

ORIGINAL ARTICLE

Clinical significance of changes in AFP, HTATIP2/TIP30, B7-H4 and inflammatory cytokines after transcatheter arterial chemoembolization

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Summary

Purpose: To explore the clinical significance of changes in alpha-fetoprotein (AFP), HIV-1 TAT interactive protein 2/TAT interactive protein 30 (HTATIP2/TIP30), B7-H4 and inflammatory cytokines after transcatheter arterial chemoembolization (TACE).

Methods: A total of 84 hepatocellular carcinoma (HCC) patients admitted to the Department of Hepatobiliary Surgery and the Department of Interventional Radiology of our hospital from January 1, 2017 to December 31, 2018 were randomly enrolled and divided into an experimental group and a control group according to treatment methods. The expression levels of AFP mRNA, HTATIP2/TIP30, B7-H4 and inflammatory cytokines were detected before and after treatment, the short-term efficacy was followed up and analyzed, and the correlation between the two was statistically analyzed.

Results: The AFP expression level in the two groups of patients was lower after treatment than before treatment, this reduction being more obvious in the experimental group (receiving TACE) than in the control group. Although the levels of serum HTATIP2/TIP30 and B7-H4 were decreased after treatment in both groups, and they were lower after treatment than those before treatment in the control group, lower levels were registered in the control group. Both groups of

patients had lower expression levels of tumor necrosis factor-alpha (TNF- α) and interleukin 6 (IL-6) after treatment compared with those before treatment, this decrease being more significant in the experimental group than in the control group. Moreover, the total short-term efficacy rate and the improvement rate of the quality of life were higher in the experimental group than in the control group, although no statistical difference in the survival rate was found between the two groups after 1-year follow-up. The serum level of B7-H4 in the group with good efficacy was lower than in the group with poor efficacy before treatment, and it declined in both groups after treatment, with a lower level in the former than in the latter. Furthermore, the group with good efficacy had a lower level of serum HTATIP2/TIP30 than the group with poor efficacy, while both groups had a decreased level after treatment, with a lower level in the former than in the latter.

Conclusion: Interventional therapy for primary HCC has good short-term efficacy. It can reduce the levels of serum HTATIP2/TIP30, B7-H4, AFP and inflammation-related indexes, improve the liver function and the patients' quality of life.

Key words: transcatheter arterial chemoembolization, AFP, HTATIP2/TIP30, B7-H4, TNF- α , IL-6, prognosis

Introduction

Hepatocellular carcinoma (HCC) is the third major cause of cancer-associated death worldwide [1]. Some patients with early HCC are asymptomatic.

Patients with early HCC undergoing radical resection had a high survival rate, while those with advanced HCC had poor prognosis [2]. There-

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Received: 11/07/2019; Accepted: 25/08/2019

fore, detecting and monitoring HCC in the early stage are essential for a successful clinical treatment. Alpha-fetoprotein (AFP) is regarded as an indicator with great significance for the screening and early diagnosis of HCC. According to previous studies, the AFP level is above 400 ng/mL in 60% of HCC patients but it may be below 20 ng/mL in over 50% of HCC patients, so AFP should not be the only screening indicator for HCC [3].

As there are not enough effective methods for early detection, patients with HCC often progress rapidly after being diagnosed in advanced stage despite treatment [4]. In addition, most primary HCC (PHC) patients have a history of hepatitis/cirrhosis. The appearance of obvious clinical symptoms indicates that the disease is already in the middle or advanced stage, when tumors are inoperable [5]. As treatment methods have developed in recent years, transcatheter arterial chemoembolization (TACE) has become one of the most mature, extensive and accurate methods of interventional therapy for HCC [6]. It has been shown that HIV-1 TAT interactive protein, 2/TAT interactive protein 30 (HTATIP2/TIP30) and B7-H4 are expressed in HCC tissues and are closely associated with tumor development [7,8]. Chronic liver inflammation induced by exposure to infectious factors (mainly hepatitis B virus) is regarded as the main risk factor for HCC progression (including carcinogenesis as well as tumor growth and progression), and it may be linked with the continuous, non-specific and inefficient activation of the immune system. The inflammatory microenvironment of HCC consists of immune cells and inflammatory cytokines. Cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin 6 (IL-6) exert crucial effects on the occurrence and development of liver cancer [9,10].

