors were controlled, including age, sex, HBV infection, hepatitis C virus infection, alcohol drinking, liver cirrhosis, Child-Turcotte-Pugh classification and INR. AOR: Adjusted odds ratio.

In addition, one significant difference was found for age (OR = 1.032; 95%CI: 1.005-1.059; *P* = 0.020).

### Sub-group analysis

As demonstrated in Table 1, among the six variables which were shown as statistically significant in univariate analysis, 5 were associated with liver cirrhosis, including age, HBV infection, hemoglobin, platelet count and INR level. To assess the effect of liver cirrhosis on our results, sub-group analysis was performed although liver cirrhosis had been controlled in the multivariable logistic analysis. We restricted our study population to those HCC patients with and without cirrhosis.

In the 199 HCC patients with liver cirrhosis, data were not available in 7 patients for INR level and 192 were included in the sub-group analysis, including 37 pa-

**Table 4 Results of multivariable analysis for sub-group analysis**

Variable <sup>1</sup>	AOR <sup>2</sup>	95%CI	<i>P</i> value
HCC patients with liver cirrhosis			
INR	5.161	1.618-16.455	0.006
HCC patients without liver cirrhosis			
INR	2.082	0.130-33.333	0.604
Age	1.060	1.015-1.107	0.008
HCC patients with HBV infection			
INR	2.508	0.860-7.315	0.092
Hemoglobin	0.984	0.970-0.998	0.024

<sup>1</sup>International normalized ratio (INR) and variables with significant difference were shown; <sup>2</sup>Adjusted odds ratio (AOR) for the confounding effect of age, systolic blood pressure, hemoglobin, platelet count, and hepatitis B virus (HBV) infection using unconditional multivariable logistic regression analyses, based on the results of univariate analysis shown in Table 1. HCC: Hepatocellular carcinoma.

tients in the diabetic group and 155 in the non-diabetic group. Univariate analysis showed that diabetic patients had a higher level of INR than non-diabetic patients ( $1.43 \pm 0.51$  vs  $1.25 \pm 0.23$ , *P* = 0.041). After controlling for the confounding effects of age, SBP, hemoglobin, platelet count and HBV infection by logistic analyses (Table 4), INR level remained as the sole independent variable (OR = 5.161; 95%CI: 1.618-16.455, *P* = 0.006).

When the study population was restricted to those patients without liver cirrhosis, no significant difference was found for INR value ( $1.12 \pm 0.19$  vs  $1.10 \pm 0.16$ , *P* = 0.721). A similar result was gained in logistic analyses (OR = 2.082; 95%CI: 0.130-33.333; *P* = 0.604). In addition, considering that more than 80% of HCC has been attributed to HBV infection in China (this number was 87.5% in our present study), we performed a sub-group analysis in those HCC patients with HBV infection. Unfortunately, no statistically significant differences were found in univariate analysis ( $1.28 \pm 0.43$  vs  $1.18 \pm 0.21$ , *P* = 0.091) and multivariable analysis (OR = 2.508; 95%CI: 0.860-7.315, *P* = 0.092).

**Table 5 Association between diabetes duration/treatment and International normalized ratio level *n* (%)**

Variable	INR < 1.20 ( <i>n</i> = 36)	INR ≥ 1.20 ( <i>n</i> = 27)	<i>P</i> value	INR < 1.50 ( <i>n</i> = 52)	INR ≥ 1.50 ( <i>n</i> = 11)	<i>P</i> value
Duration of diabetes, yr						
< 5	15 (41.70)	13 (48.10)	0.608	23 (44.20)	5 (45.50)	1.000
≥ 5	21 (58.30)	14 (51.90)	-	29 (55.80)	6 (54.50)	-
Age at diabetes diagnosis, yr						
< 50	11 (30.60)	12 (44.40)	0.257	15 (28.80)	8 (72.70)	0.016
≥ 50	25 (69.40)	15 (55.60)	-	37 (71.20)	3 (27.30)	-
Diabetes treatment						
Oral treatment						
Non-users	21 (58.30)	19 (70.40)	0.326	31 (59.60)	9 (81.80)	0.296
Users	15 (41.70)	8 (29.60)	-	21 (40.40)	2 (18.20)	-
Insulin treatment						
Non-users	27 (75.00)	25 (92.60)	0.138	43 (82.70)	9 (81.80)	1.000
Users	9 (25.00)	2 (7.40)	-	9 (17.30)	2 (18.20)	-
Diet only						
Non-users	16 (44.40)	17 (63.00)	0.145	26 (50.00)	7 (63.60)	0.411
Users	20 (55.60)	10 (37.00)	-	26 (50.00)	4 (36.40)	-
Type of oral treatment						
Biguanide						
Non-users	32 (88.90)	22 (81.50)	0.640	43 (82.70)	11 (100)	0.310
Users	4 (11.10)	5 (18.50)	-	9 (17.30)	0 (0)	-
Sulfonylureas						
Non-users	29 (80.60)	23 (85.20)	0.886	42 (80.80)	10 (90.9)	0.713
Users	7 (19.40)	4 (14.80)	-	10 (19.20)	1 (9.1)	-
α-glucosidase inhibitor						
Non-users	29 (80.60)	25 (92.60)	0.323	44 (84.60)	10 (90.9)	0.946
Users	7 (19.40)	2 (7.40)	-	8 (15.40)	1 (9.1)	-

