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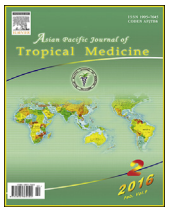

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Association of CT perfusion imaging with plasma levels of TGF-β1 and VEGF in patients with NSCLC

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ABSTRACT

Objective: To study the association of CT perfusion imaging parameters with plasma level of transforming growth factor-β1 (TGF-β1) and vascular endothelial growth (VEGF) in patients with non small cell lung cancer (NSCLC).

Methods: A total of 67 patients with NSCLC (NSCLC group) and 64 patients with benign lesion (control group) were given with CT perfusion imaging to obtain blood flow, blood volume, mean transit time, time to peal and permeability surface through CT perfusion software. The plasma levels of TGF-β1 and VEGF were tested by ELISA. The relationship between plasma levels of TGF-β1, VEGF and CT perfusion imaging parameters were analyzed.

Results: CT perfusion imaging parameters and the plasma levels of TGF-β1 and VEGF of NSCLC group were significantly higher than the control group ($P < 0.05$), while CT perfusion parameters and the levels of TGF-β1 and VEGF in NSCLC group showed significant difference in different tumor node metastasis stages ($P < 0.05$). Correlation analysis showed that the level of plasma TGF-β1 and VEGF were positively correlated with blood flow, blood volume, and mean transit time ($P < 0.05$), and negatively correlated with time to peal ($P < 0.05$). There was no significant correlation between TGF-β1 and VEGF with the permeability surface.

Conclusions: CT perfusion imaging parameters in patients with NSCLC is closely associated with plasma TGF-β1, VEGF and its biological characteristics. CT perfusion imaging is a convenient method to detect tumor blood perfusion.

1. Introduction

Nowadays, the morbidity and mortality of lung cancer is increasing year by year. Eighty percentage of patients with lung cancer have non small cell lung cancer (NSCLC) [1]. Angiopoiesis affects the occurrence and development of tumor. It can slow down growth and can terminate metastasis if angiopoiesis of new vessels is blocked. Transforming growth factor-β (TGF-β1) and vascular endothelial growth (VEGF) play important roles in regulation of angiopoiesis [2]. Multi-slice spiral computed tomography perfusion imaging is a mature functional imaging, and has been widely used in observing density and distribution of tumor vessels, evaluating association with tumor metastasis related genes. In this study, we observed CT perfusion imaging parameters, including blood flow (BF), blood volume (BV), mean transit time (MTT), time to peal (TTP) and permeability surface (PS), analyzed the relationship between these parameters with TGF-β1 and VEGF, to discuss the valued of these parameter in evaluating NSCLC.

2. Materials and methods

2.1. Objective

A total of 67 cases diagnosed as NSCLC by puncture or post-operation pathological diagnosis were selected as NSCLC group, who were admitted during January 2010 to January 2015. Another 64 cases with benign lesion were selected as control group. There were 40 males and 27 females in NSCLC group, aged 33–78 years old, with average age as (50.8 ± 10.5) years. There were 36 cases with squamous carcinoma, 23 cases with adenocarcinoma, and 8 cases with adenoid squamous cell carcinoma. Among 64 cases in control group, there were 37 cases with tuberculosis focus, 13 cases with pulmonary inflammatory pseudotumor, 9 cases with silicosis nodule, and 5 cases with

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lung infection. The general data such as age, gender, *etc* were comparable in both groups.

2.2. Inclusion and exclusion criteria

Inclusion criteria: ①Patients were diagnosed as NSCLC by bronchofibroscopy biopsy, needle biopsy or post-operation pathological diagnosis, and had no chemoradiotherapy or anti-tumor drug; ②Patients were able to have CT perfusion scanning.

Exclusion criteria: ①Sensitive to iodinated contrast agent; ②Breath-holding time were shorter than 25 s, or artifact appeared due to various reasons; ③Patients with serious dysfunction of heart, liver or kidney, or with infection diseases; ④Patients were diagnosed as small cell lung cancer by pathological examination.

2.3. CT perfusion imaging

Patients underwent CT plain scanning to identify lesion location. With the maximum lay of tumor as the center, dynamic scanning was performed in multi-level continuous dynamic movie scan model. The condition was as follows: 120 kV, 50 mA, detector width was 24 mm × 1.2 mm; coverage area of Z-axis was 40 mm; thickness of reconstruction was 5 mm; average interval time was 1.0 s; rescanned 5 s later; 320 pieces of imagings were obtained after continuous collection for 40 s. Patlak method and maximum-slope method were used to calculate parameters, including BF, BV, MTT, TTP, PS, *etc*.