Therefore, this study investigated the short-term efficacy of TACE on HTATIP2/TIP30, B7-H4 and inflammatory cytokines in HCC so as to provide a theoretical basis for clinical treatment.

Methods

Clinical data

Primary HCC patients hospitalized in our hospital from January 1, 2017 to December 31, 2018 were included in the study. The patients were diagnosed according to the clinical diagnosis and staging criteria for primary HCC.

These patients were divided into an experimental group (n=42) and a control group (n=42). The experimental group consisted of 35 males and 7 females aged 33-72 years old, with an average age of 49.32 \pm 6.43 years. The lesions were in the right lobe in 21 cases, in the left lobe

in 12 cases and in both lobes in 9 cases. In the control group, there were 36 males and 6 females aged 31-72 years old, with a mean age of 49.83 \pm 6.35 years. The lesions were in the right lobe in 26 cases, in the left lobe in 11 cases and in both lobes in 5 cases.

Inclusion and exclusion criteria

Inclusion criteria: HCC criteria were good mental state, expected survival time over 3 months and a signed informed consent.

Exclusion criteria: hepatic artery or vein occlusion, metastatic lesions outside the liver that needed liver transplantation, PHC patients diagnosed with cholangiocarcinoma, severe abnormalities in the lung, kidney or heart, mental illness, or contraindications to interventional therapy.

This study was approved by the Ethics Review Committee of our hospital, and all patients involved in this study signed a written informed consent.

Treatment methods

Control group

Patients received radiofrequency ablation treatment. The patients were placed in supine position. After local anesthesia, the puncture site was selected by preoperative imaging, and then the puncture needle was guided by ultrasound to penetrate into the liver tumor focus. By adjusting the power and frequency, the extremely cold radiofrequency therapeutic apparatus was used for sequential radiofrequency therapy.

Experimental group

Patients were treated with TACE. The patients were placed in supine position and disinfected routinely. Following local anesthesia, femoral artery puncture was completed using the modified Seldinger technique, a 5F catheter was implanted, and a 4.1F hepatic catheter was used for hepatic arteriography. X-ray pictures of the diaphragmatic artery, left gastric artery and superior mesenteric artery were taken when necessary to determine the tumor number, location, size and thrombus in the portal or hepatic vein or blood supply vessel. If necessary, a 3F microcatheter was implanted to inject chemotherapeutic drugs, and 10-20 mg epirubicin or a mixed solution of 0.25-0.5 g 5 FU and super-liquid iodized oil was applied to block the target blood vessel of the tumor. Then, local small portal veins stopped their development, or iodized oil deposition of within tumor lesions became dense.

Patients in both groups received liver protection, water supplementation, antiemetics and gastric protection.

Evaluation criteria for short-term efficacy

Complete remission: Tumor lesions disappeared and no new lesions occurred.

Partial remission: The total diameter of the liver tumor base was reduced by \geq 30%.

Stable disease: The total diameter of the liver tumor base decreased but did not qualify for partial remission.

Progressive disease: The diameter of liver tumor BAS increased $\geq 20\%$, or there were one or more new lesions.

Detection of indexes

Before and after treatment, 5 mL of peripheral venous blood was collected and centrifuged at a radius of 15 cm and 3000 r/min for 10 min, and serum was separated. Samples were stored at -20°C for detection. HTATIP2/TIP30 kits (Shanghai Yinggong Biotech Co., Ltd., Shanghai, China) and B7-H4 kits (Xitang Biotech Co., Ltd., Shanghai, China) were utilized for strict detection of serum HTATIP2/TIP30 and B7-H4 according to the instructions of a Hitachi 7600 automatic biochemical analyzer. The liver function indexes in the two groups were observed before and after treatment. Alanine aminotransferase (ALT) and serum total bilirubin (TBIL) kits were used to detect ALT and TBIL in the above samples in strict accordance with the instructions. The improvement of the quality of life in the two groups was observed using the Karnofsky performance status (KPS) scale: score improvement ≥ 10 points is defined as improvement of the quality of life, score change < 10 points is defined as stable quality of life, and score decrease ≥ 10 points represents decreased quality of life. The 1-year recurrence and survival rates were observed in the two groups.

