

Retrospective Study

Diabetes mellitus may affect the long-term survival of hepatitis B virus-related hepatocellular carcinoma patients after liver transplantation

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Author contributions: Zhang Q, Deng YL, Liu C, Huang LH and Shang L contributed equally to this study; Zhang Q and Shen ZY proposed and designed the study; Zhang Q, Deng YL, Liu C and Huang LH performed the research and wrote the first draft of the manuscript; Huang LH, Shang L, Chen XG and Wang LT collected the patient and statistical data; Shang L analyzed and reviewed the data; All authors contributed to the design, revisions and interpretation of the study and to further drafts of the manuscript; Shang L and Shen ZY are the guarantors; all authors have read and approved the final manuscript.

Supported by the National Natural Science Foundation of China, General Program, No. 81372595; and the National High Technology Research and Development Program of China (863 Program), No. 2012AA021006.

Institutional review board statement: This study was performed in compliance with the principles of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the General Hospital of the Chinese People's Armed Police Force, Tianjin First Central Hospital and the First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University

(China). Written informed consent was obtained from individual participants.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

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Manuscript source: Unsolicited manuscript

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Received: June 11, 2016

Peer-review started: June 17, 2016

First decision: July 12, 2016

Revised: August 2, 2016

Accepted: September 14, 2016

Article in press: September 14, 2016

Published online: November 21, 2016

Abstract

AIM

To determine whether diabetes mellitus (DM) affects

prognosis/recurrence after liver transplantation (LT) for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC).

METHODS

A retrospective study was conducted between January 2000 and August 2013 on 1631 patients with HBV-related HCC who underwent LT with antiviral prophylaxis. Patient data were obtained from the China Liver Transplant Registry (<https://www.cltr.org/>). To compare the outcomes and tumor recurrence in the HBV-related HCC patients with or without DM, statistical analyses were conducted using χ^2 tests, Mann-Whitney tests, the Kaplan-Meier method, log-rank tests and multivariate step-wise Cox regression analysis.

RESULTS

Univariate analysis of 1631 patients who underwent LT found overall 1-, 3- and 5-year survival rates of 79%, 73% and 71% respectively in the DM patients, and 84%, 78% and 76% in the non-DM patients respectively. Overall survival rate differences after LT between the two groups were significant ($P = 0.041$), but recurrence-free survival rates were not ($P = 0.096$). By stratified analysis, the overall survival rates in DM patients for age > 50 years ($P = 0.002$), the presence of vascular invasion ($P = 0.096$), tumors ≤ 3 cm ($P = 0.047$), two to three tumor nodules ($P = 0.007$), Child-Pugh grade B ($P = 0.018$), and pre-LT alanine aminotransferase levels between 40 and 80 IU/L ($P = 0.017$) were significantly lower than in non-DM patients. Additionally, serum α -fetoprotein level > 2000 ng/mL ($P = 0.052$) was associated with a significant survival difference trend between DM and non-DM patients. Multivariate analysis showed that the presence of DM ($P < 0.001$, HR = 1.591; 95%CI: 1.239-2.041) was an independent predictor associated with poor survival after LT.

CONCLUSION

HBV-related HCC patients with DM have decreased long-term overall survival and poor LT outcomes. Prevention strategies for HCC patients with DM are recommended.

Key words: Diabetes mellitus; Hepatocellular carcinoma; Hepatitis B virus; Liver transplantation; Survival

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Core tip: Diabetes mellitus (DM) may be associated with hepatocellular carcinoma (HCC) pathological features, old age and poor liver function, which may influence post-liver transplantation (LT) survival in hepatitis B virus (HBV)-related HCC patients. However, no direct relation between DM and HBV infection or HBV load were observed in HCC patients. The current data represent a multi-center study involving a larger number of samples, and suggest that DM may affect the long-term post-LT survival of HBV-related HCC

patients, but not of tumor recurrence. Thus, HCC patients with DM undergoing LT should be closely followed to optimize outcomes. Follow-up examinations are especially important among DM patients who are elderly or who have the presence of vascular invasion and serum α -fetoprotein levels > 2000 ng/mL pre-LT.

Zhang Q, Deng YL, Liu C, Huang LH, Shang L, Chen XG, Wang LT, Du JZ, Wang Y, Wang PX, Zhang H, Shen ZY. Diabetes mellitus may affect the long-term survival of hepatitis B virus-related hepatocellular carcinoma patients after liver transplantation. *World J Gastroenterol* 2016; 22(43): 9571-9585 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v22/i43/9571.htm> DOI: <http://dx.doi.org/10.3748/wjg.v22.i43.9571>

INTRODUCTION

Of the 350 million individuals infected with hepatitis B virus (HBV) worldwide, there are 130 million HBV carriers and 30 million chronically HBV-infected people in China^[1,2]. The incidence of hepatocellular carcinoma (HCC) in China is dramatically increased by HBV infection, which is the main cause of HCC^[3,4]. Over 70% of cases of HCC in China have been associated with HBV infection^[5].

Diabetes mellitus (DM) is the sixth leading cause of death in China, and its prevalence in the adult Chinese population increased to 11.7% in 2010^[6]. Recent studies have shown that DM may be a potential risk factor for HCC^[5,7,8], and this hypothesis has been supported by several case control^[9-12] and cohort studies^[13-17]. Additionally, increased HCC prevalence in the diabetic population has been reported, possibly due to steatohepatitis^[7,9]. However, it is unknown whether HBV infection acts directly or additively with steatohepatitis on the incidence of HCC in DM patients. Liver transplantation (LT) has been established as the only curative method for simultaneous treatment of HCC and cirrhosis^[18,19], and is generally accepted as superior for early HCC with cirrhosis compared to hepatic resection^[20,21].

There has been recent debate about DM as an independent risk factor for post-treatment HCC recurrence and poor outcomes. Some studies have suggested that DM increases the risk of HCC recurrence and poor patient outcomes in patients undergoing hepatic resection^[22]. However, other studies reported that DM does not worsen the prognosis of HCC patients after resection^[23,24]. Few studies have investigated the association between HBV, DM, tumor recurrence and long-term survival of HCC patients who underwent LT. The aim of this study was to investigate the potential associations between pre-existing DM with HBV-induced HCC, HCC recurrence and long-term survival after LT in a large cohort.

MATERIALS AND METHODS

Patient population

Between January 2000 and August 2013, 2128 patients were diagnosed with primary HCC and underwent LT at the General Hospital of Chinese People's Armed Police Force, Tianjin First Central Hospital and First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University (data from the China Liver Transplant Registry: <https://www.cltr.org/>). Among the 2128 HCC patients who underwent LT, 1631 HBV-related cirrhosis patients with antiviral prophylaxis and complete follow-up information were studied retrospectively.

Inclusion criteria were: (1) adults \geq 18-year-old; (2) fulfillment of the UCSF criteria^[25] or Hangzhou criteria (China)^[26]; (3) diagnosis of cirrhosis and HCC; and (4) diagnosis of pre-LT HBV infection. Exclusion criteria were: (1) pre-LT imaging diagnosis of HCC complicated by inferior vena cava tumor thrombus or extrahepatic tumor metastasis (including lymph node metastasis); (2) hepatitis C co-infection; (3) any history of alcohol abuse; and (4) HIV or HDV infection without adequate social support.

