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Original Article

Study of the prognosis of esophageal cancer based on microvascular model density and imaging parameters

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Abstract: With increasing incidence by years, esophageal carcinoma has now required next-generation of treatment approaches. Currently anti-angiogenesis treatment has drawn much research interest for malignant tumors. However, the single parameter of micro-vascular density may not fully reflect the formation of tumor vessels in esophagus cancer. Therefore more experimental approaches are required to detect morphological changes of micro-vessels and their angiogenesis. This study therefore investigated the prognosis of esophagus cancer by the combination of microvascular pattern, density and imaging. A total of 148 esophagus cancer patients were recruited for 64-row spiral CT before and after the surgery. Immunohistochemistry (IHC) method was employed to detect microvascular density (MVD) and expression of D34/CD105 monoclonal antibody in vascular endothelial cells. Correlation between CT perfusion parameter and MVD indexes with malignancy of tumors was analyzed. CD34 and CD105 expressions were marginally elevated in malignant tumor then benign tumor but without statistical significance ($p>0.05$). Perfusion indexes including BF, BV, and PS of malignant tumors were significantly higher than benign ones ($p<0.05$). MTT index, however, had no significant difference ($p>0.05$). Spearman analysis revealed positive correlation between BF, PS, BV, MTT and MVD. CT perfusion index can quantitatively analyze the perfusion status of tumor blood flow. MVD and CD105 can specifically target novel endothelial cells of tumors and have potency in both treatment and diagnosis of esophageal cancer.

Keywords: Esophageal cancer, CT perfusion parameters, microvascular density, CD34, CD105

Introduction

Esophageal carcinoma is quite common in China, and is the leading cause of tumor incidence and mortality in certain regions. Surveys showed that the incidence of esophageal carcinoma has been increased by 7-fold in the past 50 years, making it the most rapidly increasing malignant tumor in China, over any other epithelial cell derived cancer. Normally esophageal carcinoma has an insidious onset and is usually at terminal stage with distal metastasis at the time of diagnosis [1-3]. Currently surgical resection is still the primary choice of treatment, including endoscopy and heating-fusion method, both of which had higher safety and efficiency, but are only available in those patients with less lymph node metastasis or tumor invasion. As esophageal cancer is susceptible to focal infiltration and nerve invasion, the penetration across blood vessel wall and blood-borne metastasis lead to unfavorable prognosis, although with relatively longer tumor-bearing survival time [4, 5]. The growth of tumor is dependent on angiogenesis, during which microvascular density (MVD) can work as the effective marker evaluating blood vessel formation for further reflecting the malignancy of cancer. Little has been known, however, regarding the correlation between microvascular pattern and tumor progression, along with other high risk factors such as intravascular tumor thrombosis and focal infiltration, in addition to the prognosis. Imaging technique has now become the major approach for diagnosing esophageal cancer, as it can provide direct evidenced judging the nature and metastasis of lesion. CT perfusion imaging can also reflect the hemodynamic change of tissues, thus benefiting tumor angiogenesis [6-8]. Traditional approaches, however, cannot satisfy the need of timely and precise diagnosis of tumors. Currently anti-angiogenesis intervention has become one research focus for tumor treatment.

However, few reports had been made regarding the microvascular pattern of tumors. Due to the atypical distribution of tumor vessels, most studies only calculate the density, which, however, may not fully reflect the property of hemodynamics of tumors. A comprehensive analysis including imaging data, pathological examination and observation during surgery, along with post-op follow-ups were required but were still lacked [9]. This study thus collected information regarding preoperative imaging by CT perfusion, prognostic data and pathological records of tumor tissue samples, and investigated the evolution of blood supply and microvascular generation of esophageal carcinoma by observing the pattern of micro-vessels and examining MVD of by various endothelial markers, in order to provide evidences for studying the prognostic evaluation of esophageal cancer.

Materials and methods

Clinical information

A total of 148 esophageal cancer patients (87 males and 61 females, aging between 39 and 74 years old, average age = 55.3±10.7 years) who were diagnosed with esophageal cancer from January 2014 to January 2016 in Yanting Cancer Hospital (Mianyang, Sichuan, China) were recruited in this study. According to WHO diagnostic criteria [10], there were 40, 35, 39 and 34 cases of stage I, II, III and IV cancers. Based on pathological types, there were 51 adenoma, 50 squamous carcinoma, and 47 other tumors. All patients were randomly divided into control and experiment group (N = 74 each).

