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Original Article

The efficacy assessment of radiofrequency ablation treatment on primary hepatocellular carcinoma by contrast enhanced ultrasonography

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Abstract: Aims: This study is to evaluate the efficacy of radiofrequency ablation (RFA) treatment on primary hepatocellular carcinoma (HCC) by contrast enhanced ultrasonography (CEUS), and to evaluate the application value of CEUS before and after RFA. Methods: There were 140 cases of primary HCC patients with 176 lesions that were randomly divided into two groups (A and B). Group A was treatment group, including 70 patients with 86 lesions. Of them, there were 57 cases of even high enhancement and 29 cases of uneven high enhancement in artery phase; 75 low enhancement and 11 even enhancements in portal phase and delayed phase. Group B was control group, including 70 cases with 90 lesions. Of them, there were 64 cases of even high enhancement and 26 cases of uneven high enhancement in artery phase; 78 low enhancement and 12 even enhancements in portal phase and delayed phase. Patients underwent CEUS to evaluate tumor blood supply before RFA and residual lesions after RFA, 10 min before and after RFA, and one month after RFA. Clinical symptoms before and after RFA, tumor size, liver function, bilirubin, alanine aminotransferase (ALT) and α-fetoprotein (AFP) were analyzed to assess the safety of RFA. Results: There were 32 residual lesions after RFA, thus, second RFA was performed in Group A. There were no severe complications or death after treatment. Compared with that before surgery, postoperative ALT level increased but gradually decreased through postoperative 2 weeks. AFP, bilirubin and ALT levels were significantly lowered one month after surgery; there were more cases of Child-pugh A level; postoperative tumor size was significantly reduced. The survival rate of 6-month, 1 year and 2 year was 100%, 92.5%, and 80.7% for Group A, and 95.7%, 78.4%, and 64.0% for Group B. There were statistically significant differences between two groups ($P < 0.05$). Conclusions: CEUS is of great clinical importance to evaluate the completeness of ablation for RFA on treatment of HCC.

Keywords: Primary hepatocellular carcinoma, contrast enhanced ultrasonography, radiofrequency ablation

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent cancer in China and worldwide, and its high malignancy only ranked after lung cancer [1]. Currently, liver resection and transplantation achieved the best clinical efficacy, with 5-year survival rate only less than 50% [2]. However, due to the late diagnosis, surgery may not be optimal for many patients. For patients without surgical opportunities, palliative treatments would be applied, such as hepatic artery ligation, frozen, microwave, intratumoral injection. Radiofrequency ablation (RFA) is commonly used as non-surgical treatment for primary HCC. RFA can real-time monitor and destroy tumors in a much less invasive way with simple operation and certain efficacy, however RFA is not suitable for large tumors with diameter of > 5 cm [3]. The efficacy evaluation of RFA includes enhanced CT, MRI, and color Doppler flow imaging (CDFI), as well as contrast-enhanced ultrasound (CEUS) in recent years [4, 5] that significantly increased the detection of tissue microperfusion. In this study, CEUS was used to further evaluate the efficacy and clinical application of RFA treatment on primary HCC.

Methods

Subjects

From October 2013 to October 2015, 140 HCC patients with 176 lesions admitted in Yantai Yu Huang Ding Hospital were recruited in this study and patients were randomly divided into Group

Table 1. Demographics of all patients

	Group A	Group B
Gender (male/female)	43/27	41/29
Age (years)	34-76 (64.5 ± 11.5)	31-80 (68.5 ± 13.7)
Kps score (100/≥ 9)	62/8	67/3
Liver function (Child A/B)	29/41	34/36
Hepatitis (HBV/HCV/others)	58/10/2	62/7/1
BCLC staging* (0/A/B)	21/32/17	15/42/13
AFP level (mg/L)*	564.3 ± 97.8	612.3 ± 107.5
AFP level (> 400 or ≤ 400)	45/25	37/33
Tumor size	0.8-4.5 (1.8 ± 0.7)	1.1-4.6 (1.7 ± 0.9)
Tumor number (solitary/multiple)	57/29	67/23

BCLC*: Barcelona Clinic Liver Cancer. AFP*: Alpha-Fetoprotein.

