ORIGINAL ARTICLE

Diabetes mellitus may worsen the prognosis in hepatocellular carcinoma patients undergoing curative microwave ablation

Shi-rong Liu[1,2]*, Chao Ren[1]*, Ping Liang[1], Xiao-ling Yu[1], Zhi-gang Cheng[1], Zhi-yu Han[1]

[1]Department of Interventional Ultrasound, Chinese PLA General Hospital, Beijing, China; [2]Department of Ultrasound, Peking University Third Hospital, Beijing, China

*These authors contributed equally to this study.

Summary

Purpose: This study aimed to investigate the outcomes of hepatocellular carcinoma (HCC) patients after curative microwave ablation (MWA) with and without diabetes mellitus (DM).

Methods: A total of 308 patients with HCC were retrospectively studied from 2005 to 2012 over an 8-year period. They were all successfully treated by MWA. Progression-free survival (PFS) and overall survival (OS) were analyzed according to the status of DM. The presence of other comorbidities and tumor status were studied using multivariate analysis.

Results: Significant differences were observed both for 1-, 3-, 5- year's PFS rates (DM: 63.8, 23.0 and 15.8 vs non-DM: 72.7, 43.6 and 30.8%; p=0.013) and OS rates (DM: 87.3, 75.1 and 49.5% vs non-DM: 97.9, 82.9 and 70.5%; p=0.045) between patients with and without DM. Cox multivariate analysis identified the following factors significantly associated

with PFS: (hazard ration (HR): 1.191, 95%CI: 1.051-1,349, p=0.006), AFP (HR:1.000, 95%CI: 1.000-1.000, p=0.022), alcohol abuse (>100g/d vs ≤100g/d, HR:1.579, 95%CI:1.128-2.212, p=0.008), mean fasting plasma glucose level after initial therapy for HCC(>7.0/≤7.0, HR:2.728, 95%CI:1.414-5.265, p=0.003); and the followings associated with OS: Child-Pugh classification A against B, C (risk 1.692, 95%CI 1.065-2.689, P=0.026), tumor diameter (risk 1.251. 95%CI 1.021-1.534, P=0.031), and AFP (risk 1.000. 95%CI 1.000-1.000, P=0.000).

Conclusion: DM may affect the HCC progression and overall survival in patients undergoing curative MWA. A good control of the glucose levels after ablation may be important for improving the prognosis of HCC.

Key words: ldiabetes mellitus, hepatocellular carcinoma, microwave ablation, survival

Introduction

HCC is the sixth most common malignancy which kill more than 600,000 people annually worldwide [1]. Despite the improvements in medical and surgical therapy, the prognosis of HCC patients remains poor due to the high recurrence and metastatic rate [2]. In most cases, HCC develops on a background of chronic liver disease (70-90% of all patients), with the most common cause being virus infection of hepatitis B or C [3]. The damaged liver due to chronic liver disease may result in defects in glucose metabolism and thus a substantial proportion of HCC patients may have impaired glucose tolerance or DM [4]. As one of the co-morbid illnesses associated with HCC, DM has been increasingly recognized as an independent risk factor for HCC incidence [5]. However, the impact of DM on the prognosis of patients with HCC remains controversial. Some authors maintain that DM increases the risk of morbidity and postoperative liver failure [6,7], while others report

otherwise [8]. Some report that DM has a negative impact on HCC recurrence and long-term survival of patients undergoing hepatectomy [9], whereas others did not find a similar significant adverse effect of DM [10]. The prognostic effects of DM on HCC patients undergoing non-surgical treatments, especially MWA, are less well characterized.

The purpose of this study was to determine whether DM significantly impacts intra-hepatic recurrence and survival after potentially curative MWA of HCC.

Methods

Ethics statements

The study was approved by the Ethics Committee of the Chinese PLA General Hospital. Written informed consent was obtained from each patient before any procedures and the patients' records or information was anonymized prior to analysis.

Patients

From January 2005 to December 2012, 349 consecutive HCC patients who were newly diagnosed and radically treated with ultrasound-guided MWA were retrospectively analyzed at our Institute. Of these patients, 2 died within 30 days after the procedure (including one hepatorenal syndrome 12 days and one upper gastrointestinal hemorrhage due to server portal hypertension 16 days after ablation), 19 cases had concurrent malignancies at other sites, 5 cases had portal vein tumor thrombosis or extra-hepatic metastasis, 10 cases had a follow-up period less than 6 months and 5 were lost to follow-up. All these patients were excluded. Thus, 308 patients with 365 tumors were enrolled. The characteristics of the patients with or without DM are summarized in Table 1.