This study enrolled 1496 men and 135 women, with ages ranging between 25 and 80 years and a median age of 52.42 years. Of the 1631 patients, at the time of LT, 1336 (81.9%) were DM-negative and 295 (18.1%) had DM. Among the DM patients, 17 (5.8%) had type 1 diabetes and 278 (94.2%) had type 2 diabetes. Among the 295 DM cases, 29 (9.8%) were controlled by diet and exercise alone, 71 (24.1%) used oral sulfonylurea medications, 92 (31.2%) used oral sulfonylureas combined with insulin, and 103 (34.9%) used insulin alone. There were 101 (34.2%) patients with fasting blood glucose \leq 7.0 mmol/L and 194 (65.8%) patients with fasting blood glucose $>$ 7.0 mmol/L, out of the total of 295 DM patients. The baseline characteristics of the 1631 HBV-induced HCC patients with or without DM are summarized in Table 1.

HBV infection diagnosis and treatment

For HBV-related patients, the pre-LT HBV infection status was checked routinely using the following viral markers: hepatitis B surface antigen (HBsAg); HB surface antibody (HBsAb), HB e antigen (HBeAg); hepatitis B core antibody (HBcAb); and HBV deoxyribonucleic acid (DNA) levels. HBV infection was defined by the presence of serum HBsAg or HBV DNA positivity. Before and after LT, HBV status was assessed using HBV markers and PCR for HBV DNA. In order to identify lamivudine resistance, viral mutation tests were also conducted^[27]. Percutaneous or transjugular liver biopsies were performed to prove HBV re-infection. HBV genotyping was not performed in the majority of patients, and, therefore, was not included in the final analysis. All patients were routinely given nucleoside analogues (lamivudine, adefovir or entecavir) pre- and post-LT, and hepatitis B

immunoglobulins post-LT (Table 2).

HCC and tumor recurrence assessment

Pre-LT HCC diagnosis was confirmed by a combination of two abdominal imaging techniques [ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI)], and by measuring serum α -fetoprotein (AFP) levels, or positron emission tomography (PET), if necessary, to exclude extrahepatic tumor metastasis. HCC and cirrhosis were diagnosed by pathological examination post-LT.

During the LT follow-up, routine examinations included X-ray imaging, abdominal ultrasonography, whole-body CT scans and measurement of serial serum AFP levels. Early tumor recurrence or metastasis was assessed by monthly monitoring of AFP levels and abdominal ultrasonography, and by whole-body CT, MRI examinations and bone scintigraphy every 3 to 6 mo. Histological confirmation of recurrence was conducted using patient biopsies, if necessary. Liver function tests were regularly checked before and after LT.

DM assessment and post-LT treatment

Pre-LT DM status was determined by retrospective review and laboratory tests, and assessed using the DM history. Medical history included medical records, recent history and physical examination records of use of oral hypoglycemic agents or insulin. Patients were also asked if they had a history of physician-diagnosed DM. The diagnostic criteria for DM consisted of fasting (\geq 8 h) plasma glucose levels \geq 126 mg/dL and 2-h oral glucose tolerance test results showing \geq 200 mg/dL^[28]. Post-LT DM status was not included in this study. The treatment method for early post-LT DM was mainly insulin alone or oral sulfonylureas combined with insulin, according to fasting plasma glucose levels and 2-h oral glucose tolerance test results.

Study variables

Variables analyzed included sex, age, body mass index (BMI), DM, HBV markers, pathologic tumor characteristics (including number of tumor nodules, tumor size, venous invasion, lymph node invasion, capsular invasion and microsatellite lesions), serum AFP levels, Child-Pugh scores, model for end-stage liver disease (MELD) scores, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), triglyceride (TG), and cholesterol (CH) levels. Pre-LT antitumor therapy and post LT follow-up data included post-LT HBsAg reinfection, tumor recurrence, metastasis and survival time. These variables were collected and recorded in the databases of the China Liver Transplant Registry (<https://www.cltr.org/>).

Patient height and weight were recorded prior to LT, as well as BMI for all patients. BMI was defined as weight in kilograms divided by height² (m²). All

Table 1 Baseline characteristics of patients with diabetes mellitus and without diabetes mellitus *n* (%)

Category	Diabetic (<i>n</i> = 295)	Non-diabetic (<i>n</i> = 1336)	Value	df	<i>P</i> value
Sex			2.999	1	0.083
Male	278 (94.2)	1218 (91.2)			
Female	17 (5.8)	118 (8.8)			
Age, yr			23.690	1	0.000
≤ 50	96 (32.5)	643 (48.1)			
> 50	199 (67.5)	693 (51.9)			
Tumor size			4.333	3	0.228
≤ 3	163 (55.3)	704 (52.7)			
3-5	85 (28.8)	350 (26.2)			
5-7	28 (9.5)	157 (11.8)			
> 7	19 (6.4)	125 (9.4)			
Number of tumor nodules			0.245	2	0.885
Singer	147 (49.8)	666 (49.9)			
≥ 2, ≤ 3	72 (24.4)	341 (25.5)			
≥ 4	76 (25.8)	329 (24.6)			
Venous invasion			0.641	1	0.423
Absent	256 (86.8)	1135 (85.0)			
Present	39 (13.2)	201 (15.0)			
Lymph node invasion			1.272	1	0.304
Absent	284 (96.3)	1265 (94.7)			
Present	11 (3.7)	71 (5.3)			
Capsular invasion			0.005	1	0.942
Absent	259 (87.8)	1175 (87.9)			
Present	36 (12.2)	161 (12.1)			
Microsatellite lesions			0.430	1	0.512
Absent	234 (79.3)	1082 (81.0)			
Present	61 (20.7)	254 (19.0)			
Edmondson			4.359	2	0.108
1	12 (4.1)	27 (2.0)			
2	168 (56.9)	797 (59.7)			
3 + 4 + 5	115 (39.0)	512 (38.3)			
Serum AFP level, in ng/mL			5.518	4	0.236
< 200	231 (78.3)	966 (72.3)			
200-400	18 (6.1)	87 (6.5)			
400-1000	17 (5.8)	122 (9.1)			
1000-2000	13 (4.4)	65 (4.9)			
> 2000	16 (5.4)	96 (7.2)			
MELD score			14.184	2	0.001
< 10	97 (32.9)	582 (43.6)			
10-15	94 (31.9)	406 (30.4)			
> 15	104 (35.3)	348 (26.0)			
Child-Pugh score			12.456	2	0.002
A	125 (42.4)	715 (53.5)			
B	104 (35.3)	396 (29.6)			
C	66 (22.4)	225 (16.8)			
Fasting blood glucose, in mmol/L			678.412	1	0.000
< 7	101 (34.2)	1273 (95.3)			
≥ 7	194 (65.8)	63 (4.7)			
BMI, in kg/m ²			7.937	3	0.047
< 18.5	19 (6.4)	69 (5.2)			
18.5-24	164 (55.6)	856 (64.1)			
24-28	84 (28.5)	295 (22.1)			
> 28	28 (9.5)	116 (8.7)			
ALP, in IU/L			1.546	1	0.214
≤ 112	179 (60.7)	862 (64.5)			
> 112	116 (39.3)	474 (35.5)			
GGT, in IU/L			2.196	1	0.138
≤ 54	107 (36.3)	547 (40.9)			
> 54	188 (63.7)	789 (59.1)			
AST, in IU/L			2.682	2	0.262
≤ 40	102 (34.6)	481 (36.0)			
40-80	107 (36.3)	526 (39.4)			
> 80	86 (29.2)	329 (24.6)			
ALT, in IU/L			3.142	2	0.208
≤ 40	121 (41.0)	566 (42.4)			
40-80	105 (35.6)	517 (38.7)			
> 80	69 (23.4)	253 (18.9)			