2.4. Collection and detection of blood

2.4.1. Collection of blood

Five mL elbow venous blood were extracted at 8:00 am for fasting subjects in both groups. Anti-freezing was carried out immediately with ethylenediaminetetraacetic acid. It was centrifugated at 3600 r/min for 10 min. Supernatant was obtained by pipettor, and preserved at −80 °C.

2.4.2. Detection of TGF-β1 and VEGF

Enzyme linked immunosorbent assay was used to detect content of TGF-β1 and VEGF. The assays were provided by Aikang Biotechnology Company (Hangzhou, China). The process was in accord with instruction. Two holes were detected for every sample, and the average was calculated.

2.5. Statistical analysis

The data was processed by SPSS19.0. *t* Test was used in comparison between groups. Pearson correlation analysis was used to analyze the correlation between parameters with TGF-β1 and VEGF.

3. Results

3.1. Parameters of two groups

Table 1 showed BF (mL·100 mL^{-1}·min^{-1}), BV (mL/1000 mL), MTT (s), TTP (s) and PS (mL·100 mL^{-1}·min^{-1}) were significantly higher in NSCLC group ($P < 0.05$).

3.2. TGF-β1 and VEGF levels

Table 2 showed that TGF-β1 and VEGF levels of NSCLC group were significantly higher than those of control group ($P < 0.01$).

3.3. Parameters, TGF-β1 and VEGF levels of NSCLC patients at different stage

All 67 NSCLC patients were divided into IA–IIA stage group ($n = 23$, Group 1), IIB–IIIA stage group ($n = 27$, Group 2), IIIB–IV stage group ($n = 17$, Group 3) according to clinical and CT examination result. CT perfusion parameters, TGF-β1 and VEGF levels were observed. There were significant differences in parameters, TGF-β1 and VEGF levels between group 1 and group 2 ($P < 0.05$), and the differences were also significant between group 1 and group 3 ($P < 0.05$). However, the difference was not significant between group 2 and group 3 ($P > 0.05$) (Table 3).

3.4. Correlation between parameter with TGF-β1 and VEGF

Pearson linear correlation analysis showed that the level of plasma TGF-β1 and VEGF were positively correlated with BF, BV, and MTT ($P < 0.05$), and negatively correlated with TTP ($P < 0.05$). There was no significant correlation between TGF-β1 and VEGF with the PS (Table 4).

Table 1
Comparison of parameters between two groups.

CT perfusion imaging parameters	NSCLC group ($n = 67$)	Control group ($n = 64$)	*t* Value	*P* value
BF	61.51 ± 12.23	36.65 ± 8.51	13.445	0.000
BV	11.15 ± 2.37	7.02 ± 1.25	12.390	0.000
MTT	8.69 ± 2.03	6.87 ± 1.35	6.013	0.000
TTP	20.45 ± 4.17	16.75 ± 3.51	5.511	0.000
PS	18.38 ± 5.15	14.39 ± 4.89	4.543	0.000

Table 2
TGF-β1 and VEGF levels (pg/mL).

Indexes	NSCLC group ($n = 67$)	Control group ($n = 64$)	*t* Value	*P* value
TGF-β1	647.29 ± 71.27	443.32 ± 55.31	18.240	0.000
VEGF	187.23 ± 31.20	110.52 ± 28.24	14.731	0.000

Table 3
Parameters, TGF-β1 and VEGF levels of NSCLC patients at different stage.

Indexes	Group 1 ($n = 23$)	Group 2 ($n = 27$)	Group 3 ($n = 17$)
BF	58.23 ± 10.62	65.42 ± 12.11*	63.17 ± 12.23*
BV	7.42 ± 3.02	11.49 ± 3.56*	12.82 ± 3.58*
MTT	7.04 ± 2.54	9.90 ± 3.47*	10.12 ± 3.51*
TTP	16.75 ± 3.85	21.85 ± 4.68*	22.32 ± 5.05*
PS	14.83 ± 4.62	19.57 ± 5.72*	21.18 ± 5.65*
TGF-β1	532.10 ± 45.33	696.39 ± 64.28*	688.91 ± 60.24*
VEGF	142.53 ± 15.73	202.54 ± 22.47*	197.56 ± 21.72*

BF: mL·100 mL^{-1}·min^{-1}; BV: mL/1000 mL; MTT: s; TTP: s; PS: mL·100 mL^{-1}·min^{-1}; TGF-β1: pg/mL; VEGF: pg/mL. Compared with group 1, *$P < 0.05$.