Diagnosis and definitions

The diagnosis of HCC was made by either histopathology or typical hyper-enhancement in the arterial phase and hypo-enhancement in the venous or delay phase on at least two types of enhanced imaging, including contrast-enhanced ultrasound (CEUS), dynamic helical computed tomography (CT) or enhanced magnetic resonance imaging (MRI). Patients were divided into diabetic or non-diabetic groups based on recorded diagnosis by diabetic physicians and those with a fasting plasma glucose level of >7.0 mmol/L, or a plasma glucose level of >11.1 mmol/L at 2 hrs in a 75-g oral glucose tolerance test, or typical DM symptoms together with a casual plasma glucose level of >11.1 mmol/L [11]. Alcohol abuse was defined as cumulative ethanol consumption no less than 320 kg in females or 480 kg in males, with daily alcohol consumption ≥20 g [12].

Microwave equipment and ablation techniques

A cooled-shaft microwave system (KY-2000, Kangyou Medical, Nanjing, China) was used as treatment equipment. A detailed treatment planning, including the antennae placement, power output setting and emission time was established before each procedure. In general, for nodules <1.7 cm in diameter, a single antenna was used; for tumors or nodules ≥1.7 cm in diameter, multiple antennae were required and emitted frequency of 2450 MHz was used. The output power was set between 40W and 60 W. During the procedure, the ablation region was carefully monitored with real-time ultrasound imaging (ACUSON Sequoia, California, USA), and the treatment session was performed until the hyperechoic region on gray-scale US covered the entire target lesion. CEUS, contrast CT or MRI were performed on each patient within 3 days after ablation to evaluate the treatment response. If residual tumor was considered, an additional ablation session was performed.

Table 1. Characteristics of patients with and without diabetes

Characteristics	Patients with DM (n=64)	Patients without DM (n=244)	p value
Age, years, mean±SD	59.8±9.2 (42-83)	55.5±10.6 (29-78)	0.004
Gender (M/F)	58/6 (90.6%; 9.4%)	191/53 (78.3%;21.7%)	0.025
Etiology (HBV/HCV/Alcohol/ NBC), n	43/11/4/6	199/30/2/13	0.031
BMI (kg/m²), mean±SD (range)	25.3±3.8 (19.4-39.5)	24.4±3.0 (16.6-33.8)	0.047
Alcohol abuse (+/-)	15/49 (23.4%; 76.6%)	53/191 (21.7%; 78.3%)	0.768
Child-Pugh score (A/B/C), n	58/6/0	215/25/4	0.57
AFP (ng/mL), mean±SD	416.96±1224.07	325.70±1333.45	0.442
ALT (IU/L), mean±SD	59.61±48.72	58.21±49.63	0.775
AST [IU/L], mean±SD	61.38±52.09	62.09±56.37	0.906
Index tumors, mean±SD	1.2±0.4 (total 77)	1.2±0.4 (total 288)	0.715
Tumor diameter (cm), mean±SD (range)	2.8±1.0 (1.0-5.2)	2.6±1.1(0.7-6.6)	0.096
Differentiation grade (high/middle/low/none), n	24/24/3/13	83/79/16/66	0.624
Follow-up (months), mean±SD	39.4±20.7 (median 35.5)	43.1±23.8 (median 38.5)	0.253

DM: diabetes mellitus, M: male, F: female, HBV: hepatitis B virus, HCV: hepatitis C virus, NBC: non hepatitis B and C virus, BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: α-fetoprotein

Outcome evaluation and follow-up

Patients were followed-up by abdominal contrast imaging (CEUS, contrast CT or MRI) at 3-month intervals during the first year after ablation and then at 6-month intervals during the second year. Tumor progression was diagnosed by imaging findings, and the date of progression was defined as the date of the examination when the progression of HCC was first noted. PFS was defined as the length of time from initial HCC ablation to the tumor progression (local tumor progression or intrahepatic recurrence) or patient death. OS was defined as the time from the beginning of the initial therapy to patient death from any causes or last contact.

The mean fasting plasma glucose values and continuation of alcohol consumption during every three months' follow-up were recorded. Patients with DM were classified into the "fasting glucose within 7.0mmol/L group", which was defined as having a mean fasting plasma glucose level ≤7.0mmol/L during follow-up, or the "fasting glucose > 7.0mmol/L group". Patients without DM were included in the "fasting glucose < 7.0mmol/L group". Drinking after the initial ablation was classified into two groups: habitual drinkers who continued to drink >100g daily (continuation group) and those who were able to reduce their quantity of daily consumption to <100g (non-alcohol group). Patients who did not drink regularly were regarded as non-alcohol group.

