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Original Article

Effects of radiofrequency ablation under ultrasound guidance on immunity, tumor markers and prognosis of patients with liver cancer

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Abstract: Objective: This study is to investigate the effects of radiofrequency ablation (RFA) under ultrasound guidance on immunity, tumor markers and prognosis of patients with liver cancer. Methods: A total of 86 patients with liver cancer treated in our hospital were selected and received RFA under ultrasound guidance. The therapeutic effect was evaluated via contrast-enhanced ultrasonography (CEUS), and changes in immune function, liver function and serum tumor markers were compared before and after treatment. Moreover, patients were followed up for 5 years to analyze the overall survival result of different patients. Results: At 3 months after treatment, the complete response rate in patients was significantly increased, the partial response rate was statistically decreased, and the life quality score was also significantly elevated ($P < 0.05$). After treatment, levels of cluster of differentiation (CD)3^+ and $CD4^+$ and $CD4^+/CD8^+$ ratio were markedly increased, while the level of $CD8^+$ was obviously reduced ($P < 0.05$). In addition, levels of interleukin-2 (IL-2) and interferon (IFN)-γ were remarkably up-regulated, while levels of IL-4 and IL-10 were significantly decreased ($P < 0.05$). After treatment, the liver function of patients was significantly superior to that before treatment, and levels of tumor markers significantly declined compared with those before treatment ($P < 0.05$). Besides, 5-year follow-up revealed that the survival rate of patients with a small tumor diameter and single lesion was statistically higher than that of patients with a large tumor diameter and multiple lesions ($P < 0.05$). Conclusion: RFA under ultrasound guidance can effectively improve the immune function of patients, reduce levels of tumor markers, and increase the survival rate of some patients, which indicates favorable therapeutic effect in the treatment of liver cancer.

Keywords: Contrast-enhanced ultrasonography, radiofrequency ablation, liver cancer, immunity, tumor markers, prognosis

Introduction

Liver cancer is one of the most common malignant tumors in the digestive system, the occurrence of which is associated with various predisposing factors, such as viral infection, cirrhosis, chemical carcinogens, contaminated drinking water, smoking and drinking and genetic factors [1]. Although diverse strategies of blocking or retarding the development of chronic hepatitis into cirrhosis have been continuously implemented in recent years, the incidence rate of liver cancer still stays at a high level [2]. Liver cancer is implicated to a complex pathological process and is characterized by hidden onset, rapid progression and high mortality rate, with extremely low 5-year survival rate [3]. There are a multitude of therapeutic methods for liver cancer in clinic, including surgery, interventional therapy, chemoradiotherapy, TCM treatment and radiofrequency ablation (RFA), in which operative treatment has been so far the optimal method to improve the survival rate of patients. However, the liver cancer has often been in the middle and late stages once patients are diagnosed, optimal opportunity for operation are frequently missed, so physicians have to adopt alternative treatments [4]. RFA is a non-invasive therapy with remarkable curative effects, especially for non-surgical patients, which has been extensively used in clinic [5]. In this study, patients with liver cancer were treat-

Table 1. General data of objects of study

Item	Objects of study (n = 86)
Male [n (%)]	54 (62.79)
Female [n (%)]	32 (37.21)
Age (years old)	45-79
Average age (years old)	58.56 ± 9.43
Type of lesion [n (%)]	
Single lesion	65 (75.58)
Multiple lesions	23 (24.42)

ed with RFA under ultrasound guidance, and its effects on immune function, tumor markers and prognosis of patients were analyzed, so as to provide a basis for the non-surgical treatment of patients with liver cancer.

Materials and methods

General data

A total of 86 patients with liver cancer treated in our hospital from April 2016 to May 2017 were selected as objects of study. Inclusion criteria: 1) patients diagnosed with liver cancer according to the diagnostic criteria of the American Association for the Study of Liver Diseases (AASLD), liver biopsy and imaging examination [6], 2) patients receiving RFA, 3) patients who signed the informed consent. Exclusion criteria: 1) patients with severe dysfunction in the heart, brain, lung or kidney, 2) patients complicated with mental or neurological diseases. The general data of objects are shown in **Table 1**. This clinical trial was approved by the Ethics Committee of Jingzhou Central Hospital.