Table 4
Correlation between parameter with TGF-β1 and VEGF.

Parameters	TGF-β1		VEGF	
	γ	*P*	γ	*P*
BF	0.57	0.000	0.69	0.001
BV	0.48	0.000	0.56	0.000
MTT	0.31	0.019	0.45	0.004
TTP	−0.53	0.015	−0.62	0.012
PS	0.54	0.220	0.78	0.091

4. Discussion

NSCLC is the commonest type of lung tumors. The complicated biological processes such as occurrence, development, metastasis *etc* are associated with angiopoiesis. Angiopoiesis of tumor vessels includes angiopoiesis induced by tumor cells and intra-tumor microcirculation, which is achieved by angiopoiesis and vasculogenesis. Vasculogenesis refers to differentiation of mesoblastema into haemangioblast, which is predecessor of endothelial cell, and constitute embryo primitive vascular system. In this stage, the growth of tumor is slow, and shows no invasiveness, with few metastases [3]. Angiopoiesis refers to growth of existed capillary or of new capillary in postcapillary venule. It can obtain various nutritions for growth, and is liable to infiltration and metastasis. Therefore, it is easy for tumor to growth in area with rich new vessels and sufficient blood supply [4].

TGF-β super family-mediated signal path can regulate the occurrence, development, metastasis of tumors. It can stimulate the growth of vessels. There are three types of TGF-β for mammal animals, and the content of TGF-β1 is the highest. TGF-β1 is representative. It can stimulate vessel growth directly or indirectly, which may be correlated with angiopoiesis and development [5]. It is reported that malignant tumor cells can secret plenty of cytokines during tumor development, which lead to rapid proliferation of malignant tumor, and higher expression of TGF-β1 [6]. Besides, VEGF is a glycoprotein with many functions. It can stimulate proliferation and migration of vascular endothelial cell. And it is also the strongest vascular permeability agent. It can lead to pathological angiopoiesis, including increasing mitosis and migration of vascular endothelial cell [7,8].

CT perfusion imaging is a non-invasive method to evaluate growth of microvessels in tumor tissues, then to reflect physiological and metabolic changes via detecting various parameters. Besides, it can also indicate clinical change characters of tumors before any change in size occurs or just when tiny change occurs. Tacelli *et al.* found that APA detected by dynamic contrast-enhance spiral CT is positively correlated with VEGF expression of adenocarcinoma. BF, BV, MTT, TTP and PS can reflect microcirculation characters from various aspects [9]. This study showed that the level of plasma TGF-β1 and VEGF were positively correlated with BF, BV, and MTT, and negatively correlated with TTP. It indicates that TGF-β1 and VEGF expressions are increased, new vessels are increased and circulation is also increased. From macroscopic aspect, CT imaging is characterized as increased blood flow in per unit volume of tumor tissue per unit time (BF), increased blood volume in per unit volume of tumor tissue (BV), and delayed time for contrast agent passing tissue capillary [10]. However, PS which indicates permeability surface and integrity of capillary, shows no significant correlation with TGF-β1 or VEGF. Tumor node metastasis staging reflects tumor progress degree. The higher the stage is, the severer the progress is, the wider the affected tissue. This progress is closely correlated with angiopoiesis. Our study showed the difference in parameters between IIB–IIIA and IIIB–IV is more significant than IA–IIA. This trend is consistent with TGF-β1 and VEGF levels in plasma. It indicates that CT perfusion imaging not only can be used in diagnosis and differentiation of benign and malignant tumors, but also can be used in NSCLC staging and prognosis.

In conclusion, a plenty of angiogenesis occurs during tumor development. It can lead to increased TGF-β1 and VEGF levels. CT perfusion imaging can reflect density and structure of microvascular system from imaging aspect. The combination of both is valuable in diagnosis, differentiation, treatment and prognosis.

Conflict of interest statement

The authors have no conflicts of interest.

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