Statistics

The quantitative variables are shown as mean ± standard deviation (SD). Mean quantitative values were compared by the Student's t-test. Nonparametric data were compared using the Mann–Whitney U test. Differences in proportions were tested by the chi-square or Fisher's exact test. The Kaplan-Meier method was used to estimate PFS and OS and the log-rank test was used to compare differences. Univariate analysis of factors related to survival was estimated by Kaplan-Meier method, and variables with p value less than 0.1 entered in multivariate regression analysis. Cox proportional hazards model was used to identify independent prognostic factors. The data analysis was performed using SPSS (Version 17.0). All p values derived from two-tailed tests, and a level of <0.05 was considered to be statistically significant.

Results

A total of 308 patients with newly diagnosed HCC who underwent primary treatment with MWA were enrolled. Sixty four out of 308 (20.8%) had DM prior to MWA. Baseline characteristics for both DM and non-DM patients are shown in Table 1.

Table 2. Univariate analysis of factors related to survival of HCC patients who underwent curative MWA

Variables	No.	PFS		OS	
		x^2	p value	x^2	p value
Age, years (>60/ ≤60)	108/200	5.512	0.019	5.084	0.024
Sex (M/ F)	249/59	0.482	0.487	2.166	0.141
Stage of underlying disease (non cirrhosis, Child-Pugh A/ Child-Pugh B,C)	273/35	0.746	0.388	7.392	0.007
Differentiation grade (high/middle/low)	107/103/29	7.086	0.029	10.114	0.006
Index tumors (1/ 2 or more)	258/50	11.307	0.001	9.255	0.002
Tumor diameter (<2cm/ ≥2cm)	58/250	14.990	0.000	2.489	0.115
HBs-Ag (positive/ negative)	242/66	0.407	0.524	0.202	0.653
HCV-Ab (positive/ negative)	42/266	3.992	0.046	1.785	0.182
AST (<63/ ≥63 IU/L)	209/99	7.001	0.008	7.253	0.007
ALT (<49/ ≥49 IU/L)	184/124	14.591	0.000	6.538	0.011
AFP (<41/ ≥41 ng/mL)	195/113	13.939	0.000	8.034	0.005
Alcohol abuse (yes/no)	68/240	7.698	0.006	8.978	0.003
Uninterrupted alcohol consumption after initial therapy for HCC (yes/no)	36/32	0.932	0.334	1.510	0.219
BMI (<25/ ≥25kg/m²)	172/136	0.182	0.670	0.221	0.638
Presence of DM (yes/no)	64/244	3.609	0.057	3.150	0.076
Mean fasting plasma glucose level after initial therapy for HCC (>7.0/≤7.0)	37/271	23.442	0.000	11.780	0.001

PFS: progression-free survival, OS: overall survival, M: male, F: female, Child: Child-Pugh classification, HBs-Ag: surface antigen of the hepatitis B virus, HCV-Ab: hepatitis C antibody, AST: aspartate aminotransferase, ALT: alanine aminotransferase, AFP: α-fetoprotein, BMI: body mass index, DM: diabetes mellitus
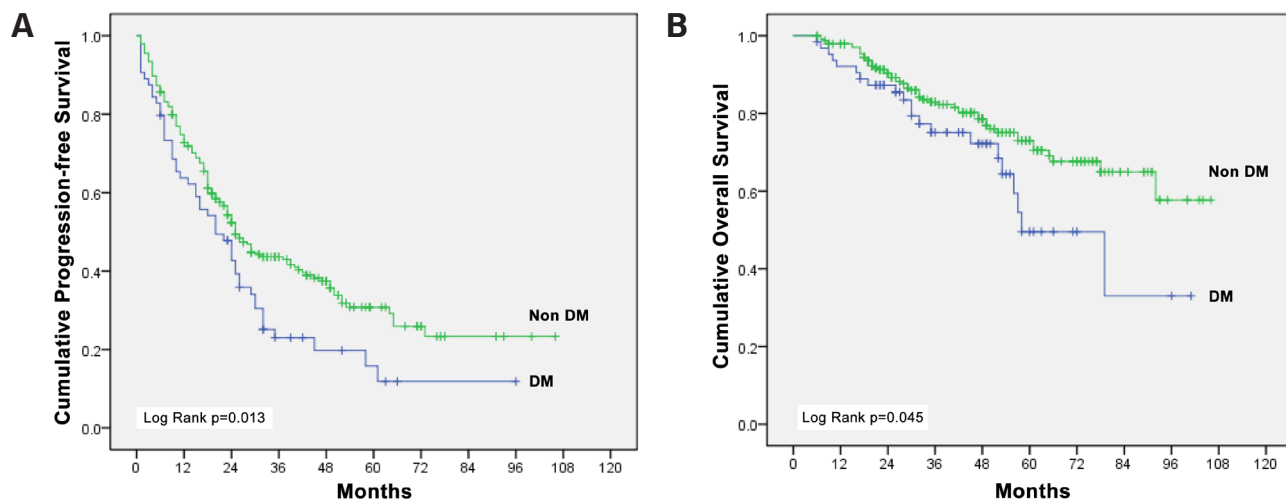
Progression-free survival rate after MWA