TC, in mmol/L			22.536	1	0.000
≤ 1.7	250 (84.7)	1245 (93.2)			
> 1.7	45 (15.3)	91 (6.8)			
CH, in mmol/L			3.840	2	0.147
< 3.88	185 (62.7)	865 (64.7)			
3.88-5.2	67 (22.7)	329 (24.6)			
> 5.2	43 (14.6)	142 (10.6)			
Pre-LT antitumor therapy			0.451	2	0.798
No	219 (74.5)	1016 (76.2)			
local therapy	48 (16.3)	209 (15.7)			
Resection	27 (9.2)	109 (8.2)			
HBeAg			0.670	1	0.413
Negative	232 (78.6)	1021 (76.4)			
Positive	63 (21.4)	315 (23.6)			
HBV DNA, in IU/mL			6.140	1	0.013
Negative, ≤ 10 ³	191 (64.7)	760 (56.9)			
Positive, ≥ 10 ³	104 (35.3)	576 (43.1)			
Post-LT HBV recurrence			0.217	1	0.740
No	285 (96.6)	1283 (96.0)			
Yes	10 (3.4)	53 (4.0)			

BMI: Body mass index; DM: Diabetes mellitus; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; TG: Triglyceride; CH: Cholesterol; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; LT: Liver transplantation.

Table 2 Antiviral prophylaxis before and after liver transplantation for hepatitis B virus reinfection

Patients with high risk of HBV reinfection [HBV-DNA ≥ 10⁵ copies/mL or HBeAg(+)]	Patients with low risk of HBV reinfection [HBV-DNA < 10⁵ copies/mL or HBeAg(-)]
Pre-LT: nucleoside analogues, qd 2-4 w	Pre-LT: nucleoside analogues, qd 0-2 w
Intraoperative: HBIG 4000 IU, iv	Intraoperative: HBIG 2000 IU, iv
Post-LT: HBIG 1000 IU, iv, qd, 1-7 d	Post-LT: HBIG 1000 IU, iv, qd, 1-7d
After 7 d, HBIG 1000 IU, iv, once a week; or HBIG 400 IU, im, qd or qod or twice a week. Adjust frequency of HBIG administration to reach target therapeutic concentration	After 7 d, HBIG 1000 IU, iv, once a week; or HBIG 400 IU, im, qd or qod or twice a week. Adjust frequency of HBIG administration to reach target therapeutic concentration
Target therapeutic concentration post-LT	Target therapeutic concentration post-LT
≤ 6 mo post-LT: anti-HBs titer ≥ 500 IU/L	≤ 6 mo post-LT: anti-HBs titer ≥ 300 IU/L
6-12 mo post-LT: anti-HBs titer ≥ 200 IU/L	6-12 mo post-LT: anti-HBs titer ≥ 200 IU/L
≥ 12 mo post-LT: anti-HBs titer ≥ 100 IU/L	≥ 12 mo post-LT: anti-HBs titer ≥ 100 IU/L

HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; LT: Liver transplantation.

included patients tested positive for HBsAg and showed detectable HBV DNA levels within the 3 mo prior to LT. Liver function levels and blood fat levels were measured at the time of transplant. The tumor nodule number, tumor nodule size (maximum diameter), vascular invasion, perihepatic lymph node invasion, capsular invasion, microsatellite lesions and tumor differentiation were determined by experienced pathologists.

Statistical analysis

Categorical variables and non-normal distribution quantitative data were analyzed to compare the differences between non-diabetic and diabetic groups using χ^2 tests and Mann-Whitney tests, respectively. From the date of LT to the date of death or to the most recent follow-up, survival time was calculated. The Kaplan-Meier method was used to analyze overall and recurrence-free survival. The log-rank test (Mantel-Cox) was used to compare group survival curves. Additionally, multivariate step-wise Cox regression was performed to analyze survival and

tumor recurrence risk factors among LT patients. Statistical calculations were carried out using SPSS 17.0 software (SPSS Inc., Chicago, IL, United States). Two-sided *P* of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristic comparisons between HBV-HCC patients with or without DM

The mean ages in patients with or without DM were 54.1 or 50.8, respectively ($t = 6.597$, $P < 0.001$). Mean patient BMI with or without DM was 24.2 for both ($t = 0.036$, $P = 0.971$). Mean fasting plasma glucose levels were 9.23 in DM patients, and 5.20 in non-DM patients ($t = 16.849$, $P < 0.001$). The mean TC level ($t = 2.342$, $P = 0.019$) and CH level ($t = 1.106$, $P = 0.269$) were 1.12 or 4.46 in DM patients and 0.92 or 3.82 in non-DM patients, respectively.

In the current study, patients with DM exhibited a higher proportion of individuals with age > 50-years-old (67.5% vs 51.9%, $\chi^2 = 23.690$, $P < 0.001$), Child-

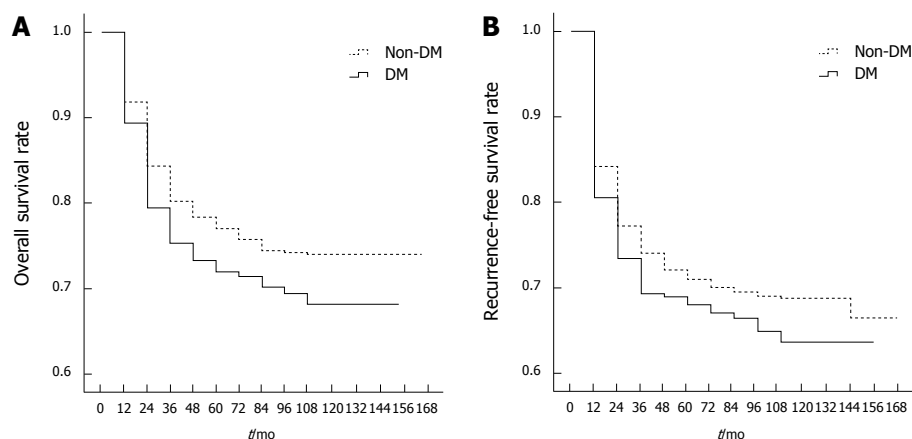


Figure 1 Overall (A) and recurrence-free (B) survival rates of diabetes mellitus and non-diabetes mellitus patients with hepatocellular carcinoma. DM: Diabetes mellitus.