These experiments were repeated three times.

Statistics

Experimental data were analyzed by SPSS 22.0 (IBM, Armonk, NY, USA). Measurement data were assessed using the t-test. The data in the same group were compared with the paired sample t-test, while those between two groups were compared using the independent-samples t-test. Survival curves were plotted according to Kaplan-Meier method and log-rank test was utilized to compare survival between groups. $P < 0.05$ indicated that the difference was statistically significant.

Results

Changes in AFP in the two groups of patients before and after treatment

AFP levels were measured in the control and experimental groups before and after treatment. The results revealed that the AFP expression level in the two groups of patients was lower after treatment than before treatment ($p < 0.05$), this decrease being more obvious in the experimental group treated with TACE than in the control group (Table 1).

Changes in HTATIP2/TIP30 and B7-H4 in the two groups of patients before and after treatment

As shown in Table 2, there were no statistical differences in the levels of serum HTATIP2/TIP30 and B7-H4 between the two groups before treatment ($t = 0.7947, 0.2848, p > 0.05$). After treatment, the levels of serum HTATIP2/TIP30 and B7-H4 were reduced in both groups (control group: $t = 17.1838, 18.9795$, experimental group: $t = 8.3787, 10.6393, p < 0.05$). The levels of serum HTATIP2/TIP30 and B7-H4 were lower after treatment than before treatment in the control group ($t = 12.2975, 10.5361, p < 0.05$).

Changes in inflammatory cytokines in the two groups of patients before and after treatment

The levels of inflammatory cytokines TNF- α and IL-6 were detected in the two groups of patients before and after treatment. It was found that the expression levels of TNF- α and IL-6 in the two groups of patients were lower after treatment than before treatment ($p < 0.05$), this descending trend being sharper in the experimental group than in the control group (Figure 1).

Table 1. AFP levels in the control and experimental groups before and after treatment (mean \pm SD)

Group	AFP level (ng/mL)				p
	Before treatment	At 3 d after treatment	At 1 week after treatment	At 4 weeks after treatment	
Experimental group	19.90 \pm 3.32	17.7 \pm 9.6	13.41 \pm 4.71	1.05 \pm 2.51	0.023
Control group	21.01 \pm 2.82	12.7 \pm 6.6	9.41 \pm 3.63	3.05 \pm 1.55	0.033
p	0.423	0.032	0.011	0.001	

Table 2. Changes in HTATIP2/TIP30 and B7-H4 in the two groups of patients before and after treatment (mean \pm SD)

Group	HTATIP2/TIP30 (ng/mL)			B7-H4 (ng/mL)		
	Before treatment	At 4 weeks after treatment	p	Before treatment	At 4 weeks after treatment	p
Control group	7.53 \pm 1.60	4.89 \pm 3.14	0.032	57.53 \pm 3.60	41.54 \pm 5.60	0.011
Experimental group	8.73 \pm 1.23	3.73 \pm 2.22	0.042	59.22 \pm 3.60	47.54 \pm 2.70	0.021
p	0.321	0.031		0.061	0.046	

