Original Article
Changes of Th1/Th2 cytokines in patients with primary hepatocellular carcinoma after ultrasound-guided ablation

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Abstract: Liver cancer is a malignancy of the digestive system and has a high morbidity and mortality rate. Local intervention has become a viable option in identifying liver treatment. The aim of the present study was to analyze the changes of Th1/Th2 cytokines in patients with primary hepatocellular carcinoma (HCC) after radiofrequency ablation (RFA) treatment. 26 patients with stage III-IV liver cancers and 25 healthy controls were selected to participate in the study. HCC patients were initiated with RFA treatment and the serum levels of alpha fetoprotein (AFP) and Th1/Th2 cytokines were valued. We found that with the level of AFP decreased, the levels of Th1 cytokines including interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were significantly increased after treatment with RFA ($P<0.05$). Meanwhile, the levels of Th2 cytokines consist of interleukin-4 (IL-4), IL-6 and IL-10 were decreased markedly on the contrary, and the differences were statistically significant ($P<0.05$). In conclusion, the levels of Th1/Th2 cytokines were correlated with the change of AFP in patients of HCC after treatment with RFA, which might be an important guiding significance for the prognosis of HCC.

Keywords: Radiofrequency ablation, hepatocellular carcinoma, Th1/Th2 cytokines, alpha fetoprotein, immunity

Introduction

Liver cancer consists of a wide range of different histologically primary liver cancers including bile duct cystadenocarcinoma, cholangiocarcinoma, haemangiosarcoma, hepatoblastoma and hepatocellular carcinoma (HCC) [1]. However, HCC, a type of hepatocyte epithelial tumor, is the most commonly seen cancer among of all these tumors, constituting 83% of all the incidences [2]. Furthermore, HCC has become one of the most widespread and lethal cancers, which posed as a worldwide public health issue [3]. Besides, approximately 6% of the existing human cancers are induced by HCC, which is the third most frequent mortality causing malignancy accounting for 85%-90% of primary liver cancers [4]. What is more, the occurrence of HCC cases have achieved over half a million worldwide annually, which makes it the fifth most widespread cancer globally [5, 6].

Although surgery has always been considered as the first therapeutic choice for treatment in HCC patients, difficulties of surgical resection may be related to the number of tumors, the size and site, vascular and extrahepatic involvement, as well as the poor liver function [7]. An effective and less invasive technique is needed for treatment of unresectable hepatic malignancies. In the past ten years, several kinds of local ablative techniques have been reported to be effective in HCC patients, which are considered for liver-directed therapies [8, 9]. In particular, radiofrequency ablation (RFA), a minimal invasiveness puncture treatment, has resulted in a higher rate of complete necrosis of metastatic lesions in the liver. In the clinical application, RFA has displayed a good survival and positive impacts on the clinical management of patients with liver metastasis [10].

Cytokines are potent immune-modulatory molecules and major players, which are produced
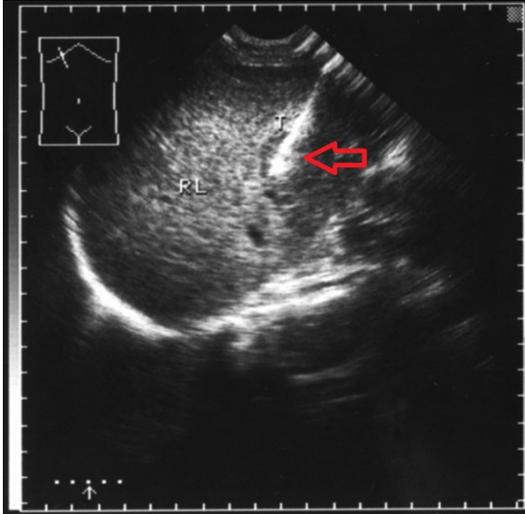
Figure 1. The process of radiofrequency ablation.

chiefly by immune/non-immune cells in physiological conditions, cytokines protect against viral infection via either analyzing the host response pattern or by inhibiting viral replication [11]. Cytokines are generally consisted of two groups: Th1 cytokines: IL-1, IL-2, IL-12 and non-ILs like TNF-α and IFN-γ [12, 13]. Th1 cytokines cause stimulation of virus-specific CD8-positive cytolytic T lymphocytes, leading to viral clearance. Th2 cytokines: IL-4, IL-6 and IL-10. These cytokines induce Th1 cytokines and stimulate activation/differentiation of B cells [14, 15]. The effects of cytokines are closely related to their circulating levels, especially in HCC, even they can be activated at very low concentrations. A present study reported that cytokines play a key determinant in regulating the immune response in HCC patients, which are quite relevant for cytokine gene polymorphisms and HCC. Hepatocellular carcinoma (HCC) is a commonly seen malignant tumor in China, and the state of Th1/Th2 in this condition is controversial [16].