Methods

Treatment: Before operation, color Doppler ultrasonography was performed for the upper abdomen to observe the tumor diameter, number and position, and locate and mark the entry path and depth. The operation was explained 1 d before operation, and the venous channel was opened with fasting for solids and liquids at 8 h before operation. Patients were instructed to take a supine position, a cold-circulation radiofrequency needle was inserted under the ultrasound guidance through the marked entry point after local anesthesia, and a different number of radiofrequency needles were inserted according to the size of lesion: 1 needle if tumor diameter < 3 cm, and 2 needles if tumor diameter ≥ 3 cm. When the tip of needle reached the bottom of tumor, the LDRF-120S multi-polar RFA instrument (MianyangLide Electronic Technology Co., Ltd.) started to work for treatment, accompanied by real-time ultrasound monitoring. The entire process of RFA lasted for 10-20 min, accompanied by real-time ultrasound monitoring, and changes in tumor tissues were observed.

Contrast-enhanced ultrasonography (CEUS)

CEUS was performed at 1 month and 3 months after treatment using the Philips U22 color Doppler ultrasound instrument (Philips, Netherlands) to evaluate the therapeutic effect of RFA (frequency of frequency-conversion probe: 3.0 MHz, harmonic frequency: 3.5 MHz, and speed range: 0.06-0.12 m/s). SonoVue (manufacturer: Bracco, Milan, registration No.: H20080059) was used as the contrast agent, and 1 piece of SonoVue and 5 mL 9% sodium chloride solution were mixed to obtain the suspension (2×10^8/mL sulfur hexafluoride microbubbles). The ultrasound probe was fixed at the target region, the ultrasound contrast agent was injected via peripheral vein, and timing started. When the contrast agent reached the target, the lesion was slowly scanned in a fan-shaped way, and the enhancement and perfusion of contrast agent was observed in real time.

Determination of indexes

Before treatment and at 3 months after treatment, 3-5 mL fasting venous blood was drawn from patients in both groups. The serum was collected, and the anti-cluster of differentiation 3 (CD3), CD4 and CD8 antibodies were added for incubation for 30 min in a dark place at 4°C, followed by detection using a flow cytometer (BD, USA) and calculation of $CD4^+/CD8^+$ ratio. Levels of interleukin-2 (IL-2), interferon (IFN)-γ, IL-4 and IL-10 were detected via enzyme-linked immunosorbent assay (ELISA) using related kits provided by ADLITTERAM, USA. After incubation at room temperature (20°C) for 15 min in a dark place, 50 μL stop buffer was added. The optical density (OD) value was read using a full-automatic multi-functional microplate reader (Thermo Fisher Scientific, USA) within 15 min, and the concentrations of IL-2, IFN-γ, IL-4 and IL-10 were calculated. The concentrations of carcino-embryonic antigen (CEA), carbohy-

Table 2. Therapeutic effects and quality of life at different time points after treatment

Item	1 month after treatment	3 months after treatment	t/χ^2	*P*
Complete response rate [n (%)]	21 (23.81)	51 (59.30)	20.091	< 0.001
Partial response rate [n (%)]	63 (73.26)	34 (39.53)	18.822	< 0.001
FACT-G score	70.29 ± 3.74	83.67 ± 3.82	23.210	< 0.001

Table 3. Comparisons of levels of T lymphocyte subsets before and after treatment

Time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Before treatment	71.52 ± 6.73	42.75 ± 7.26	23.47 ± 6.35	1.83 ± 0.56
After treatment	64.36 ± 6.42	37.43 ± 7.63	27.22 ± 6.15	1.45 ± 0.53
t	7.139	4.684	3.934	3.343
P	< 0.001	< 0.001	< 0.001	< 0.001

drate antigen 199 (CA199), CA125, neuron-specific enolase (NSE) and cytokeratin 19 fragment (CYFRA21-1) in patients were detected using the electrochemiluminescence method strictly according to instructions of related kits (Roche).