A and B. Clinical data of patients were shown in **Table 1**. Inclusion criteria include 1) solitary tumor; 2) max tumor diameter ≤ 5 cm or number of tumors ≤ 3; 3) no intrahepatic vascular thrombosis and no invasion of adjacent organs; 4) liver function of Child-pugh A or B level. Exclusion criteria include 1) Kps score < 90; 2) ascites; 3) abnormal coagulation; 4) ALT > 3 times of normal upper limit. Group A was experiment group, there were 43 male and 27 female, with average age of 64.5 ± 11.5 years, ranging from 34 to 76 years, and max tumor diameter of 1.8 ± 0.7 cm, ranging from 0.8 to 4.5 cm. Group B was control group, there were 41 male and 29 female, with average age of 68.5 ± 13.7 years, ranging from 31 to 80 years, and max tumor diameter of 1.7 ± 0.9 cm, ranging from 1.1 to 4.6 cm. There were 124 patients of solitary tumor, and 52 patients of multiple tumors. There were no statistical differences between two groups in terms of gender, age, liver function grade and tumor size. In this study, 86 patients were diagnosed by pathology, and 54 patients were diagnosed by combination of medical history, laboratory tests and radiology imaging. All patients were of Kps tumor score (Karnofsky) ≥ 90 points. Prior written and informed consent were obtained from every patient's family and the study was approved by the ethics review board of Yantai Yu Huang Ding Hospital.

CEUS imaging

Color Doppler ultrasound instrument (Philips-iU22, Amsterdam, Netherlands) with pulse subtraction harmonic synthesis technology was used, with central probe frequency of 4-9 MHz and mechanical index (MI) of 0.07 to 0.10. Two-dimensional ultrasound (Philips-iU22, Amsterdam, Netherlands) was used to detect tumor morphology, size, location, echo, blood flow and resistance index. MI, depth, gain, and depth gain compensation (DGC) remain unchanged in all patients during angiography. The contrast agent (SonoVue, Bracco, Italy) was diluted by 5 ml saline solution, and bolus injected through elbow superficial veins rapidly. After injection, real-time CEUS was used to dynamically monitor the tumor size and invasion. Tumor location was precisely positioned and marked the best entry point on skin. There were 3 main phases of CEUS, including artery phase, which is 10-35 s after contrast agent injection; portal phase, 35-120 s after injection; and delayed phase, 120 s after injection until injection micro bubbles disappeared (about 240-360 s). With CEUS, micro bubble break was recorded, and liver MI ultrasound was performed every 10 min. All images were analyzed by the two independent sonographers.

RFA procedures

Radiofrequency instrument (RITA 1500, California, US) was used with maximum output power of 150 W, STAR Burst TMXL sheathed needle, and cluster radial needle group (5 electrodes) with diameter of 5.0 cm or Uniblat monopolar needle with size 3.0 cm × 2.5 cm. Each patient was anaesthetized using pethidine (50 mg) by intramuscular injection. With ultrasound guidance and ECG monitor, RFA was performed based on tumor size and number of tumors, with each site of 10 min. When tumor turned hyperechoic, CEUS was performed to determine whether there was blood supply in tumor. After RFA, treatment of anti bleeding, liver protection and nutrition support was given to all patients.

Ultrasound diagnostic criteria of HCC

The ultrasound diagnostic criteria of HCC [6] include: ultrasound detection of artery blood flow in tumor, RI (resistance index) ≥ 0.60;

Table 2. Comparison of clinical indicators between the two groups

Time	AFP (ng/L)	Bilirubin (μmol/L)	ALT (U/L)	Child classification		Tumor size
				A	B	
Preoperative	564.3 ± 97.8	35.2 ± 11.8	84.7 ± 17.3	29	41	1.88 ± 0.74
Postoperative 3 days	98.5 ± 20.7	21.8 ± 9.7	100.8 ± 28.7	18	52	1.42 ± 0.56
Postoperative 2 weeks	81.7 ± 16.4	18.5 ± 10.3	80.6 ± 16.3	20	50	1.39 ± 0.43
Postoperative 1 month	56.3 ± 11.8	16.5 ± 6.7	62.4 ± 10.2	36	34	0.97 ± 0.13

Table 3. CEUS evaluation of solitary and multiple lesions after RFA

Lesion	Number	CEUS evaluation (N/%)		*P* value
		Complete ablation	Residual lesions	
Solitary	59	36 (61.0)	23 (38.9)	0.001
Multiple	27	18 (66.6)	9 (33.3)	0.036
Total	86	54 (62.7)	32 (37.2)	

CEUS detection of high enhancement in artery phase, and low or no enhancement in portal phase and delayed phase.

Analysis of lab tests and imaging

Postoperative 3 days, patients underwent lab tests and ultrasound; postoperative 2 weeks, patients underwent CEUS to assess the efficacy of RFA. If the tumor and surrounding tissues showed no enhancement, suggesting complete tumor ablation; if the tumor or surrounding area showed enhancement, suggesting residual tumor that requires second RFA. Decreased alpha fetoprotein (AFP), reduced pain, increased appetite and weight all indicated clinical improvements. Child classification for liver function was used to assess liver reserve function by evaluation of general condition, ascites, serum bilirubin, serum albumin concentration and prothrombin time. The above 5 parameters were scored as 1, 2, 3 and liver reserve function were categorized as three levels: A (score of 5-6), B (score of 7-9), and C (score of 10-15). The higher the score, the worse the liver reserve function.