The study protocol was approved by the Research Ethics Committee of Yanting Cancer Hospital (Mianyang, Sichuan, China), and all patients gave their informed consent before study commencement.

Treatment

All patients were primarily diagnosed with cancer and received surgeries in Affiliated Hospital of Weifang Medical College. Among those there were 85 cases using surgery only, and 63 cases receiving other complimentary treatment such as radiotherapy. A total of 12 cases received microwave treatment. Ten patients had the lymph node clearance of upper chest, and 5 of them had lymph node clearance plus partial resection of sternum. In all 101 cases of upper esophageal tumor, 38 of them had complete removal of esophagus, and 34 of them had expanded resection. In 20 patients with lower esophageal tumor, 8 of them had the removal of esophagus, and 8 patients underwent combined radical surgery, plus 2 of them received lymph node clearance and 2 cases of esophagus resection plus lymph node clearance. In all 63 patients having radiotherapy, opposite radiation fields were applied in chest-neck region. A total of 24 of them had radical chest-neck radiation and 15 of them had preventive radiation. The radiation dose of focal lymph node is between 50 Gy and 70 Gy. The dose of primary lesion was 30~70 Gy. In all 68 patients having recurrence, there were 32 cases of surgical resection, among those 15 patients also received radiotherapy.

IHC staining

Paraffin-based tissues sections were de-waxed and re-hydrated. After incubation in 3% H_2O_2-methanol for 10 minutes to quench endogenous activity of peroxidase, slices were firstly rinsed in 0.01 M PBS for 5 minutes, and were processed by antigen-retrieval buffer. 10% normal goat serum (NGS) was used to block non-specific binding sites. Primary antibody was added for 4°C overnight incubation. On the next day, biotin-labelled anti-IgG secondary antibody was added after rinsing in PBS. Horseradish peroxidase (HRP)-labelled streptavidin working solution was added for 30-minute incubation at 37°C. DAB chromogenic substrate was then added for 5-minutes development. The reaction was stopped by rinsing in tap water. Slices were counter-stained, dehydrated and mounted with coverslips.

CT perfusion examination

Normal CT scan was firstly performed to locate the center of tumors. The area of interest was selected. Contrast reagent (300 mg/mL iodixanol) was introduced into elbow vein (4 mL/s, 50 mL total, with 5~15 sec lag time). After 50-sec persistent scan, perfusion scan was performed using lesion maximal layer as the center, with 5 mm thickness for 8 layers. Consecutive dynamic scan for 50 times were carried out. Results were analyzed by Perfusion 3 software on AW4.22 work station (GE, US) to obtain CT perfusion references including BF, BV, PS and MTT.

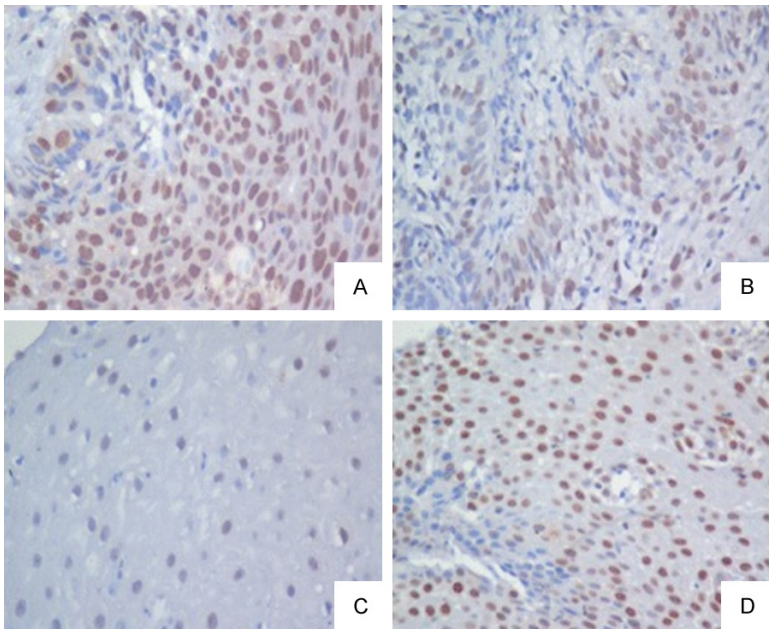

Figure 1. Expression of CD34 and CD105 in esophageal carcinoma (× 200). A and B. CD34; C and D. CD105. A and C. Malignant tumor; B and D. Benign tumor.