Evaluation criteria

Evaluation criteria of the therapeutic effect of RFA are as follows [7]: 1) complete response: Myoma volume declined by more than 50%, and 2) partial response: Myoma volume declined by less than 50%. The quality of life of patients in both groups was evaluated using the Functional Assessment of Cancer Therapy-General (FACT-G) scale [8]. 5-level scoring method was used for evaluation from the physiological status, social/family status, emotional status and functional status. The total score is positively correlated with the quality of life of patients.

Before treatment and at 3 months after treatment, 3-5 mL fasting venous blood was drawn from patients in both groups, and the serum was isolated and extracted. Levels of T lymphocyte subsets $CD3^+$, $CD4^+$ and $CD8^+$, and $CD4^+/CD8^+$ ratio were detected using the flow cytometer (BD, USA). Levels of serum IL-2, IFN-γ, IL-4 and IL-10 were detected via ELISA. Concentrations of tumor markers CEA, CA199, CA125, NSE and CYFRA21-1 were detected using the electrochemiluminescence method. The liver function of patients was examined before and after treatment.

Statistical analysis

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for data processing. Measurement data were presented as mean ± standard deviation ($\bar{x}$ ± sd), and *t* test was used. Enumeration data were presented as ratio, and chi-square test was correspondingly adopted. Survival analysis was performed using Kaplan-Meier analysis. $P < 0.05$ suggested that the difference was statistically significant.

Results

Therapeutic effects and quality of life at different time points after RFA

At 3 months after treatment, the complete response rate in patients was significantly elevated, the partial response rate was decreased, and the life quality score was also significantly increased, compared to those at 1 month after treatment ($P < 0.05$) (**Table 2**).

Comparisons of levels of T lymphocyte subsets at 3 months after treatment

After treatment, levels of $CD3^+$ and $CD4^+$ and $CD4^+/CD8^+$ ratio were apparently up-regulated, while the level of $CD8^+$ was obviously reduced, compared to those before treatment ($P < 0.05$) (**Table 3**).

Comparisons of levels of serum inflammatory indexes at 3 months after treatment

We compared the inflammatory factors in serum before and after the treatment. Of note, after treatment, levels of IL-2 and IFN-γ were remarkably increased, while levels of IL-4 and IL-10 were remarkably decreased ($P < 0.05$) (**Table 4**).

Table 4. Comparisons of levels of inflammatory indexes before and after treatment

Time	IL-2 (μmol/L)	IL-4 (μmol/L)	IL-10 (μmol/L)	IFN-γ (μmol/L)
Before treatment	45.73 ± 3.27	27.53 ± 3.32	52.32 ± 3.43	13.87 ± 3.24
After treatment	56.28 ± 3.46	18.82 ± 3.35	35.45 ± 3.56	19.35 ± 3.43
t	20.551	17.126	31.647	10.771
P	< 0.001	< 0.001	< 0.001	< 0.001

Table 5. Comparison of liver function of patients before and after treatment

Time	ALT (g/L)	AST (U/L)	TBIL (μmol/L)	ALB (U/L)
Before treatment	41.38 ± 3.27	48.48 ± 3.37	29.12 ± 3.43	35.57 ± 3.54
After treatment	26.46 ± 3.16	30.62 ± 3.35	18.45 ± 3.52	39.34 ± 3.23
t	30.427	34.856	20.133	7.296
P	< 0.001	< 0.001	< 0.001	< 0.001

Table 6. Comparisons of tumor markers in patients before and after treatment

Time	CEA (ng/mL)	CA199 (U/mL)	CA125 (U/mL)	NSE (ng/mL)	CYFRA21-1 (ng/mL)
Before treatment	3.26 ± 1.12	14.65 ± 2.14	13.65 ± 2.15	17.94 ± 2.14	2.78 ± 0.62
After treatment	2.36 ± 0.37	8.13 ± 1.02	7.03 ± 1.08	10.03 ± 1.07	2.02 ± 0.26
t	7.076	25.505	25.516	30.659	10.483
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Liver function of patients before and after treatment

We evaluated the effect of the RFA under ultrasound guidance on liver function of the patients. After treatment, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) were significantly decreased, while albumin (ALB) was significantly increased ($P < 0.05$) (**Table 5**).