The bilirubin, ATL, ALT, and AFP was detected in fasting blood serum. The AFP was determined by chemiluminescence detection, and bilirubin, ATL and ALT were determined by enzyme assay.

Statistical analysis

SPSS 17.0 (US, Microsoft) was used to analyze data. Qualitative data was presented as mean ± standard deviation, and its group comparison used T test. Categorical data used chi-square test. $P < 0.05$ was considered statistically significant.

Results

Clinical improvement indicators after RFA

To determine the postoperative information, follow up and laboratory tests were performed. As shown in **Table 2**, compared with that before surgery, the clinical symptoms of 68 patients in Group A relieved, including increased appetite and reduced fatigue; though postoperative ALT increased, AST gradually decrease through postoperative 2 weeks; the postoperative one month AFP, bilirubin and ATL levels was significantly lower; more cases of Child-pugh A level; postoperative tumor size was significantly reduced. Patients' general conditions and laboratory tests deteriorated. These results indicate that the general condition and laboratory tests of patients underwent RFA improved.

Postoperative diagnostic evaluation

To determine the postoperative differences between two groups, CEUS was performed. In Group A, there were 57 cases of even high enhancement and 29 cases of uneven high enhancement in artery phase; 75 low enhancement and 11 even enhancements in portal phase and delayed phase. After RFA, CEUS showed high enhancement in early artery phase, low or no enhancement in portal phase and delayed phase, categorized as "fast forward and fast backward". Of them, there were 54 (62.7%) complete ablated lesions and 32 (37.2%) residual lesions. However, in Group B, CEUS showed lesion increased. There were significant differences of residual lesion rate between the two groups ($P < 0.05$), as shown in **Table 3**. These results indicate that CEUS can be used in post operation evaluation.

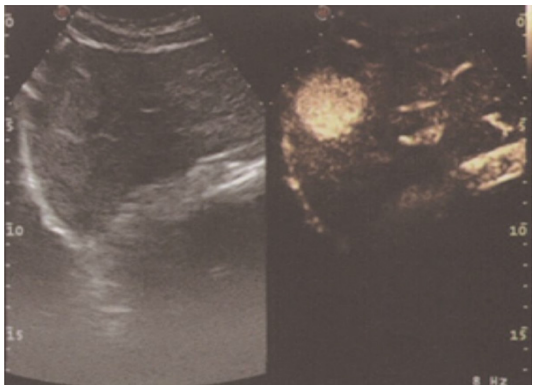

Figure 1. CEUS before RFA.

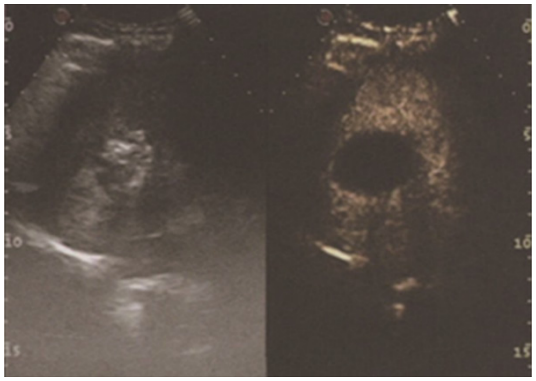

Figure 2. CEUS two weeks after RFA.

Imaging analysis

To determine the postoperative lesion change, CEUS was performed. Preoperative CEUS showed 86 lesions of high enhancement as "fast forward and fast backward" in **Figure 1**. Postoperative two weeks, 54 lesions and surrounding tissues showed no enhancement and no blood flow in CDFI, as shown in **Figure 2**. While 32 lesions showed residual lesions, which are partial high enhancements of lesion or uneven enhancement of surrounding tissues in early artery phase, as shown in **Figure 3**. Second RFA was performed on these 32 lesions and CEUS showed no blood flow in lesions. These results indicate that different treatments should be provided for patients with different postoperative CEUS to improve the prognosis.

Survival rate comparison

All patients were followed up by telephone for 2 years. There were 3 patients lost of follow-up in year 1 and 5 patients in year 2 in Group A; 3 patients in year 1 and 6 patients in year 2 in Group B. The survival rate of 6-month, 1 year and 2 year was 100%, 92.5%, 80.7% for Group A, and 95.7%, 78.4%, 64.0% for Group B. There were statistically significant differences between two groups ($P < 0.05$), as shown in **Table 4**.

