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Effect of high−intensity focused ultrasound (HIFU) combined with radiotherapy on tumor malignancy in patients with advanced pancreatic cancer and evaluation of side effects

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ABSTRACT

Objective: To study the effect of high-intensity focused ultrasound (HIFU) combined with radiotherapy on tumor malignancy in patients with advanced pancreatic cancer and the corresponding side effects. **Methods:** A total of 84 patients with advanced pancreatic cancer treated in our hospital between May 2013 and March 2016 were selected and randomly divided into HIFU group and IGRT group, HIFU group accepted high-intensity focused ultrasound combined with radiotherapy and IGRT group received radiotherapy alone. 4 weeks after treatment, the levels of tumor markers, liver and kidney function indexes, perineural invasion-related molecules and cytokines in serum as well as the levels of immune cells in peripheral blood were determined. **Results:** 4 weeks after treatment, serum CA199, CA242, OPN, NGAL, RBP4, NGF, TrkA, p75, BDNF and TrkB levels of HIFU group were significantly lower than those of IGRT group, serum IL-2, TNF-α, IFN-γ and IL-13 levels as well as peripheral blood NKT cell and CD4+T cell levels were significantly higher than those of IGRT group, and serum ALT, AST, Cr and BUN levels were not significantly different from those of IGRT group. **Conclusion:** HIFU combined with radiotherapy treatment of advanced pancreatic cancer can more effectively kill cancer cells, inhibit pancreatic cancer cell invasion to the peripheral nerve and enhance the antitumor immune response mediated by NKT cells and CD4+T cells.

1. Introduction

Pancreatic cancer is one of malignant tumors of digestive system with the high malignant degree and poor prognosis, and the average survival is less than 6 months after diagnosis. The current preferred way for clinical treatment of pancreatic cancer is still surgical resection, but the clinical symptoms and signs of pancreatic cancer are relatively hidden, so the early diagnosis is difficult and the majority of patients has developed to the advanced stage at the time of diagnosis and cannot receive surgical resection[1]. Relevant epidemiological data show that the surgical resection rate of pancreatic cancer is less than 20%. In clinical practice, radiotherapy and chemotherapy is the major means to treat advanced pancreatic cancer, but the overall effect is not ideal[2,3]. High-intensity focused ultrasound (HIFU) is a new minimally invasive treatment for malignant tumors developed in recent years, which focuses the high-intensity ultrasonic wave on the local tumor, produces instantaneous high temperature in the focused area, and cause coagulation necrosis of the target tissue[4]. Domestic scholars have reported that HIFU treatment of advanced pancreatic cancer can prolong survival time and relieve cancerous pain, but the molecular-level changes in patients with pancreatic cancer after HIFU treatment are still not clear[5]. In the following study, the effect of high-intensity focused ultrasound (HIFU) combined with radiotherapy on tumor malignancy in patients with advanced pancreatic cancer and the corresponding side effects were analyzed.

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2. Subjects and methods

2.1 Research subjects

A total of 84 patients with advanced pancreatic cancer treated in our hospital between May 2013 and March 2016 were selected as research subjects, all patients were diagnosed with pancreatic cancer after pathological biopsy, they were at III-IV and couldn't receive surgical, and the expected survival time was >3 months. Random number table was used to divide the included patients into HIFU group and IGRT group, 42 cases in each group. HIFU group accepted high-intensity focused ultrasound combined with image-guided radiotherapy, 29 cases were male and 13 cases were female, they were 50-65 years old, 31 cases were at clinical III stage and 11 cases were at IV stage; IGRT group received image-guided radiotherapy alone, 27 cases were male and 15 cases were female, they were 51-65 years old, 28 cases were at clinical III stage and 14 cases were at IV stage. The two groups of patients were not significantly different in general information (P>0.05).

2.2 Treatment methods

Both groups of patients accepted image-guided radiotherapy (IGRT) according to the following methods: the patients took supine position and received CT location scan, CT images were referred to sketch the low-density pancreatic lesions, peripheral accented edges and enlarged lymph node as GTV, the area externally expanding by 0.5 cm on the basis of GTV was the CTV, the area externally expanding by 1cm on the basis of CTV was the PTV, PTV was used as standard for radiotherapy, 95% isodose curve should cover the PTV, and total dose of radiotherapy was 50-60 Gy, 1.8-2.0 Gy/time, five times a week. HIFU group received high-intensity focused ultrasound therapy on the basis of IGRT, and the method was as follows: external ultrasonic probe was used to determine the tumor location, then built-in ultrasonic probe was guided by laser positioning for treatment, pulse time was 0.15-0.2 s, the time interval was 0.3-0.4 s, number of single transmitter was 8-16, the probe was moved with the distance of 3 mm/layer from the tumor edge layer by layer to make the focused ultrasound pulse cover the entire tumor lesions, and the treatment was conducted once every Monday, Wednesday and Friday for a total of 4 weeks.