Comparisons of tumor markers in patients before and after treatment

The relevant tumor markers in patients were detected before and after the treatment. At 3 months after treatment, serum CEA, CA199, CA125, NSE and CYFRA21-1 levels remarkably declined, indicating that RFA under ultrasound guidance reduces the risk of hepatocellular carcinoma ($P < 0.05$) (**Table 6**).

Comparison of overall survival of different patients

The survival rate of patients with tumor diameter < 3 cm and single lesion was significantly higher than that of patients with tumor diameter ≥ 3 cm and multiple lesions ($P < 0.05$) (**Figure 1A**, **1B**).

Discussion

Liver cancer is one of the most common malignant tumors in the digestive system, and its mortality rate ranks 2nd in tumors in China. Liver cancer frequently occurs in intrahepatic bile ducts or hepatocytes, and intrahepatic cholangiocarcinoma is the most common type, accounting for more than 80% in liver cancer, with incidence and mortality rates among the top three of all malignant tumors [9]. The clinical manifestations of patients with liver cancer are loss of appetite, hepatalgia, weight loss and weakness, ascites and jaundice, and even coma and systemic failure [10]. The pathogenesis of liver cancer is complex, the process of which is related to dynamic change and long-time accumulation under action of various factors. The interaction between genetic polymorphism and environmental factors plays an important role in the occurrence and development of liver cancer. At present, the therapeutic intervention of liver cancer remains unsatisfactory. The deficiency of early diagnosis and missing the optimal opportunity for surgery such as radical excision result in low survival rate of patients [11].

RFA represents a kind of minimally-invasive treatment characterized by simple operation,

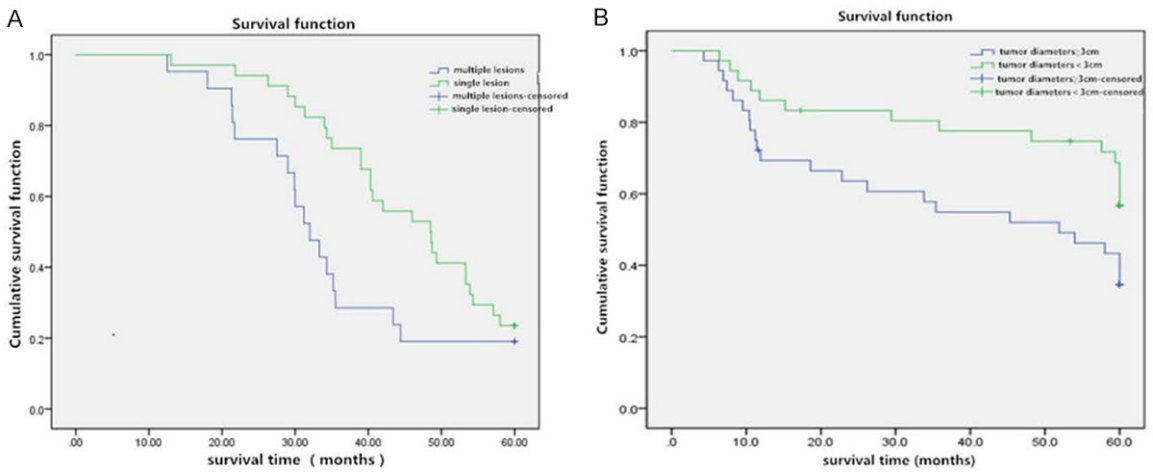

Figure 1. The survival rate of patients. The survival rates of patients with different lesions (A) and tumor diameters (B) were analyzed and compared.

no radioactive damage and repeatability. In RFA, the biological heat is generated by high-frequency alternating electromagnetic waves, and it causes the rise of temperature in tumor, leading to protein denaturation in cells and coagulative necrosis of lesion tissues. In addition, coagulation reaction occurs around tumor tissues, and effectively prevents the further spread of tumor cells [12, 13]. Results of this study revealed that, compared with those at 1 month after treatment, the complete response rate in patients was significantly increased, the partial response rate was decreased, and the life quality score was also significantly elevated at 3 months after treatment, indicating that RFA under ultrasound guidance can ablate the lesion under direct vision. CEUS also showed that the tumor volume in patients was reduced progressively, and there was no obvious damage in surrounding normal tissues. We found that the ablation effect was significant, and the quality of life of patients was continuously improved.