Patients and methods

Patient selection and eligibility

Between April 2014 and October 2016, 26 patients diagnosed with stage III-IV (Union for International Cancer Control stages) liver cancer were selected. The study conformed to interventional treatment testimony. The inclusion criteria for the study were: i) Patients were mentally conscious; ii) All tumors were <5 cm in diameter and the total number of tumors was <4; and iii) It was the first time patients were treated using RFA; iiii) The immunosuppressive agents were not used in the last half year, and without immune system diseases. The exclusion criteria for the study were: i) Patients with hepatic metastasis; ii) Pregnant patients and those with serious coagulation mechanism disorders as well as those with medical histories of liver cancer resection and chemoradiotherapy and serious cachexia; iii) Patients who had <6 months survival expectancy; and iv) Patients who were intolerant to surgery and those with interrupted treatments, and missed follow ups.

This study included 15 male and 11 female HCC patients with liver cirrhosis (average age 49.7 years, range 37-69 years). All of the patients were diagnosed pathologically as having hepatocellular carcinoma (19 patients) or cholangiocarcinoma of the liver (7). The diameters of the tumors were 5-12 cm. The level of AFP was >600 ng/ml in 11 patients, <200 ng/ml in 4, and between 200 ng/ml and 600 ng/ml in 11. 25 healthy subjects were selected as control group (average age 53.6 years, range 42-73 years).

Treatment

RFA was performed using percutaneous puncture needling guided by ultrasound (Aloka SSD-1100 Color Doppler Ultrasound, Siemens AG). The cool-tip RF System (Valleylab, Boulder, CO, USA), uni-polar cold circulation system, mating electrode wire and plate electrode were used during the process. The radiofrequency power was started from 60 W each time, and it was gradually elevated until impedance effectively increased and power was automatically weakened. During RFA, output frequency was adjusted to 450 kHz, the maximum power was 150 W and the needle used was a 14 G trocar, and a total of 6-12 fine needle electrodes were set on top of the inner needles, which formed 5.0 cm spherical heat coagulation after stretching energization. The temperature was set at 100°C and the unit was set to 90 W for RFA actual burning power. The temperature was maintained at 100°C for 15 min. The position of the needle electrodes was adjusted according to the tumor size for repeated ablation, in order to cover the whole target tumor in the echo area, and the ablation area surpassed tumor lesions

Table 1. Expression of AFP and Th1/Th2 cytokines before RFA ($\bar{x} \pm s$)

Groups	n	AFP (µg/L)	Th1 (µmol/L)			Th2 (µmol/L)		
			IL-2	TNF-α	IFN-γ	IL-4	IL-6	IL-10
Normal health	25	5.29±1.08	35.74±4.98	5.14±1.58	149.64±12.07	57.69±14.38	7.86±1.23	4.15±0.89
HCC patients	26	704.33±76.91**	17.35±3.17*	0.73±0.43**	71.28±10.79*	132.47±13.59**	20.77±2.96*	13.24±0.98**

Notes: *$P<0.05$, **$P<0.01$, compared with Normal health group. IL-2, 4, 6, 10, Interleukin-2, 4, 6, 10; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon-γ.