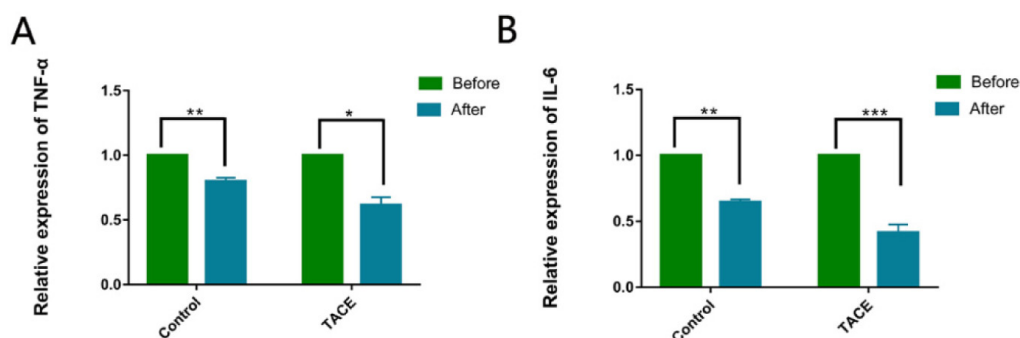


Figure 1. Changes in inflammatory cytokines in the two groups of patients before and after treatment: **A:** Changes in the expression levels of TNF- α in the two groups of patients before and after treatment: The expression levels of TNF- α declined in the two groups of patients before and after treatment ($p > 0.05$). **B:** Changes in the expression levels of IL-6 in the two groups of patients before and after treatment: The expression level of IL-6 was lower in the two groups of patients after treatment than before treatment ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3. Comparison of efficacy between the two groups of patients

Group	n	Complete remission n (%)	Partial remission n (%)	Stable disease n (%)	Progressive disease n (%)	Total effective rate n (%)
Experimental group	42	10 (32.26)	12 (38.71)	4 (12.90)	5 (16.13)	22 (70.97)
Control group	42	4 (12.90)	8 (25.81)	10 (23.26)	9 (29.03)	12 (38.71)
t						6.2342
p						<0.05

Table 4. Comparison of the quality of life between the two groups of patients

Group	n	Increase n (%)	Stable n (%)	Decrease n (%)
Experimental group	42	31 (80.65)	8 (12.90)	3 (6.45)
Control group	42	23 (54.84)	11 (25.81)	8 (19.35)
χ^2	-	4.7238	1.6533	1.2917
p	-	<0.05	>0.05	>0.05

Short-term efficacy of in the two groups of patients

Analysis results of short-term efficacy in the two groups (Table 3) showed that the total short-term effective rate was higher in the experimental group (70.97%) than in the control group (38.71%) ($p < 0.05$). The experimental group had a higher improvement rate of the quality of life (80.65%) than the control group (54.84%) ($p < 0.05$) (Table 4), and no statistical difference in the survival rate was found between the two groups after 1-year follow-up ($p > 0.05$) (Table 5).

Comparisons of the levels of serum B7-H4 and HTATIP2/TIP30 between groups with different TACE efficacy in liver cancer and between those before and after treatment

Further analysis of changes in indexes between groups with different TACE efficacy revealed that the serum B7-H4 level was lower in the group with

Table 5. Comparison of the survival rate between the two groups of patients after 1-year follow-up

Group	n	Survival rate n (%)
Control group	42	31 (80.65)
Experimental group	42	25 (67.74)
χ^2		1.3478
p		>0.05

a good efficacy than in the group with poor efficacy before treatment ($p < 0.01$), being decreased in both groups after treatment, with a lower level in the former than in the latter. Furthermore, the group with good efficacy had a lower level of serum HTATIP2/TIP30 than the group with poor efficacy, while both groups had a decreased level after treatment, with a lower level in the former than in the latter (Table 6).

Table 6. Levels of serum B7-H4 and HTATIP2/TIP30 between groups with different efficacy of TACE before and after treatment (mean±SD)

Group		n	B7-H4 (ng/mL)	HTATIP2/TIP30 (ng/mL)
Good efficacy	Before treatment	29	45.33±4.22	7.19±2.44
	At 4 weeks after treatment		36.03±2.99 ^{#*}	4.94±3.33 [#]
Poor efficacy	Before treatment	13	58.14±4.04	7.12±1.10
	At 4 weeks after treatment		4.94±3.33 [#]	5.81±1.11 [#]

[#]p<0.01 vs. the same group before treatment, *p<0.01 vs. group with poor efficacy