For the DM group, the 1-, 3-, 5- year's cumulative PFS rates were 63.8, 23.0 and 15.8% respectively, which were significantly lower compared with the non-DM group (72.7, 43.6, 30.8%; p=0.013; Figure 1A). The factors associated with the progression of HCC were investigated in all 308 patients. Univariate analysis identified the following factors that influenced the rate of HCC progression: age (>60/≤60 years; p=0.019), differentiation grade (high/middle/low; p=0.029), tumor index (1/2 or more; p=0.001), tumor diameter in cm (<2/≥2; p=0.000), HCV-Ab (positive/negative; p=0.046), AST (<63/≥63 IU/L; p=0.008), ALT (<49/≥49 IU/L; p=0.000), AFP (<41/≥41 ng/Ml; p=0.000), alcohol abuse (yes/no; p=0.006), mean fasting plasma glucose level after initial therapy for HCC (>7.0/≤7.0; p=0.000) (Table 2). These parameters entered into the multivariate Cox regression analysis. Tumor

diameter (hazard ratio (HR): 1.191, p=0.006), AFP (HR:1.000, p=0.022), alcohol abuse (>100g/d vs ≤100g/d, HR:1.579, p=0.008), mean fasting plasma glucose level after initial therapy for HCC (>7.0/≤7.0, HR:2.728, p=0.003) (Table 3A).

Overall survival rate after MWA

The cumulative OS rates at 1, 3, and 5 years were 87.3, 75.1 and 49.5% for the DM group and 97.9, 82.9 and 70.5% for the non-DM group respectively (p=0.045; Figure 1B). Univariate analysis identified the following variables as factors significantly contributing to HCC OS after MWA: age (>60/≤60; p=0.024), stage of underlying disease (non cirrhosis, Child-Pugh A/Child-Pugh B, C; p=0.007), differentiation grade (high/middle/low; p=0.006), tumor index (1/2 or more; p=0.002), AST (<63/≥63 IU/L; p=0.007), ALT (<49/≥49 IU/L; p=0.011), AFP (<41/≥41 ng/mL; p=0.005), alcohol

Figure 1. Kaplan-Meier curves for progression-free survival **(A)** and overall survival **(B)** of DM vs non-DM patients. Significant differences between the two groups were noticed.

Table 3. Multivariate analysis for progression-free and overall survival in HCC patients receiving curative MWA

Characteristics	RR	95% CI	p value
(A) Progression-free survival			
Tumor diameter	1.191	1.051-1.349	0.006
AFP	1.000	1.000-1.000	0.022
Alcohol abuse >100g/d	1.579	1.128-2.212	0.008
Mean fasting plasma glucose level after initial therapy for HCC>7.0	2.728	1.414-5.265	0.003
(B) Overall survival			
Child-Pugh classification (A/B,C)	1.692	1.065-2.689	0.026
Tumor diameter	1.251	1.021-1.534	0.031
AFP	1.000	1.000-1.000	0.000

RR: relative risk, 95% CI: 95% confidence Interval

abuse (yes/no; p=0.003), presence of DM (yes/no; p=0.076), mean fasting plasma glucose level after initial therapy for HCC (>7.0/no; p=0.003) (Table 2). Multivariate analysis showed that the Child-Pugh classification A versus B, C (risk 1.692, p=0.026), tumor diameter (risk 1.251, p=0.031), and AFP (risk 1.000, p=0.000) were significant factors impacting HCC OS after MWA (Table 3B).