Table 2. Expression of AFP and Th1/Th2 cytokines with AFP level decreased after RFA ($\bar{x} \pm s$)

Groups	n	AFP (µg/L)	Th1 (µmol/L)			Th2 (µmol/L)		
			IL-2	TNF-α	IFN-γ	IL-4	IL-6	IL-10
Normal health	25	5.29±1.08	35.74±4.98	5.14±1.58	149.64±12.07	57.69±14.38	7.86±1.23	4.15±0.89
HCC patients	21							
Before RFA		713.88±81.07**	16.98±3.64*	0.89±0.51**	76.94±11.77*	138.94±14.22**	21.07±3.14*	12.79±1.06**
15 days after RFA		27.68±1.14*,#	32.17±4.21#	4.97±1.27##	116.53±12.08#	67.94±10.23*,##	9.27±1.38#	6.07±0.89**,#
30 days after RFA		18.97±1.06*,#	33.59±4.67#	5.06±1.35##	137.66±14.07#	60.81±8.89##	8.65±1.15#	5.47±0.86##

Notes: *$P<0.05$, **$P<0.01$, compared with Normal health group; #$P<0.05$, ##$P<0.01$, compared with HCC patients before RFA group. IL-2, 4, 6, 10, Interleukin-2, 4, 6, 10; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon-γ.

by 0.5-1 cm. The scope of vaporization of strong echo covering all boundaries of the tumors was used to determine when to terminate the treatment. A few days after RFA, the response was assessed by performing 3-phase contrast-enhanced CT, with interpretation of the scans by consensus between 2 experienced radiologists (**Figure 1**).

Quantification of serum levels of cytokines

Patient peripheral blood was collected within one day before ablation and within 15 days and 30 days after ablation. The following cytokines: IL-2, TNF-α, IFN-γ and IL-4, IL-6, IL-10 in serum were assayed using the Quansys human 9-plex assay kit (Quansys Biosciences, Utah, USA), which was performed according to the manufacturer's instructions. Serum samples were 1:1.5 diluted and conducted in duplicate. Final concentration of each cytokines was calculated based on standard curves by using the Q-view software (Quansys Biosciences, Utah, USA).

Statistical analysis

SPSS 18.0 software (IBM SPSS, Armonk, NY, USA) was used for statistical analyses. Measurement data were presented by mean ± standard deviation. The statistical comparisons of the AFP and Th1/Th2 cytokines levels were performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A *P*-value <0.05 was considered statistically significant.

Results

None of the cases were converted to open surgery. The median follow-up period for all patients was 1 month. Before RFA, the cytokines levels of Th1, including IL-2, TNF-α, IFN-γ were significantly down-regulated, compared with normal health (*P*<0.05). While, the cytokines levels of Th2, including IL-4, IL-6 and IL-10 were markedly up-regulated in comparison with normal health (*P*<0.05) (**Table 1**). During follow-up, cytokines were observed in 26 patients, and there were 21 cases in which AFP level had significantly declined. The cytokines levels of Th1, including IL-2, TNF-α, IFN-γ were significantly up-regulated along with the decline of AFP level (*P*<0.05). In contrast, the cytokines levels of Th2, including IL-4, IL-6 and IL-10 were markedly down-regulated at the same time (*P*<0.05) (**Table 2**). Besides, there were 4 cases in which AFP level had significantly risen. The cytokines levels of Th1, including IL-2, TNF-α, IFN-γ were significantly down-regulated along with the risen of AFP level (*P*<0.05). In contrast, the cytokines levels of Th2, including IL-4, IL-6 and IL-10 were markedly up-regulated on the contrary (*P*<0.05) (**Table 3**).

Discussion

There are many advantages of ultrasound guidance technology compared with traditional surgical treatment, such as real-time monitoring, negligible trauma, accurate guidance, effectiveness and safety, easy operation and repeatabil-

Table 3. Expressions of AFP and Th1/Th2 cytokines with AFP level increased after RFA ($\bar{x}$ ±s)

Groups	n	AFP (µg/L)	Th1 (µmol/L)			Th2 (µmol/L)		
			IL-2	TNF-α	IFN-γ	IL-4	IL-6	IL-10
Normal health	25	5.29±1.08	35.74±4.98	5.14±1.58	149.64±12.07	57.69±14.38	7.86±1.23	4.15±0.89
HCC patients	4							
Before RFA		726.91±94.27**	17.06±2.99*	0.92±0.37**	81.27±9.63*	144.99±15.08**	23.94±4.15*	14.76±2.07**
15 days after RFA		923.49±100.32**,#	17.38±3.64*	1.06±0.42**	78.44±11.19*	159.62±16.17*,#	29.18±5.27*,#	18.37±2.56**,#
30 days after RFA		1105.38±99.14**,#	16.59±2.49*	0.94±0.53**	75.92±8.75*	179.94,±14.86*,#	37.14±3.78*,##	25.63±1.99**,#