2.3 Serum index detection methods

4 weeks after treatment, 5 mL of peripheral blood sample was collected from two groups of patients and centrifuged to separate serum, then electrochemiluminescence kits were used to detect CA199 and CA242 content, enzyme-linked immunosorbent assay kits were used to determine OPN, NGAL, RBP4, NGF, TrkA, p75, BDNF, TrkB, IL-2, TNF-α, IFN-γ and IL-13 content, and automatic biochemical analyzer was used to detect the content of ALT, AST, Cr and BUN.

2.4 Peripheral blood index detection methods

4 weeks after treatment, 5 mL of peripheral blood sample was collected from two groups of patients, one tube of blood was used to incubate monoclonal antibody of CD3, Vα and Vβ, the other tube of blood was used to incubate monoclonal antibody of CD3 and CD4, permeabilization reagent was added after 15 min, the blood continued to be incubated for 10 min and washed with PBS buffer twice, and flow cytometer was used to determine the content of CD3+Vα+Vβ+ cells (NKT cells) and CD3+CD4+T cells.

2.5 Statistical methods

SPSS 19.0 software was used to input the detected data, measurement data between two groups was by t test and P<0.05 indicated statistical significance in the differences.

3. Results

3.1 Serum tumor marker levels

4 weeks after treatment, analysis of serum tumor markers CA199, CA242, OPN, NGAL and RBP4 between two groups of patients was as follows: serum CA199, CA242, OPN, NGAL and RBP4 levels of HIFU group were significantly lower than those of IGRT group. Differences in serum CA199, CA242, OPN, NGAL and RBP4 levels were statistically significant between two groups of patients 4 weeks

Table 1.

Comparison of serum tumor marker levels between two groups of patients after treatment.

Groups	n	CA199 (U/mL)	CA242 (U/mL)	OPN (ng/mL)	NGAL (ng/mL)	RBP4 (ng/mL)
HIFU	42	28.89±5.67	9.38±1.03	29.15±3.67	39.63±5.56	64.27±7.91
IGRT	42	51.32±7.82	15.63±2.10	43.59±6.72	55.25±7.29	98.34±10.36
T		9.238	7.758	7.139	7.348	8.301
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

Table 2.

Comparison of serum liver and kidney function indexes between two groups of patients after treatment.

Groups	*n*	Liver function (U/L)		Kidney function	
		ALT	AST	Cr (μmol/L)	BUN (mmol/L)
HIFU	42	42.62±6.51	47.26±7.15	93.51±10.25	8.35±0.93
IGRT	42	43.31±5.98	48.03±6.79	95.13±10.77	8.61±0.98
T		0.395	0.412	0.682	0.228
P		＞0.05	＞0.05	＞0.05	＞0.05

Table 3.

Comparison of perineural invasion-related molecules between two groups of patients after treatment (ng/mL).

Groups	*n*	NGF	TrkA	p75	BDNF	TrkB
HIFU	42	6.39±0.83	2.48±0.34	3.14±0.41	8.32±0.94	4.28±0.55
IGRT	42	11.32±1.48	5.57±0.79	5.93±0.84	17.53±2.04	8.15±0.92
T		9.498	11.348	8.137	12.038	9.228
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

Table 4.

Comparison of peripheral blood immune cell and serum cytokine levels between two groups of patients after treatment.

Groups	*n*	Immune cells		Cytokines			
		NKT cell	CD4+T cell	IL-2	TNF- α	IFN- γ	IL-12
HIFU	42	9.38±1.02	39.52±5.52	17.65±2.23	38.76±5.25	25.36±3.36	9.48±1.03
IGRT	42	5.63±0.78	27.65±3.41	9.38±1.03	21.32±2.84	11.38±1.74	4.42±0.55
T		8.937	6.584	9.228	7.658	12.492	11.038
P		＜0.05	＜0.05	＜0.05	＜0.05	＜0.05	＜0.05

after treatment (P<0.05).

3.2 Serum liver and kidney function index levels

4 weeks after treatment, analysis of serum liver and kidney function indexes ALT, AST, Cr and BUN between two groups of patients was as follows: serum ALT, AST, Cr and BUN levels of HIFU group were not significantly different from those of IGRT group. Differences in serum ALT, AST, Cr and BUN levels were not statistically significant between two groups of patients 4 weeks after treatment (P>0.05).

3.3 Serum perineural invasion–related molecule levels

4 weeks after treatment, analysis of serum perineural invasion-related molecules NGF, TrkA, p75, BDNF and TrkB between two groups of patients was as follows: serum NGF, TrkA, p75, BDNF and TrkB levels of HIFU group were significantly lower than those of IGRT group. Differences in serum NGF, TrkA, p75, BDNF and TrkB levels were statistically significant between two groups of patients 4 weeks after treatment (P<0.05).

3.4 Peripheral blood immune cell and serum cytokine levels

4 weeks after treatment, analysis of peripheral blood immune cells NKT cells and CD4+T cells as well as cytokines IL-2, TNF-α, IFN-γ and IL-13 between two groups of patients was as follows: peripheral blood NKT cell and CD4+T cell levels as well as serum IL-2, TNF-α, IFN-γ and IL-13 levels of HIFU group were significantly higher than those of IGRT group. Differences in peripheral blood NKT cell and CD4+T cell levels as well as serum IL-2, TNF-α, IFN-γ and IL-13 levels were statistically significant between two groups of patients 4 weeks after treatment (P<0.05).