Patients with liver cancer generally suffer from immune dysfunction or hypoimmunity, and T lymphocytes can be involved in the body's immune response and regulates the immune function. $CD3^+$ represents the total number of T lymphocytes in the body, $CD4^+$ is a helper T lymphocyte that enhances the regulatory effect on the immune response, and $CD8^+$ is an inhibitory T lymphocyte subset that can destroy and kill infected cells [14, 15]. Immune disorder in patients with liver cancer is basically due to the imbalance of Th1/Th2 cytokines. T lymphocyte subsets can be generally divided into Th1 and Th2 types. The main function of Th1 type is to secrete IFN-γ and IL-2 that can induce macrophage activation and produce immunoglobulin. IFN-γ exerts potent effects of anti-virus, anti-infection, enhancement of host defense and involvement in immune regulation. IL-2 is a growth factor of T cell subsets and an important factor regulating immune response, which can promote activation and proliferation of B cells [16]. The main function of Th2 type is to secrete IL-4 and IL-10 that can induce growth of mastocytes and macrophages. Results of this study demonstrated that at 3 months after treatment, levels of $CD3^+$ and $CD4^+$ and $CD4^+/CD8^+$ ratio were obviously increased, while the level of $CD8^+$ was evidently reduced. Levels of IL-2 and IFN-γ were remarkably increased, while levels of IL-4 and IL-10 were dramatically decreased. The possible reason is that RFA under ultrasound guidance kills tumor cells, effectively blocks the secretion of secretory factors, and alleviates the body's immune injury, thereby benefitting the self-repair of patients' immune function. At the same time, RFA can also correct the imbalance of Th1/Th2 cytokines.

Tumor markers include NSE, CYFRA21-1, CA-199, CA125 and CEA, which are secreted by or exfoliate from tumor cells and can be detected in the serum of patients, providing a basis for tumor diagnosis and treatment [17]. CEA is a kind of antigen in tumor tissues, which is used most widely in clinic [18]. NSE is a kind of

enzyme in neurons and neuroendocrine cells, which can not only serve as a marker for tumor diagnosis, but also judge the patient's prognosis sensitively [19]. CYFRA21-1 is consisted of two fragments of cytokeratin 19 in the cytoplasm of tumor cells. CA199 and CA125 are common in the diagnosis of hepatobiliary cancer, which present positive significance in evaluating the therapeutic effect on the tumor [20]. Our data demonstrated that at 3 months after treatment, serum CEA, CA199, CA125, NSE and CYFRA21-1 levels were significantly decreased, and the survival rate of patients with tumor diameter < 3 cm and single lesion was noticeably higher than that of patients with tumor diameter ≥ 3 cm and multiple lesions. We propose that RFA under ultrasound guidance with accurate positioning can effectively inhibit and kill liver cancer cells, and there are significant changes in tumor markers. However, the tumor in patients with a large tumor diameter and multiple lesions is adjacent to important organs and large vessels, so the actual effect on the tumor border cannot be precisely guaranteed in RFA. To avoid damage to adjacent organs, complete ablation is often difficult to be performed, only partial ablation can be performed instead, which may causes local recurrence after operation, and thus affects the survival time of patients. It is proposed that other therapeutic regimens should be selected for patients with a larger tumor diameter and multiple lesions after comprehensive evaluation.

Conclusion

In conclusion, our data indicate that RFA under ultrasound guidance can effectively improve the immune and liver functions of patients, reduce the levels of tumor markers, and increase the survival rate of patients with tumor diameter < 3 cm and single lesions in the treatment of liver cancer, which findings provide basis for the development of anti-liver cancer strategy in the future.

Disclosure of conflict of interest

None.

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