Pugh grade B (35.3% vs 29.6%, $\chi^2 = 13.456$, $P = 0.002$) and C (22.4% vs 16.8%, $\chi^2 = 13.456$, $P = 0.002$), MELD scores > 15 (35.3% vs 26.0%, $\chi^2 = 14.184$, $P = 0.001$), BMI of 24-28 (28.5% vs 22.1%, $\chi^2 = 7.937$, $P = 0.047$), TC > 1.7 mmol/L (15.3% vs 6.8%, $\chi^2 = 22.536$, $P < 0.001$) and HBV DNA $\leq 10^3$ IU/mL (64.7% vs 56.9%, $\chi^2 = 6.140$, $P = 0.013$), when compared with non-DM patients (Table 1).

Analysis of DM and non-DM patients showed significant differences in age ($P < 0.001$), MELD scores ($P = 0.001$), Child-Pugh grade ($P = 0.002$), BMI ($P = 0.047$), TC levels ($P < 0.001$) and HBV DNA ($P = 0.013$; Table 1). No significant differences were observed between patients with and without DM for other factors analyzed (Table 1).

Overall survival and tumor recurrence comparisons by univariate analysis between HBV-HCC patients with or without DM

Complete follow-up monitoring of the 1631 HBV-associated HCC patients who underwent LT was conducted. The median survival time was 62.0 ± 39.1 mo (0 to 152 mo) in DM patients and 67.0 ± 38.9 mo (0 to 165 mo) in non-DM patients. Univariate analysis of 1631 patients showed that 1-, 3- and 5-year overall survival rates post-LT were 79%, 73% and 71% in DM patients, respectively, and 84%, 78% and 76% in non-DM patients, respectively. Overall survival rates post-LT were significantly different between the HCC patients with DM and those without DM ($P = 0.041$; Figure 1A).

The median tumor recurrence-free survival time was 55.0 ± 40.9 mo in DM patients and 62.0 ± 41.3 mo (0 to 165 mo) in non-DM patients. Univariate analysis of 1631 patients found that post-LT 1-, 3- and 5-year recurrence-free survival rates were 73%, 69% and 67% in DM patients, respectively, and 77%, 72% and 70% in non-DM patients, respectively. However, there were no significant differences in tumor recurrence-free survival rates between the two groups ($P = 0.096$; Figure 1B).

Risk factors for overall survival and recurrence-free survival comparisons by univariate analysis between HBV-HCC patients with or without DM

Table 3 shows the results of a stratified analysis of risk factors for overall survival of HBV-related HCC patients with and without DM. Significant survival differences were found between DM and non-DM patients in relation to age > 50 years ($P = 0.002$; Figure 2A), tumor size ≤ 3 cm ($P = 0.047$), number of tumor nodules between two and three ($P = 0.007$), vascular invasion ($P = 0.033$; Figure 2B), Child-Pugh grade B ($P = 0.018$), BMI of 18.5-24 ($P = 0.048$), ALT levels of 40-80 IU/L ($P = 0.017$; Figure 2C), AST levels of 40-80 IU/L ($P = 0.038$), TC levels ≤ 1.7 mmol/L ($P = 0.021$), no pre-LT antitumor therapy ($P = 0.020$), and absence of post-LT HBsAg reinfection ($P = 0.045$). When compared with non-DM patients, DM patients with these factors had poorer overall 1-, 3- and 5-year post-LT survival rates. Analysis of patients with vascular invasion showed that the 1-, 3- and 5-year post-LT survival rates were 45%, 28% and 25% in DM patients, respectively, compared to 63%, 47% and 42% in non-DM patients, respectively. DM patients > 50 years of age had 1-, 3- and 5-year post-LT survival rates of 79%, 73% and 71%, respectively, compared to 88%, 83% and 80% in non-DM patients, respectively. DM patients with ALT levels of 40 to 80 IU/L had overall 1-, 3- and 5-year post-LT survival rates of 76%, 71% and 70%, respectively, compared to 85%, 78% and 76% in non-DM patients, respectively.

Additionally, a trend was observed in survival differences between DM and non-DM patients in relation to tumor size > 7 cm ($P = 0.055$), no capsular invasion ($P = 0.050$), serum AFP levels > 2000 ng/mL ($P = 0.052$) and HBeAg negativity ($P = 0.055$).

Stratified analysis of risk factors for the tumor recurrence-free survival between DM and non-DM patients (Table 4) showed that age > 50 years ($P = 0.015$; Figure 3A), two to three tumor nodules ($P =$

Table 3 Univariate analysis of overall 1-, 3- and 5-year survival rates post-liver transplantation between patients with diabetes mellitus and without diabetes mellitus

Variables	DM, %			Non-DM, %			P value
	1 yr	3 yr	5 yr	1 yr	3 yr	5 yr	
Sex							
Male	80	74	72	84	77	75	0.140
Female	76	62	62	90	88	86	0.016
Age, yr							
≤ 50	80	73	73	80	74	71	0.970
> 50	79	73	71	88	83	80	0.002
Tumor size, in cm							
≤ 3	83	79	79	90	86	83	0.047
3-5	86	79	74	87	79	77	0.689
5-7	66	49	43	71	65	63	0.171
> 7	36	30	30	63	49	48	0.055
Number of tumor nodules							
Single	85	82	79	90	84	82	0.325
2-3	73	68	68	86	81	79	0.007
≥ 4	76	62	60	72	64	61	0.961
Venous invasion							
Absent	85	80	79	88	84	82	0.113
Present	45	28	25	63	47	42	0.033
Lymph node invasion							
Absent	80	74	72	85	79	76	0.032
Present	73	64	64	78	70	66	0.979
Capsular invasion							
Absent	80	74	72	85	79	77	0.050
Present	75	61	61	79	72	70	0.554
Edmondson							
1	91	91	91	100	100	100	0.134
2	82	77	75	87	81	78	0.124
3 + 4 + 5	74	66	64	79	73	71	0.229
Serum AFP level, in ng/mL							
< 200	85	81	79	88	83	82	0.161
200-400	64	64	64	78	68	66	0.502
400-1000	71	51	42	82	72	66	0.189
1000-2000	68	41	41	73	61	55	0.454
> 2000	34	26	26	62	56	51	0.052
MELD score							
< 10	79	68	68	84	77	74	0.101
10-15	78	70	70	84	78	76	0.197
> 15	82	80	75	85	80	78	0.423
Child-Pugh class							
A (5-6)	83	78	78	85	79	76	0.792
B (7-9)	75	67	63	83	78	75	0.018
C (≥ 9)	79	74	72	82	77	75	0.479
Fasting blood glucose, in mmol/L							
≤ 7	77	73	71	85	79	76	0.145
> 7	80	73	71	80	73	67	0.955
BMI, in kg/m ²							
< 18.5	68	62	62	81	79	76	0.156
18.5-24	77	72	70	84	78	76	0.048
24-28	86	78	76	85	79	76	0.951
> 28	81	72	72	85	80	74	0.656
ALP							
≤ 112	83	78	76	87	81	78	0.281
> 112	74	66	65	80	74	72	0.102
GGT							
≤ 54	87	86	85	92	88	85	0.559
> 54	75	66	63	79	72	69	0.104
ALT, in IU/L							
≤ 40	86	81	80	89	85	83	0.339
40-80	73	68	65	83	77	74	0.017
> 80	77	68	66	75	68	64	0.633
AST, in IU/L							
≤ 40	88	82	80	91	86	84	0.307
40-80	76	71	70	85	78	76	0.038
> 80	74	67	64	74	68	64	0.872