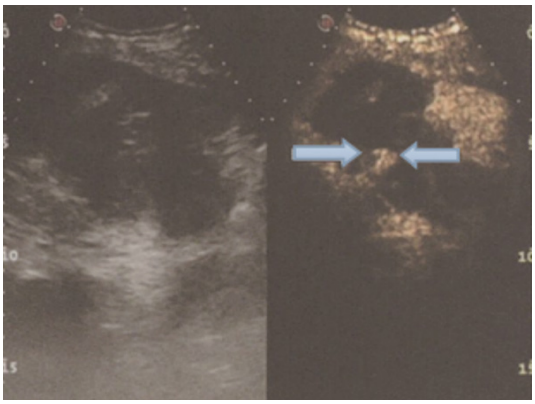

Figure 3. Residual lesions in CEUS two weeks after RFA.

Table 4. Survival rate comparison between groups

Follow up	RFA treatment group	Control group
6 months	70/70 (100)	67/70 (95.7)
1 year	62/67 (92.5)	51/65 (78.4)
2 year	46/57 (80.7)	32/50 (64.0)

Note: there is statistical difference between treatment group and control group at all time points ($P < 0.05$).

Discussion

Primary HCC is one of the most prevalent malignant tumors [7]. The incidence is increasing, especially in patients with cirrhosis, with prevalence of about 6% to 11% [8]. Because of its insidious onset, about 75% of patients were in late stage upon diagnosis, thus lost surgical opportunities [9]. Due to its minimal invasion and rapid development, in recent years, RFA has become an important HCC treatment, especially used as focal ablation for patients with no surgical opportunities [10]. Currently, RFA can eliminate 5-7 cm lesions [1]. In this study, all lesions were of diameter ≤ 5 cm, thus, theoretically it met safety requirements [1] of 1 cm edge to achieve complete ablate lesions. The theory of RFA treatment principle is focal high temperature of 80-100°C, leading to tumor tissue coagulation and necrosis [11].

Currently, clinical evaluation of RFA efficacy includes enhanced CT and MRI. CT scan showed decreased CT value in lesions, no enhancement in artery phase and portal phase target indicated no blood supply, with only thin layer of enhancement of tumor edge. Dynamic contrast-enhanced MRI scan showed no blood supply of tumor lesion, and thin layer of enhancement of tumor edge in delayed phase. Both methods are not optimal for real-time evaluation of tumor treatment [12]. Traditional ultrasound is not sensitive for detection of low blood flow, which is susceptible to breathing and heart rate, thus cannot detect blood flow inside lesion. CEUS can show tumor blood supply or even capillary supply. It is shown [13] that CEUS increased the detection rate of HCC with diameter ≤ 1.0 cm from 54% to 96%, and its differential diagnostic value was better than spiral CT in general [14-16]. CEUS is also better than enhanced MRI, especially for the detection and diagnosis of small lesions [17, 18]. Moreover, CEUS can perform second treatment for residual lesions [19]. Preoperative evaluation is essential for RFA treatment of patients with liver tumors. After injection of contrast agent, different enhancement indicates different types of liver tumors because CEUS is closely associated with tumor blood supply.

In this study, it showed that in treatment group, compared with that before surgery, clinical symptoms relieved in 70 cases of HCC after RFA treatment, including transient increased and gradually decreased postoperative ALT levels; AFP, bilirubin and ATL levels significantly lowered after one month; there were more cases of Child-pugh A level; maximum lesion diameter significantly reduced. All the above showed that RFA is one of the most effective treatment for HCC diameter < 5 cm. Assessed by CEUS and lab tests, the complete ablation rate and residual lesion rate in treatment group was significantly lowered than that of control group ($p < 0.05$). In the same time, CEUS can determine the residual lesion that can real-time reflect the efficacy of tumor ablation. In the treatment group, there were 32 residual lesions, which is partial high enhancement of the lesion or uneven enhancement of surrounding tissues in early artery phase. Second RFA was performed on these 32 lesions and CEUS showed no blood flow in lesions. The survival rate of 6-month, 1 year and 2 year was 100%, 92.5%, 80.7% for Group A, and 95.7%, 78.4%, 64.0% for Group B. The survival rate of all times in Group A was significantly higher than that in Group B ($P < 0.05$).

CEUS can not only indicate whether there is residual lesion, but also can perform second treatment that significantly reduced the number of residual lesion, relieved clinical symptoms, and effectively prolonged the survival time. Therefore, CEUS can be used to assess efficacy of RFA on primary HCC, but its further application required multi-center randomized control clinical trials with larger sample size.

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Disclosure of conflict of interest

None.

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