Table 1. Expression of CD34 and CD105

Group	N	MVD (Cell number per 100 × field, as $\bar{x}$ ± s)	
		CD34	CD105
Malignant tumor	72	24.8±10.3	16.8±8.2
Benign tumor	76	30.6±11.1	21.6±8.4
t value		1.236	1.365
P value		0.243	0.184

Intervention and measures

Pathologists were recruited to confirm tissue slices. IHC staining for CD31 and CD105 was performed using paraffin-based tissue block to label MVD and observe the growth pattern of microvessels. A 64-row spiral CT perfusion scan was performed before surgery. In brief, Normal CT scan was firstly performed to locate the center of tumors. The area of interest was selected. Contrast reagent (300 mg/mL iodixanol) was introduced into elbow vein (4 mL/s, 50 mL total, with 5~15 sec lag time). After 50-sec persistent scan, perfusion scan was performed using lesion maximal layer as the center, with 5 mm thickness for 8 layers. Consecutive dynamic scan for 50 times were carried out. Results were analyzed by Perfusion 3 software on AW4.22 work station (GE, US) to obtain CT perfusion references including blood volume (BV), blood flow (BF), mean time of contrast (MTT), permeability surface (PS) and potentiated height (PH).

Information collection

This study has obtained written consent from all patients and their families. The optimal surgical plan was designed, with complete recording of procedures. MVD with different labels were recorded to collect microvascular parameters under CT imaging. Meanwhile clinical information such as treatment plan of each patient was collected. Post-operative follow-up was performed with full documentation.

Statistical analysis

SPSS 18.0 software package was used to process all collected data. Student t-test was used to analyze the differential CT perfusion index and pathological parameters. Multiple Chi-square test was employed to observe the differential microvascular pattern and pathological indexes. The comparison of MVD between CD105 and CD31 labels was carried in t-test. Spearman analysis was recruited to analyze the correlation between CT perfusion parameter BF, BV, PS, MTT and MVD. A statistical significance was defined when $p<0.05$.

Results

Expression of CD34 and CD105 in esophagus cancer

The expression level of CD34 and CD105 was higher in tumor group compared to control ones, whereas, no significant differences were observed between two groups ($p>0.05$, **Figure 1** and **Table 1**).

Perfusion indexes of tumors

Perfusion indexes including BF (211.3±19.7), BV (28.1±5.9) and PS (30.6±5.1) were significantly higher in malignant tumors compared to

Table 2. Perfusion index of benign tumors and malignant tumors

Index	Malignant	Benign	t value	*P* value
BF (ml × 100 g^{-1} × min^{-1})	211.3±19.7	144.0±27.2	8.215	0.000
BV (ml × 100 g^{-1})	28.1±5.9	16.3±5.5	6.945	0.000
MTT (s)	12.8±1.3	12.0±6.3	0.097	0.975
PS (ml × 100 g^{-1} × min^{-1})	30.6±5.1	25.1±4.8	2.964	0.001

Table 3. MVD comparison and perfusion indexes

Index	CD34 labelled MVD rs	*P* value	CD105 labelled MVD rs	*P* value
BF	0.63	0.01	0.75	0.01
BV	0.53	0.02	0.63	0.01
MTT	0.47	0.03	0.52	0.02
PS	0.15	0.36	0.16	0.35

benign tumors (144.0±27.2 for BF; 16.3±5.5 for BV and 25.1±4.8 for PS) ($p<0.05$, **Table 2**) while the MTT value had no significant difference between two groups (12.8±1.3 for Malignant tumors and 12.0±6.3 for Benign tumors) ($p>0.05$, **Table 2**).