TC, in mmol/L							
≤ 1.7	79	72	71	85	79	77	0.021
> 1.7	82	79	76	79	71	64	0.473
CH, in mmol/L							
≤ 3.88	81	75	73	85	80	77	0.151
3.88-5.2	74	69	67	86	78	75	0.058
> 5.2	83	71	71	77	71	68	0.737
Pre-LT antitumor therapy							
No	77	73	71	85	79	76	0.020
local therapy	83	71	71	79	73	71	0.910
Resection	92	80	72	88	84	80	0.809
HBeAg							
Negative	79	73	71	85	78	76	0.055
Positive	82	75	73	83	79	76	0.446
HBV-DNA, in × 10 ³ U/mL							
< 1	80	73	71	85	79	78	0.072
> 1	77	73	72	84	77	74	0.236
Post-LT HBsAg reinfection							
Negative	79	73	72	84	79	76	0.045
Positive	80	70	60	87	73	69	0.678

BMI: Body mass index; DM: Diabetes mellitus; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; TG: Triglyceride; CH: Cholesterol; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; LT: Liver transplantation.

Table 4 Univariate analysis of tumor recurrence-free 1-, 3- and 5-year survival rates post-liver transplantation between patients with diabetes mellitus and without diabetes mellitus

Variables	DM, %			Non-DM, %			P value
	1 yr	3 yr	5 yr	1 yr	3 yr	5 yr	
Sex							
Male	73	69	67	77	71	69	0.204
Female	76	63	63	83	82	80	0.110
Age, yr							
≤ 50	73	68	68	73	69	67	0.940
> 50	73	69	66	81	75	73	0.015
Tumor size, in cm							
≤ 3	78	75	75	85	81	79	0.076
3-5	82	75	71	80	73	71	0.823
5-7	52	43	31	58	54	53	0.174
> 7	31	25	25	47	39	39	0.087
Number of tumor nodules							
Single	81	77	75	83	79	77	0.475
2-3	67	64	64	79	75	73	0.031
≥ 4	64	57	55	63	55	53	0.825
Venous invasion							
Absent	80	76	74	82	78	76	0.301
Present	28	22	19	48	37	35	0.005
Lymph node invasion							
Absent	74	69	67	78	73	71	0.058
Present	64	64	64	67	59	55	0.580
Capsular invasion							
Absent	75	71	69	78	73	71	0.201
Present	60	55	55	69	67	62	0.192
Microsatellite lesions							
Absent	79	74	72	80	75	73	0.542
Present	52	49	46	63	60	58	0.039
Edmonton							
1	91	91	91	100	100	100	0.134
2	77	73	72	80	74	72	0.375
3 + 4 + 5	67	59	57	72	67	66	0.175
Serum AFP level, in ng/mL							
< 200	80	76	75	82	78	76	0.272
200-400	59	59	59	72	63	62	0.484
400-1000	65	46	37	77	65	63	0.251
1000-2000	61	43	43	62	55	49	0.682
> 2000	17	17	17	44	42	39	0.055
MELD score							
< 10	68	60	58	75	69	67	0.058
10-15	72	70	70	78	73	70	0.412

> 15	79	76	72	79	76	74	0.534
Child-Pugh class							
A	75	71	69	78	72	70	0.672
B	69	65	61	77	72	70	0.052
C	76	71	71	76	73	71	0.610
Fasting blood glucose, in mmol/L							
≤ 7	72	68	67	77	72	70	0.154
> 7	74	69	67	70	68	63	0.914
BMI, in kg/m ²							
< 18.5	63	57	57	80	72	69	0.223
18.5-24	73	70	68	77	72	70	0.216
24-28	76	69	69	78	73	71	0.524
> 28	77	69	63	76	71	67	0.774
ALP, in IU/L							
≤ 112	80	75	73	81	75	73	0.747
> 112	63	60	57	71	67	65	0.056
GGT, in IU/L							
≤ 54	85	84	82	87	82	80	0.957
> 54	67	60	58	70	65	63	0.101
AST, in IU/L							
≤ 40	81	76	73	84	79	78	0.201
40-80	69	65	65	78	72	70	0.103
> 80	70	65	62	66	61	58	0.767
ALT, in IU/L							
≤ 40	79	76	75	82	79	77	0.318
40-80	68	63	60	77	70	68	0.033
> 80	72	65	64	65	60	58	0.376
TC, in mmol/L							
≤ 1.7	74	69	67	78	73	71	0.113
> 1.7	71	68	65	66	63	60	0.894
CH, in mmol/L							
< 3.88	75	70	68	78	74	72	0.175
3.88-5.2	68	66	64	79	72	71	0.092
> 5.2	76	68	68	66	60	57	0.415
Pre-LT antitumor therapy							
No	73	68	67	78	72	70	0.061
local therapy	68	66	66	70	67	64	0.939
Resection	81	77	70	83	79	76	0.758
HBeAg							
Negative	72	69	66	78	71	70	0.095
Positive	77	69	69	75	74	71	0.633
HBV-DNA, in × 10 ³ IU/mL							
< 1	75	69	68	78	74	72	0.165
> 1	71	68	65	76	70	67	0.256
Post-LT HBsAg reinfection							
Negative	73	69	67	77	73	70	0.092
Positive	70	60	60	72	60	58	0.899

BMI: Body mass index; DM: Diabetes mellitus; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transpeptidase; TG: Triglyceride; CH: Cholesterol; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; LT: Liver transplantation.

0.031), presence of vascular invasion ($P = 0.005$; Figure 3B), presence of microsatellite lesions ($P = 0.039$) and ALT levels of 40-80 IU/L ($P = 0.033$; Figure 3C) were associated with significant tumor recurrence-free survival differences. DM patients with these factors had poorer 1-, 3- and 5-year recurrence-free survival rates post-LT when compared with non-DM patients (Table 4). Analysis of the presence of vascular invasion in DM patients found that 1-, 3- and 5-year recurrence-free post-LT survival rates were 28%, 22% and 19%, respectively, but 48%, 37% and 35% in non-DM patients, respectively. DM patients > 50 years of age had 1-, 3- and 5-year post-LT survival rates of 73%, 69% and 66%, respectively, while non-DM patients had post-LT survival rates of 81%, 75% and 73%, respectively. DM patients with ALT levels

between 40 and 80 IU/L had 1-, 3- and 5-year post-LT survival rates of 68%, 63% and 60%, respectively, compared to post-LT survival rates of 77%, 70% and 68% in non-DM patients, respectively.

Trends were observed in tumor recurrence-free survival differences between DM and non-DM patients with AFP levels > 2000 ng/mL ($P = 0.055$) and with AST levels > 112 IU/L ($P = 0.056$).