4. Discussion

Advanced pancreatic cancer is a difficulty of clinical treatment, patients with pancreatic cancer are with low early diagnostic rate, so most patients have developed to middle-advanced stage at the time of diagnosis and are with poor prognosis, and for patients with advanced pancreatic cancer who are unable to receive surgical resection, in particular, the average survival time is less than 3 months after diagnosis. Radiotherapy is a routine treatment for patients with advanced pancreatic cancer, IGRT technology can make the radioactive rays highly concentrate in the lesions and strengthen the killing effect of radioactive rays on malignant tumors. However, the structures around pancreatic tissue are complex, and radiotherapy will inevitably cause the surrounding tissue damage and affect the effect of radiotherapy. HIFU is a new minimally invasive treatment for malignant tumors developed in recent years, and it can make the high-intensity ultrasound wave focus on the lesions and

generate instantaneous high temperature, and then kill cancer cells through the thermal effect, mechanical effect and immune effect[6,7]. In addition, the thermal effect produced by HIFU treatment can also enhance the killing effect of radiotherapy on cancer cells and reduce the surrounding tissue damage caused by radiotherapy[8,9].

In recent years, domestic research has reported that HIFU treatment of advanced pancreatic cancer can prolong survival time and relieve the degree of pain, but the molecular-level changes in patients with advanced pancreatic cancer after HIFU treatment are not clear[10]. In order to specify the killing effect of HIFU on pancreatic cancer cells, the levels of serum tumor markers were analyzed at first in the study, and the results showed that serum CA199, CA242, OPN, NGAL and RBP4 levels of HIFU group were significantly lower than those of IGRT group. This means that HIFU combined with radiotherapy can more effectively kill pancreatic cancer cells and reduce the content of serum tumor markers. On this basis, the side effect extent of HIFU was further evaluated, the target organs most commonly involved by the adverse reactions of radiotherapy and chemotherapy include the liver and kidney, and analysis of serum liver and kidney function indexes showed that serum ALT, AST, Cr and BUN levels were not significantly different between HIFU group and IGRT group. This means that HIFU treatment has extremely precise targeting and can ensure that the treatment focus is within the lesions and will not increase the damage degree of surrounding tissue.

In the development of pancreatic cancer, perineural invasion is a striking feature of advanced pancreatic cancer, it is characterized by cancer cell growth along the nerve or growth within the nerve sheath, and it can cause persistent cancerous pain in patients[11]. Nerve growth factor and its receptor play a crucial role in the process of perineural invasion of pancreatic cancer, and two nerve growth factors NGF and BDNF have been confirmed to participate in the process of pancreatic cancer cell invasion to the peripheral nerve. Receptors corresponding to NGF include high-affinity tyrosine kinase receptor TrkA and low-affinity tumor necrosis factor receptor p75, and the main receptor corresponding to BDNF is tyrosine kinase receptor TrkB[12,13]. NGF and BDNF secreted by cancer cells can be combined with the corresponding receptors on nerve cells and then cause cancer cell adhesion, migration and invasion to the nerve cells and promote the development of perineural invasion of pancreatic cancer. In the study, analysis of serum levels of these perineural invasion-related molecules showed that serum NGF, TrkA, p75, BDNF and TrkB levels of HIFU group were significantly lower than those of IGRT group. This means that HIFU combined with radiotherapy can restrain the pancreatic cancer cell invasion to the peripheral nerves and relieve the pain caused by perineural invasion in patients with advanced pancreatic cancer.

Study about HIFU in recent years has shown that the treatment not only has killing effect on cancer cells, but can also adjust the immune response and enhance antitumor immune response. In the occurrence and development of pancreatic cancer, immune escape is an important mechanism causing cancer cell proliferation and invasion, and enhancing the immune response is conducive to killing cancer cells[14]. T lymphocyte is the important cell mass mediating antitumor immune responses in the body, and the NKT cells and CD4+T cells play an important role in antitumor immune response[15]. IFN- γ , IL-13 and other cytokines secreted by NKT cells as well as the IL-2, TNF- α and other cytokines secreted by CD4+T lymphocytes can mediate cellular immune response and generate cytotoxic effect[16,17]. Analysis of the effect of HIFU treatment on the immune function of patients with advanced pancreatic cancer in the study showed that peripheral blood NKT cell and CD4+T cell levels as well as serum IL-2, TNF- α , IFN- γ and IL-13 levels of HIFU group were significantly higher than those of IGRT group. This means that HIFU combined with radiotherapy can enhance the antitumor immune response mediated by NKT cells and CD4+T cells in patients with advanced pancreatic cancer.

To sum up, HIFU combined with radiotherapy can more effectively kill cancer cells in patients with advanced pancreatic cancer, and it can also inhibit pancreatic cancer cell invasion to the peripheral nerve and enhance the antitumor immune response mediated by NKT cells and CD4+T cells.

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