INR: International normalized ratio.

### Association of INR and fasting glucose level

A Pearson correlation test was used to determine the relationship between INR level and fasting glucose. In the entire study population of 367 patients (data were not available in 8 patients for INR), the mean value of fasting glucose was  $8.83 \pm 3.12$  mmol/L for diabetic patients whereas the value was  $5.21 \pm 1.07$  mmol/L for non-diabetic patients ( $P < 0.001$ ). However, no significant difference was shown by Pearson test ( $r = 0.070$ ,  $P = 0.184$ ). Even after the analysis was restricted to diabetics only, the same result was obtained.

### Association between diabetes duration/treatment and INR level

Considering the potentially different effects of diabetes duration and anti-diabetic agents, we studied the association between DM duration/treatment and INR level. Among the 63 diabetic patients (Table 5), 35 (55.6%) patients had been diagnosed with DM for more than 5 years, 23 (36.5%) received oral anti-diabetic regimens, 11 (17.5%) received insulin, and 30 (47.6%) reported relying on diet alone to control serum glucose level. The cutoff values of 1.20 and 1.50 were determined based on the mean value and range of the normal value.  $\chi^2$  test, continuity correction  $\chi^2$  tests or Fisher's exact test were used to determine the association. As shown in Table 5, no statistically significant differences were found for the association between DM duration/treatment and INR level, except for the age at diabetes diagnosis.

## DISCUSSION

Our study showed that the INR level was an independent variable associated with DM in HCC patients (OR = 3.650; 95%CI: 1.372-9.714;  $P = 0.010$ ), compared with those HCC patients without DM, after controlling for the confounding effect of age, SBP, hemoglobin, platelet and HBV infection by logistic analyses. Considering the effect of liver cirrhosis on results, a sub-group analysis was performed and the study population was restricted to those HCC patients with cirrhosis. A similar result was obtained (OR = 5.161; 95%CI: 1.618-16.455,  $P = 0.006$ ). These results indicated that INR level was increased in HCC patients with DM and this is independent of liver cirrhosis.

No information was available for the association between DM and coagulation disorders in HCC patients, to the best of our knowledge. For the first time, our study was designed to determine the association of DM and INR in HCC patients. INR, developed by the World Health Organization to standardize PT reporting in the early 1980s, is used worldwide to monitor oral anticoagulation therapy<sup>[22,23]</sup>. The INR level is used to measure the extrinsic pathway of the coagulation cascade and influenced by coagulation factors I (fibrinogen), II (prothrombin), V, VII, and X. This index has been also recommended to evaluate the survival of patients with severe liver disease, and been included in some prognostic models, such as CTP score and MELD score.

Previous studies have reported that DM has a role in the activation of coagulation factors and subsequently increases thrombotic tendency and cardiovascular risk<sup>[28-30]</sup>. However, no information was available in HCC patients. Our study included 375 HCC patients based on the diagnostic, inclusion and exclusion criteria, and the unconditional multivariable logistic regression analysis used to determine the association. Our results indicated that coagulation disorders could also be found in HCC with DM, but the effect may be different from previously published literature.

Patients with DM have higher levels of circulating tissue factor (TF), which initiate the extrinsic pathway of the coagulation cascade by binding and activating factor VII<sup>[28,29]</sup>. The levels of TF are directly modulated by glucose and insulin, as well as by nuclear factor kappa B which is activated by the formation of advanced glycation end products and reactive oxygen species<sup>[34,35]</sup>. Levels of factor VII are influenced by triglyceride levels, which often increase in poorly controlled diabetic patients. Plasma levels of other coagulation factors, such as fibrinogen and prothrombin, are also elevated in diabetic patients. In addition to the changes in levels of coagulation factors, DM induces quantitative modifications of those factors, which also increases thrombosis risk.