Risk factors for overall survival and tumor recurrence comparisons by multivariate analysis between HBV-HCC patients with or without DM

The multivariate Cox modeling for overall survival included sex, age, BMI, DM, number of tumor nodules, tumor size, venous invasion, lymph node invasion, capsular invasion, microsatellite lesions, Edmondson

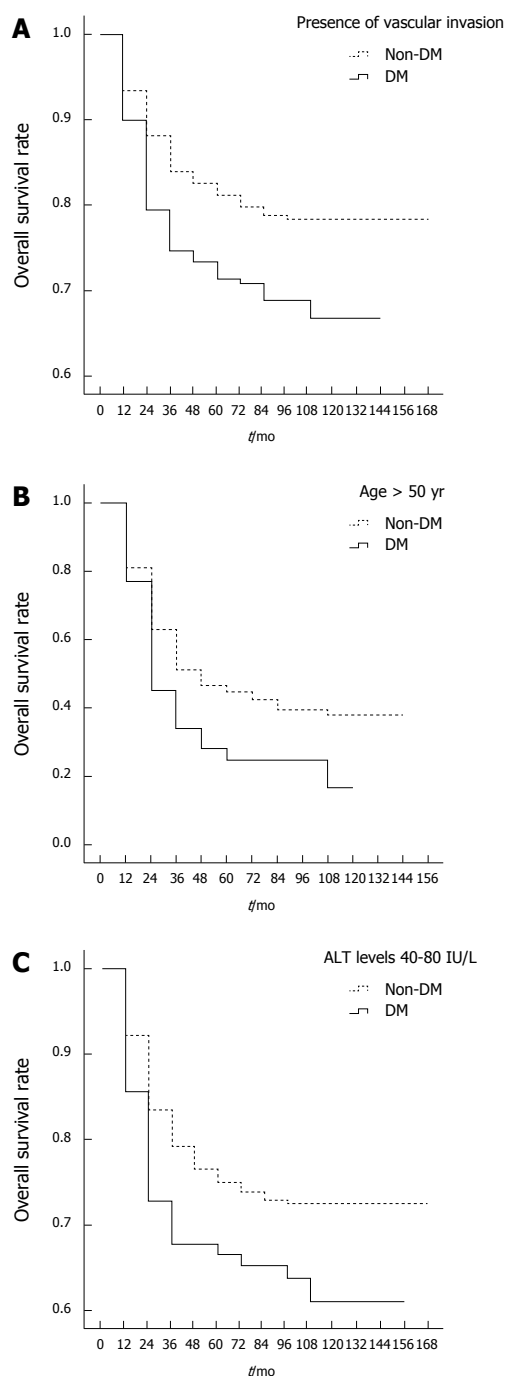


Figure 2 Overall survival rates of diabetes mellitus and non-diabetes mellitus hepatocellular carcinoma patients with presence of vascular invasion (A), age > 50 years (B), alanine aminotransferase levels > 40, ≤ 80 IU/L (C). ALT: Alanine aminotransferase; DM: Diabetes mellitus.

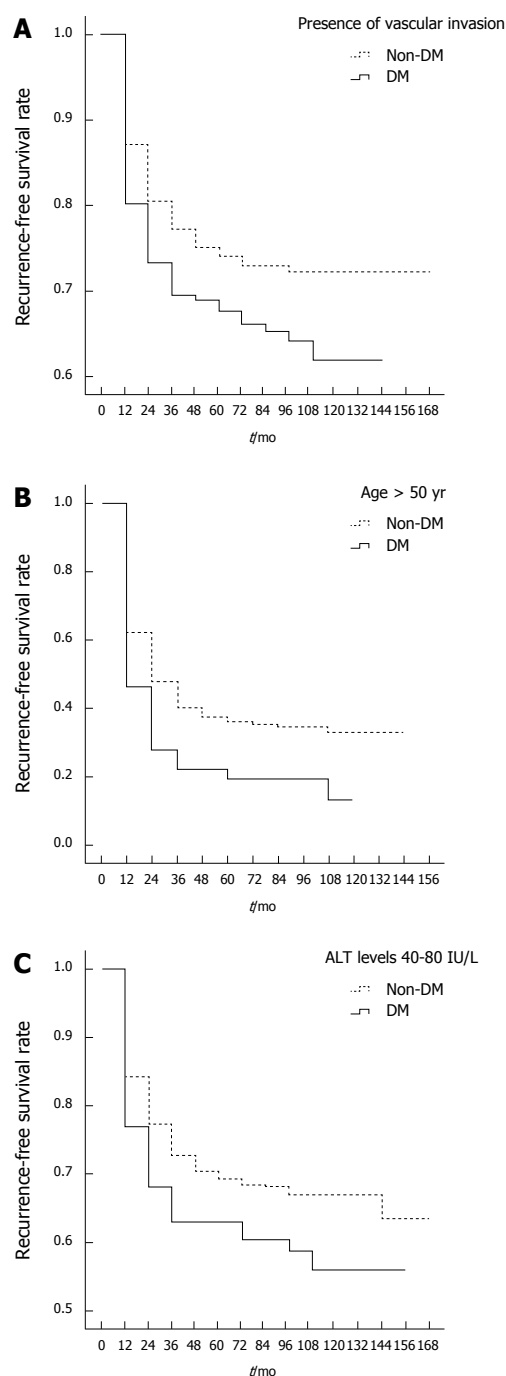


Figure 3 Recurrence-free survival rates of diabetes mellitus and non-diabetes mellitus hepatocellular carcinoma patients with vascular invasion and (A), age > 50 years (B), alanine aminotransferase levels between 40 and 80 IU/L (C). ALT: Alanine aminotransferase; DM: Diabetes mellitus.

grade, AFP level, Child-Pugh score, MELD score, ALP, GGT, AST ALT, TC, CH, HBeAg, HBV DNA, pre-LT antitumor therapy and post-LT HBsAg reinfection. Multivariate analysis (Table 5) revealed that DM [hazard rate (HR) = 1.591; 95% confidence interval (CI): 1.239-2.041, $P < 0.001$] was an independent predictor of poor survival, and that age (HR = 0.781, $P = 0.017$), vascular invasion ($P < 0.001$), number of tumor nodules ≥ 2 (HR = 1.695-2.342, $P < 0.001$), tumor size > 5 cm (HR = 2.093-3.496, $P < 0.001$), AFP level

pre-LT > 1000 ng/mL (HR = 1.860-2.065, $P \leq 0.001$), ALT level > 40 IU/L (HR = 1.103-1.246, $P \leq 0.006$), GGT level > 54 IU/L (HR = 1.394, $P = 0.010$) and ALP level > 112 IU/L (HR = 0.618, $P = 0.018$) were independent predictors of poor survival. Additionally, antitumor local therapy pre-LT was an independent predictor of good survival.