Discussion

PHC development is usually due to cirrhosis or progressive liver disease, so it has a poor prognosis. Surgical resection and liver transplantation are specific therapies for PHC [11]. Due to increased difficulty level and risks of the procedure, including transplant recipient status, donor sources, perioperative recovery, surgical difficulties and long-term anti-rejection treatment after operation, liver transplantation has not been extensively used as HCC treatment [12]. Although surgical resection is the most extensively utilized method, its outcome depends on the patient's general condition, tumor location, tumor size and liver function reserve [13]. TACE, a minimally invasive surgery, has the advantages of fewer complications, good efficacy, less damage, less pain, low cost and practicality. It is able to enhance the immune function of the body and prolong survival, thus providing opportunities for HCC patients who do not meet the criteria for surgical resection. In recent years, interventional therapy for HCC has attracted increasingly more attention, being regarded as the optimal surgical method for HCC. Studies have proved the clear short-term efficacy of TACE. The findings of this study revealed that the total short-term effective rate was higher in the experimental group than in the control group, indicating that TACE has a good short-term efficacy. Besides, the experimental group had lower ALT and TBIL levels than the control group after treatment, suggesting that TACE can improve liver function. The improvement rate of the quality of life was higher in the experimental group than in the control group, which implies that TACE has the ability to improve the quality of life.

HTATIP2/TIP30 is a binding protein in the process of HIV transcription *in vitro*, with a molecular weight of 30000 daltons. HTATIP2/TIP30, as a co-factor of HIV transcription, exhibits an extensive expression in some tumor tissues. In addition, HTATIP2/TIP30 can interact with angiogenesis inhibitors and tyrosine kinase inhibitors in cell apoptosis and cell cycle. In particular, it exerts crucial effects in the regulation of cell apoptosis

and metastasis [14,15]. Moreover, HTATIP2/TIP30 is capable of binding to DNA or RNA polymerase II in order to adjust cell proliferation and apoptosis as well as angiogenesis-related genes, thus stimulating tumor metastasis [16]. As a novel member of the B7 family, B7-H4 shows a high expression in different human tumors and has close association with tumor development. Additionally, it has been confirmed by some researchers that B7-H4 exists in serum in a soluble form [17]. B7-H4 is reported to regulate T cell-mediated immune responses and expressions in HCC by suppressing cytokine secretion and T cell proliferation, which is related to tumor progression. B7-H4 is derived from mesenchymal cells in the tumor microenvironment or tumor tissues. After TACE treatment, apoptosis and necrosis occur in tumor cells, tumor microenvironment is improved, and B7-H4 secretion is reduced, which explains why serum B7-H4 shows a downward trend [18,19]. This study discovered that the levels of serum HTATIP2/TIP30 and B7-H4 were lower in the experimental group than in the control group. Therefore, interventional therapy for HCC can remarkably decrease the levels of serum HTATIP2/TIP30 and B7-H4.

The influence of inflammatory cytokines on the progression of HCC has been widely researched. Studies illustrated that TNF- α can accelerate the death of tumor cells being related to the progression and prognosis of HCC [20]. IL-6, IL-1 and IL-12 are also common inflammatory cytokines in the inflammatory microenvironment of HCC. A high level of serum IL-6 is considered to be a risk factor for HCC, at the same time being associated with disease prognosis. According to multiple studies, an increased level of serum IL-8 is related to the occurrence of HCC. Moreover, IL-2 is also found to be a common inflammatory cytokine in the inflammatory microenvironment of HCC [21,22]. T cell factors play pivotal roles in liver function as well as in the tumor characteristics and prognosis of liver cancer patients. IL-6 is one of the most vital predictors of short OS in TACE patients, and its level has a good immune effect on the prognosis of HCC. This study highlighted the underlying

function of various cytokines as HCC biomarkers. In the upcoming immunotherapy era, the need for various cytokine biomarkers will increase.

Conclusions

In conclusion, the results of this study proved that interventional therapy for PHC has good short-term efficacy, as it can reduce the levels of serum HTATIP2/TIP30 and B7-H4, enhance liver function, improve the quality of life, and prolong survival time, thus having relatively high research and ap-

plication value. However, the study has some limitations such as small sample size, short observation time and lower observation frequency. Hence, a multi-center clinical study on a large sample size needs to be performed in the future, so as to provide a reliable basis for clinical interventional therapy for HCC.

Conflict of interests

The authors declare no conflict of interests.

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