However, our study showed that the INR level was increased in HCC patients with DM. The exact mechanism remains as yet unclearly understood and it was deduced as follows. The first was due to the liver itself. Diabetes plays its role in increasing the levels of coagulation factors in the circulation mainly through increased synthesis of the liver. When liver function is impaired, the synthesis of coagulation factors would also be impaired. In addition, our study found that a similar result was obtained when the population was restricted to those HCC patients with liver cirrhosis, whereas no significant difference was observed for those patients without cirrhosis. Increased INR levels in HCC patients with DM may be partly associated with liver cirrhosis.

Secondly, activation of inflammatory and coagulation pathways is important in the pathogenesis of coronary artery disease that worsens the prognosis of HCC patients<sup>[36]</sup>. There is ample evidence that extensive cross-talk between these two systems exists, whereby inflammation not only leads to activation of coagulation, but coagulation also markedly affects inflammatory activity. The main interfaces linking coagulation and inflammation are beyond the tissue factor pathway and thrombin, the protein C system and the fibrinolytic (or plasminogen-plasmin) system. Proinflammatory cytokines (mainly IL-6) and chemokines can affect all these coagulation mechanisms, and vice versa, activated coagulation proteases and physiological anticoagulants or components of the plasminogen-plasmin system can modulate inflammation by specific cell receptors. The intricate relationship between inflammation and coagulation is extremely clear in nonalcoholic fatty liver disease (NAFLD)<sup>[36]</sup>.

Thirdly, angiogenesis is required for tumor growth

and metastasis. Activation of the coagulation pathway also enhances tumor growth and metastasis<sup>[37]</sup>. Procoagulants involved in angiogenesis include TF and thrombin. Vascular endothelial growth factor, the most potent pro-angiogenic factor, is an indirect procoagulant; it is capable of inducing vascular hyperpermeability and of increasing TF expression on endothelial cells. Vascular hyperpermeability results in leakage of plasma proteins, including prothrombin and fibrinogen, into the extracellular matrix. Prothrombin converted into thrombin by the activated coagulation pathway may result in platelet activation and the production of fibrin from fibrinogen<sup>[37]</sup>.

In our study, we found that, compared with patients without DM, HCC patients with diabetes were older. The reason remains as yet unclearly understood. It was deduced to be possibly related to the duration, treatment and monitoring of diabetes. Some limitations should also be acknowledged. The first limitation is the case-control design, which could not provide definite evidence to clarify the causal association. To clarify the causal relationship, well-designed prospective studies are required. The second is that most of the HCC and cirrhotic 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, not on an oral glucose tolerance test. Thus the role of DM could be underestimated. However, we followed strictly 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 third limitation was due to the nature of our case-control study, which meant that some data could not be obtained and some possible factors could not be adjusted. For example, NAFLD and NASH have been regarded as risk factors for HCC, but we could not assess these changes. However, biopsy was unnecessary for these confirmed HCC patients and was not recommended. In addition, activated partial thromboplastin time is another commonly used screening test for evaluating coagulation disorders. Unfortunately, data were not available for some patients and the analysis could not be performed. However, in clinical practice, this parameter is not frequently used in liver disease, compared with PT and the INR.

Our present study also raises several questions for future research. Firstly, how DM affects the coagulation system in HCC patients; secondly, whether increased INR in diabetic patients has a clinical meaning or can affect the prognosis of HCC patients; and last but not least, whether antidiabetic treatment can improve coagulation function in HCC. To answer these questions, further pre-clinical and clinical studies are needed.

In conclusion, our study showed that INR level was increased in HCC patients with DM and these patients should be monitored for coagulation function in clinical practice. More studies are required for a better understanding of this change.

## COMMENTS

**Background**

Diabetes mellitus (DM) is a potential risk factor for hepatocellular carcinoma (HCC). HCC patients with impaired liver function have complex coagulation disorders, especially in those with liver cirrhosis. However, no information was available for the association between DM and coagulation disorders in HCC patients.

**Research frontiers**

International normalized ratio (INR) has been included in some prognostic models of HCC and liver cirrhosis, such as Child-Turcotte-Pugh score and the model for end stage liver disease. This study was designed to determine the association of DM and INR in HCC patients, considering that no information was available for the effect of DM on INR in HCC.

**Innovations and breakthroughs**

The authors found that the INR level was increased in HCC patients with DM which is independent of liver cirrhosis. This is the first time that information was available for the effect of DM on the INR level in HCC.

**Applications**

HCC patients with DM should be monitored for coagulation function in clinical practice.

**Peer review**

The effect of DM in INR levels in HCC is quite interesting with clinical therapeutic implications. Nice paper to be published after minor revision.

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