In multivariate Cox modeling for tumor recurrence, the variables analyzed were the same as those used for multivariate analysis for overall survival. The

Table 5 Multivariate proportional hazard model for overall survival after liver transplantation in 1631 patients with hepatitis B virus-related hepatocellular carcinoma

Variables	B	SE	Wald	P value	OR (95%CI)
Venous invasion, absent <i>vs</i> present	0.893	0.117	57.882	0.000	2.442 (1.940-3.074)
Pre-LT antitumor therapy			7.129	0.032	
Local therapy	-0.319	0.138	5.368	0.021	1.375 (1.050-1.801)
Resection	-0.227	0.212	1.146	0.284	0.797 (0.526-1.208)
Age, ≤ 50 <i>vs</i> > 50	-0.248	0.104	5.655	0.017	0.781 (0.636-0.957)
ALP, ≤ 112 IU/L <i>vs</i> > 112 IU/L	-0.264	0.111	5.630	0.018	0.768 (0.618-0.955)
GGT, ≤ 54 IU/L <i>vs</i> > 54 IU/L	0.332	0.128	6.681	0.010	1.394 (1.084-1.793)
ALT			13.416	0.001	
≤ 40 IU/L <i>vs</i> 40-80 IU/L	0.340	0.123	7.575	0.006	1.404 (1.103-1.789)
≤ 40 IU/L <i>vs</i> > 80 IU/L	0.496	0.141	12.396	0.000	1.642 (1.246-2.164)
Serum AFP level, in ng/mL			30.829	0.000	
< 200 <i>vs</i> 200-400	0.525	0.185	8.097	0.004	1.691 (1.178-2.427)
< 200 <i>vs</i> 400-1000	0.399	0.163	6.012	0.014	1.491 (1.083-2.052)
< 200 <i>vs</i> 1000-2000	0.621	0.189	10.804	0.001	1.860 (1.285-2.693)
< 200 <i>vs</i> > 2000	0.725	0.160	20.569	0.000	2.065 (1.509-2.825)
Number of tumor nodules			47.936	0.000	
Single <i>vs</i> 2-3	0.528	0.138	14.631	0.000	1.695 (1.293-2.221)
Single <i>vs</i> ≥ 4	0.851	0.124	47.265	0.000	2.342 (1.837-2.984)
Tumor size, in cm			78.643	0.000	
≤ 3 <i>vs</i> 3-5	0.152	0.132	1.314	0.252	1.164 (0.898-1.509)
≤ 3 <i>vs</i> 5-7	0.739	0.149	24.596	0.000	2.093 (1.563-2.802)
≤ 3 <i>vs</i> > 7	1.252	0.152	67.571	0.000	3.496 (2.594-4.711)
DM, yes <i>vs</i> no	0.464	0.128	13.230	0.000	1.591 (1.239-2.043)

LT: Liver transplantation; GGT: Gamma-glutamyl transpeptidase; AFP: α -fetoprotein; DM: Diabetes mellitus; ALT: Alanine aminotransferase.

Table 6 Multivariate proportional hazard model for tumor recurrence after liver transplantation in 1631 patients with hepatitis B virus-related hepatocellular carcinoma

Variables	B	SE	Wald	P value	OR (95%CI)
Venous invasion, absent <i>vs</i> present	0.780	0.124	39.825	0.000	2.182 (1.712-2.780)
Pre-LT antitumor therapy			11.093	0.005	
Local therapy	-0.404	0.138	8.555	0.003	1.498 (1.143-1.964)
Resection	-0.285	0.230	1.536	0.215	0.752 (0.480-1.180)
GGT, ≤ 54 IU/L <i>vs</i> > 54 IU/L	0.360	0.131	7.559	0.006	1.433 (1.109-1.851)
TC, ≤ 1.7 mmol/L <i>vs</i> > 1.7 mmol/L	0.425	0.165	6.682	0.010	1.530 (1.108-2.113)
Serum AFP level, in ng/mL			27.634	0.000	
< 200 <i>vs</i> 200-400	0.330	0.198	2.767	0.096	1.391 (0.943-2.051)
< 200 <i>vs</i> 400-1000	0.031	0.193	0.026	0.872	1.032 (0.707-1.506)
< 200 <i>vs</i> 1000-2000	0.378	0.212	3.161	0.075	1.459 (0.962-2.212)
< 200 <i>vs</i> > 2000	0.794	0.158	25.372	0.000	2.213 (1.625-3.015)
Number of tumor nodules			34.680	0.000	
Single <i>vs</i> 2-3	0.377	0.144	6.885	0.009	1.457 (1.100-1.931)
Single <i>vs</i> ≥ 4	0.763	0.130	34.674	0.000	2.144 (1.664-2.764)
Tumor size, in cm			110.647	0.000	
≤ 3 <i>vs</i> 3-5	0.143	0.146	0.953	0.329	1.154 (0.866-1.537)
≤ 3 <i>vs</i> 5-7	1.112	0.150	55.206	0.000	3.042 (2.268-4.079)
≤ 3 <i>vs</i> > 7	1.402	0.158	78.831	0.000	4.065 (2.983-5.540)

LT: Liver transplantation; GGT: Gamma-glutamyl transpeptidase; AFP: α -fetoprotein.

multivariate analysis (Table 6) indicated that tumor size > 5 cm (HR = 3.042-4.065, $P < 0.001$), tumor nodule number ≥ 2 (HR = 1.457-2.144, $P \leq 0.009$), presence of vascular invasion (HR = 2.182, $P < 0.001$), AFP level > 2000 ng/mL (HR = 2.213, $P < 0.001$), GGT level > 54 IU/L (HR = 1.433, $P = 0.006$) and TC > 1.7 mmol/L (HR = 1.530, $P = 0.010$) were independent predictors of tumor recurrence post-LT. However, multivariate analysis indicated that DM was not an independent predictor of post-LT tumor

recurrence ($P > 0.05$).

DISCUSSION

Among the 1631 HCC patients enrolled in this study with HBV-induced cirrhosis who underwent LT in multiple institutions, univariate analysis indicated that patients with DM had worse overall survival rates than patients without DM. There was no significant difference in tumor recurrence-free survival between

patients with and without DM. Multivariate analysis also indicated that DM was a significant independent predictor of poor survival, but not of HCC recurrence post-LT. This finding is consistent with previous studies that reported decreased post-operative survival rates in HCC patients with DM^[23,24,29-32].

To our knowledge, only two studies have compared the impact of pre-LT DM on survival and tumor recurrence in HCC patients after LT^[23,24]. Connolly *et al.*^[23] studied 191 LT-treated HCC patients (12 HBV-related, 104 HCV-related and 81 other HCCs) and determined that the median survival was 78.3 mo for the non-DM patients, but only 31.1 mo for the DM patients. However, no significant differences were observed in survival between the two groups. Siegel *et al.*^[24] studied 342 patients with HBV-related HCC (16%) and HCV-related (70%) HCC. They reported that DM patients had worse overall survival than those without diabetes. The reason for the different outcomes in these studies is not clear, but the differences may be partly attributable to the smaller patient populations with HBV-related HCC in the Connolly *et al.*^[23] study. Nonetheless, both studies determined that there was no difference in tumor recurrence between DM and non-DM patients. This finding is consistent with the results of the current study.

Additionally, some studies have investigated the post-surgical impact of DM on HBV- and HCV-related HCC patient outcomes^[33-35]. Huo *et al.*^[34] showed that DM patients with HBV (but not HCV) had poor long-term outcomes. However, Komura *et al.*^[33] reported the opposite results: DM patients with HCV (but not HBV) exhibited poor long-term outcomes. Other studies reported that DM had a similar impact on HCC patients with HBV- and HCV-infection after surgical resection^[35]. The reason for these different results is not clear. Our current report is a multi-center study involving a larger number of samples, and we reported that DM had a significant effect on long-term survival post-LT in HBV-related HCC patients, but not on HCC recurrence.