Notes: *$P<0.05$, **$P<0.01$, compared with Normal health group; #$P<0.05$, ##$P<0.01$, compared with HCC patients before RFA group. IL-2, 4, 6, 10, Interleukin-2, 4, 6, 10; TNF-α, tumor necrosis factor-α; IFN-γ, Interferon-γ.

ity, which have led to be considered as one of the three major types of treatment for liver cancer together with surgery and regional vascular intervention [17, 18]. It aims to treat tumors or cytoreduction, reducing clinical symptoms, improving life quality and even extending survival time. The mechanism of ultrasound-guided local interstitial thermal ablation method is consist of conducting energy into the tumor to inactivate tumor cells in situ, and it includes radiofrequency (RFA), laser, microwave, focused ultrasound and refrigeration [19]. Rossi et al [20] were the first to find that RFA has comparable clinical results compared to PEI in treating small hepatocellular carcinoma. Besides, RFA can also be used as a supplementary method for the treatment of large liver tumors and for those patients that cannot tolerate surgery or those unwilling as well [21].

RFA treatment of primary hepatocellular carcinoma (HCC) has been confirmed efficiency. Research reported that the immune function could be enhanced of HCC patients after RFA treatment [22]. T-helper cells play a vital role in tumor immunity. T-helper cells are classified into 2 types: Th1 and Th2. TNF-α, IFN-γ and IL-2 are produced by Th1 cells, which can promote T-cell-mediated immune responses. IL-4, IL-6 and IL-10, produced by Th2 cells, inhibit Th1 cytokine release and promote immunoglobulin production [23]. Accordingly, the predominant expression of Th1 cytokines is important for anti-tumor immunity. Among Th1 cytokines, IL-2 can inhibit the invasive and metastatic potential of hepatocellular carcinoma cells. Increased serum levels of Th2 cytokines have been associated with poor prognosis in patients with primary tumors [24]. In normal physiological conditions, Th1- and Th2-type cytokines keep a stable balance. In the past few years, it has been revealed that Th2 cytokines might be activated predominantly in patients with HCC [25,

26]. Research showed the same result in pancreatic cancer, colorectal cancer, gastric cancer, urinary system cancer, malignant melanoma, Hodgkin lymphoma and glioblastoma. The underline mechanism might be that tumors escape the immune response, because cell immunity dominates in anti-tumor immunity, and the shift towards Th2 can depress this antitumor action [27, 28]. Cao et al [29] studied cytokine protein expression in mononuclear cells, and they found that Th1-type cytokines were expressed predominantly in stage I hepatic carcinoma patients with hepatic cirrhosis in 31 cases of HCC patients. But the elevated IL-10 concentrations in serum suggested a shift from a Th1 to a Th2 cytokine profile [30].

In the present study, we found that the cytokines levels of Th1, including IL-2, TNF-α, IFN-γ were significantly down-regulated, while the cytokines levels of Th2, including IL-4, IL-6 and IL-10 were markedly up-regulated before RFA, which indicates that the immune function was suppressed in HCC patients. After RFA, the shift towards Th1 was more evident along with the serum level of AFP increased, and the ratio of Th1/Th2 was markedly suppressed as well. In contrast, the cytokines levels of Th1 were effectively up-regulated and cytokines in Th2 were significantly down-regulated along with the serum level of AFP decreased, which indicates that treatment with radiofrequency ablation could increase the expression of Th1 cytokines, promoting the transform of cytokines form Th2 to Th1. Potential mechanisms may be related to the followings: 1) Malignant tumor cells could be killed directly by radiofrequency ablation and the immunologic function could be relieved with the disappearance of cancer cells. 2) Heat shock protein could be activated by radiofrequency ablation treatment, which mediates tumor antigen presentation and the activation of T lymphocytes.

Researches on HCC Th1/Th2 cytokine profiles are controversial. The cytokine network may be different in the pathogenesis of HCC because the liver is an immune preferential organ. In brief, the mechanism of the Th1/Th2 shift in HCC is not clear and needs further study.

Disclosure of conflict of interest

None.

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