Discussion

Although whether DM worsens the prognosis of post-treatment HCC patients remains controversial, an increasing number of reports examine this topic [6-10]. However, only little information regarding DM and non-surgical treatment methods; especially on MWA in HCC patients is not available [13,14]. It may be worthwhile to investigate the impact of DM on the long-term prognosis in patients undergoing MWA.

In the present study, long-term prognosis of a relatively large cohort of HCC patients was invastigated. Significant differences were observed both in PFS (p=0.013) and OS rate (p=0.045) between these two groups, suggesting that DM may worsen the long-term outcome of HCC. The current results are consistent with results of previous studies [15-18]. Although Huo et al. and Ikeda et al. included only surgical patients [15,18], Shau et al. included surgical and non-surgical patients undergoing radiofrequency ablation or percutaneous ethanol injection [16]. None was subjected to MWA, yet similar results were obtained.

In this study, although multivariate analysis showed that presence of DM prior to initial MWA did not independently affect HCC prognosis, postoperative poor blood glucose control was an independent risk factor for tumor progression after curative MWA for HCC in patients with DM, probably because good plasma glucose control may help delay progression in patients with DM, which can be equalized to patients without DM. Kaneda et al. [19] considered that poor glycemic control increases the risk of postoperative tumor recurrence by 3.551-fold in patients with DM. This is in accordance with the Abe et al. study [12], in that a higher plasma glucose levels after initial therapy may lead to both enhanced carcinogenesis of the liver and deterioration of liver function. However, the mechanism for the association of DM and HCC prognosis is still unclear. Several potential mechanisms were postulated, which are as follows: 1) DM as a metabolic factor may lead the body into a state of chronic low-grade inflammation, which can initiate or expedite oncogenic transformation. Meanwhile, changes in tumor cells can generate an inflammatory environment which supports HCC progression and tumorigenesis [20,21]; 2) the environment of hyperinsulinemia and hyperglycemia caused by DM may promote tumor cell proliferation and metastasis by increasing endothelial cell permeability and increased generation of reactive oxygen species (ROS) [22], and structural changes in the basement membrane, which may be associated with an increased risk of metastasis [23,24], and by the phosphorylation and activation of AKT and ERK pathways via interaction with the insulin receptor, which may play important roles in cancer cell promotion and tumor growth [25]; 3) the underlying liver dysfunction may cause rapid progression to liver failure or other morbidities [10,26].

Recent studies suggest that metformin, a commonly used anti-diabetic agent, may reduce carcinogenesis of HCC and improve its prognosis [27]. Chen et al. presented the results on 135 individuals who underwent RFA for early HCC and concluded that the use of metformin was associated with better survival [13]. Two large meta-analyses also showed that DM patients who were taking metformin had lower risk of developing liver cancer [28,29]. However, its mechanism remains relatively unknown. Several basic investigations have shown that metformin inhibits the proliferation and tumor growth probably by its ability to activate 5-adenosine monophosphate-activated protein kinase, preventing the gluconeogenesis in the liver and stimulating glucose uptake in muscle, and the potential to inhibition of its downstream target mammalian target of rapamycin (mTOR) activity. Inhibition of mTOR signaling leads to reduction in the phosphorylation of its key downstream effectors involved in mRNA translation and consequent inhibition of global protein synthesis [30]. In addition, metformin was also demonstrated to reduced mTOR signaling through inhibition of Rag GTPase, which activates mTOR, or by decreasing the levels of insulin-like growth factor I [31]. However, factors conducing to better result in metformin takers, such as genetic polymorphisms, are still to be clarified [32].

We acknowledge that the present study has some limitations. Firstly, unlike typical populations of HCC patients in most Western countries, most of the individuals in our study (78.6%) had chronic HBV infection, so our results may not be generalized to all HCC patient populations. Secondly, according to the blood glucose level before treatment, we divided the patients into those with or without DM, but in a relatively long follow-up period, some non-DM patients might change into DM patients. Therefore, this may cause certain confu-

sion in the statistical results. Thirdly, the causes of death in different groups failed to be distinguished, because it is difficult to clarify whether individuals with HCC died of HCC progression or hepatic failure. Moreover, the distinguished effects on the types of hypoglycemic drugs need to be investigated because drugs stimulating insulin secretion may affect the prognosis of HCC. These limitations argue for larger prospective studies to establish the prognostic role of DM in HCC patients.

Conflict of interests

The authors declare no conflict of interests.

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