Vascular invasion, tumor size > 5 cm, and tumor nodule number > 1 were also demonstrated by the current study to be independent prognostic factors of poor survival. These findings are also consistent with previous studies which reported that vascular invasion, tumor size (≤ 3 cm or ≤ 5 cm, or > 3 cm) and the number of tumor lesions appears to affect prognosis in DM patients compared with non-DM patients^[23,29,31,33]. The current findings suggest that DM may be related to pathological features of tumors that affect long-term survival post-LT in HBV-related HCC patients.

DM, obesity and metabolic syndrome (MS) are related to each other, and are key pathogenetic factors for non-alcoholic fatty liver disease (NAFLD)^[36]. It has been reported that "metabolic epidemics" are probably involved in the rise in incidence of HCC, and NAFLD has been cited as the major causative factor^[36]. However, although MS has been shown to predispose to NAFLD,

and the latter has been associated with an increased incidence of HCC, it has not been shown that MS is a direct etiological factor for development of HCC. While impaired glucose metabolism may be involved in hepato-carcinogenesis, its role in the development of HCC has not been proven^[37,38]. In the current study, DM patients with vascular invasion or microsatellite lesions had worse tumor recurrence-free survival rates when compared with non-DM patients. Connolly *et al.*^[23] analyzed 191 HCC patients treated with LT, in which 84 (44%) of the patients had DM, and found macrovascular invasion in 20.2% of the DM patients and 9.3% of the non-DM patients. By multivariate analysis, they also found that DM was associated with macrovascular invasion in HCC patients who underwent LT. This supports the current findings that DM may impact the risk of vascular invasion in HCC patients treated with LT.

The current study suggests that DM may be related to older age (> 50 years) and poor liver function (ALT or AST levels 40-80 IU/L, Child-Pugh score B), which may affect outcomes post-LT. A previous study of 389 patients with HCC and cirrhosis by Ting *et al.*^[31] reported that DM, Child-Pugh scores (B and C) and AST levels were significant risk factors affecting HCC prognosis after surgical resection. Huo *et al.*^[32] reported that elderly and DM patients were associated with hepatic decompensation. Some previous studies have suggested that DM patients with HCC have an increased cumulative risk of hepatic decompensation after partial hepatectomy^[24]. However, unlike partial liver resection, LT offers a more radical oncologic treatment: the complete replacement of a poorly functioning liver with a normal one. Moreover, large doses of hormones and calcineurin inhibitor immunosuppressant drugs (used as anti-rejection therapy post-LT) can increase blood glucose and may induce or aggravate DM or make DM difficult to control. This treatment regimen and its associated side effects may contribute to an elevated incidence of diabetic complications, and decrease survival post-LT for HCC patients with DM (especially among older patients). Thus, we suggest that factors contributing to poor outcomes post-LT in DM patients compared with non-DM patients may include advanced age, reduced liver function and an increase in diabetes-associated complications.

Additionally, the current study observed a survival difference trend between DM and non-DM patients with pre-LT AFP levels > 2000 ng/mL. The exact cause of this result is not clear. To our knowledge, no previous studies have reported this relationship between DM and elevated serum AFP levels. Some data suggest that hepatocyte damage or regeneration play a role in increasing AFP levels^[39,40]. Elevated serum AFP may reflect both HCC progress and the severity of chronic liver damage^[40-42]. Chronic liver disease has been reported to predispose patients to DM^[40,41]. In our

study, pre-LT DM patients had a higher proportion of Child-Pugh grades B and C, and higher MELD scores compared to non-DM patients. These findings suggest that DM may be related to liver necrosis severity or poor liver function, and that HBV-related HCC patients with both DM and AFP levels > 2000 ng/mL may have worse survival post-LT.

Several limitations of this study must be considered. First, this study was retrospective. Second, DM was determined based on medical history, fasting glucose, medication use, oral glucose tolerance test results or glycohemoglobin level. However, there was no analysis of glycohemoglobin levels because glycohemoglobin levels were not tested in some pre-LT patients. Third, the HBV DNA assay kits used varied between the hospitals in this study and, therefore, the specific kits used in each institution may have had variable sensitivity and detection limits. However, these results represent a large multi-center sample that suggests that DM is a risk predictor for decreased long-term survival after LT in HBV-related HCC patients.

In conclusion, compared with non-DM patients, DM patients exhibited decreased post-LT survival rates. DM may be associated with various tumor pathological features, older age and poor liver function, and these factors may influence post-LT survival in HBV-related HCC patients. However, no direct relation between DM and HBV infection or HBV load were observed in HCC patients. The current data suggest that DM may affect the long-term post-LT survival of HBV-related HCC patients, but not tumor recurrence. Thus, HBV-related HCC patients with DM undergoing LT should be closely followed to optimize postoperative outcomes. Follow-up examinations are especially important among elderly DM patients, or those who have vascular invasion and AFP levels > 2000 ng/mL pre-LT. These factors need to be carefully considered when selecting HCC patients for LT. Furthermore, a preventive strategy pre- and post-LT for HCC patients with DM should be established. A future prospective cohort study with larger a sample size should be conducted to confirm these observations.

ACKNOWLEDGMENTS

We thank the staff of the China Liver Transplant Registry for medical data assistance and the patients who participated in this study.

COMMENTS

Background

In China, the major risk factor for the development of hepatocellular carcinoma (HCC) is chronic hepatitis B virus (HBV) infection. Recently, an increased incidence of HCC in the diabetic population has been reported, possibly due to an increased incidence steatohepatitis. However, it is unknown whether diabetes mellitus (DM) acts directly in the development of HCC in HBV-related cirrhosis patients. Few studies have investigated the association between HBV, DM, tumor recurrence and survival after liver transplantation (LT) in HCC patients.

Research frontiers

Recently, it has been confirmed that non-alcoholic fatty liver disease (NAFLD) is related to diabetes, obesity and metabolic syndrome, and has been cited as a causative factor for the development of HCC. DM may be a potential risk factor for tumor development.

Innovations and breakthroughs

This is the first report of a multi-center study in a large cohort to investigate the potential associations between pre-existing DM with HBV-induced HCC, tumor recurrence and long-term survival post-LT in patients without alcoholic fatty liver disease.

Applications

DM may be associated with tumor pathological features, old age and poor liver function, and may be a predictor of poor survival post-LT in HBV-related HCC patients. The current data suggest that DM may affect the long-term survival post-LT for HBV-related HCC patients, but not tumor recurrence. HCC patients with DM undergoing LT should be closely monitored to optimize postoperative outcomes during follow-up.

Terminology

NAFLD, the abnormal accumulation of fat in the liver, is related to obesity, diabetes and metabolic syndrome. NAFLD has been linked clinically and pathophysiologically to obesity, insulin resistance and type 2 DM, and can be considered a new criterion of metabolic syndrome.

Peer-review

In this study, the authors investigated the potential associations between DM with HBV-induced HCC, HCC recurrence and long-term survival after LT in a large cohort. It is an interesting study and well-written manuscript. The results can support the conclusion.

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P- Reviewer: Liu SH, Tarantino G **S- Editor:** Gong ZM
L- Editor: Filipodia **E- Editor:** Wang CH





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ISSN 1007-9327



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