MVD comparison with CD34 or CD105 labels

Spearman analysis was used to analyze the correlation between tumor perfusion indexes and MVD. Results showed positive correlation between BF, BV, MTT, and MVD, as shown in **Table 3**.

Discussion

Esophageal carcinoma is one common chest tumor and is also the most popular cancer in digestive tract. With a five-year survival rate at 20%, it has a high frequence 3 of local infiltration or neural invasion, both of which require the angiogenesis of micro vessels [11]. Recently the incidence of esophagus cancer is increased by years. Although the survival rate of patient has been improved with advancement of treatment approach, the tumor reoccurrence and metastasis are still major reason causing mortality [12]. Significant progresses have been obtained regarding the study of tumor genetics, early diagnosis, novel surgical procedure and radio-/chemo-therapy, but still cannot remarkably suppress either incidence or mortality rate of esophagus cancer, suggesting the lack of major achievement in this field [13-15]. As one common malignant tumor, esophagus cancer severely affects people health. Therefore, the finding of early biological marker and sensitive diagnostic indicator for metastasis and/or reoccurrence of esophagus cancer is critical for effectively managing esophagus cancer, improving prognosis and increasing survival rate. Therefore future study should focus on the early screening and diagnosis of esophagus cancer [16].

Classical diagnosis and treatment methods cannot satisfy the requirement of patients. Currently the anti-angiogenesis treatment has drawn increasing research interests. The study of tumor microvascular pattern, however, has been sparsely reported. Due to the irregular distribution of tumor micro-vessels, most studies only calculated the density of micro-vessels. However, only density value cannot fully describe the property of micro-vessels, which need the combined evaluation based on imaging, pathological and surgical observations. This study thus provided important evidences for pre-operative evaluation of tumor property, for surgical treatment and for evaluation of diagnostic efficiency [17, 18]. Imaging results were collected from preoperative esophageal cancer patients, who had complete pathological information and follow-up records. By observing the pattern of micro-vessels, measuring MVD with different endothelial markers, the dynamic change of blood supply of esophageal cancer and angiogenesis of novel micro-vessels was investigated, in order to provide further information for prognostic evaluation.

As one necessary factor during angiogenesis, CD105 function is closely correlated with TGF and its receptor, and can act as agonist or antagonist in the formation of vessels. It can facilitate the proliferation of endothelial cells for angiogenesis. Study has found the correlation between CD105 and the prognosis and multiple malignant tumors, and its critical role in the early diagnosis of tumors. CD34 is one of factors with over-expression in vascular endothelial cells at atherosclerosis. This factor can specifically target tumor tissues with high density [19, 20]. This study found elevated but not statistically significant expression of CD34 and CD105 in malignant tumor tissues compared to benign ones. Further large-scale study is thus required. Therefore the single index of MOCD cannot completely represent the angiogenesis of esophagus cancer. The 64-row CT has inherent advantage reflecting tumor angio-

genesis, thus improving accuracy of diagnosis. CT perfusion scan can better reflect hemodynamic of focal tissues. These results show significantly elevated perfusion indexes including BF, BV, and PS than benign group. MTT index, however, had no statistical significance, probably due to higher viscosity of blood and irregular tube for tumor, both of which lead to increased friction. Therefore, compared to benign tumor, malignant tumor had high blood volume and hyper-tension. The correlation analysis revealed positive correlation between MVD label and BF, PS, BV, and MTT. This study focused the on the dysfunction of angiogenesis, structural abnormality or incompletes, plus the co-existence of higher permeability and artery-vein short circuits, leading to the bias of blood supply.

In summary, a larger sample study is required for labelling MVD. CT perfusion imaging technology has certain values for diagnosis and confirming pathological subtype of esophagus carcinoma. The CT perfusion imaging can reflect angiogenesis inside tumors. For example, there were significantly differences regarding CT perfusion index. MVD is positively correlated with perfusion indexes. The study of microvascular pattern can provide evidences for esophagus cancer treatment, and can provide new diagnostic criteria via the evaluating imaging results and screening prognostic factors.

Disclosure of conflict of interest

None.

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