

# DW-MRI for esophageal squamous cell carcinoma, correlations between ADC values with histologic differentiation and VEGF expression: A retrospective study

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**Abstract.** The aim of the present study was to assess the correlations between diffusion-weighted magnetic resonance imaging (DW-MRI) features with the histologic differentiation and the expression of vascular endothelial growth factor (VEGF) in esophageal squamous cell carcinoma (ESCC). A total of 52 patients with ESCC included in the present study received radiotherapy, and all patients underwent contrast enhanced MRI and DW-MRI prior to and following radiotherapy. The diffusion sensitivity coefficient (*b* value) was set as 800 s/mm<sup>2</sup>. Apparent diffusion coefficient (ADC) values were automatically computed. VEGF expression was evaluated by immunohistochemical staining. The results demonstrated that the pathological grading of ESCC was positively correlated with ADC values ( $r=0.635$ ,  $P=0.0007$ ), and the VEGF expression was inversely correlated with ADC values ( $r=-0.321$ ,  $P=0.008$ ). However, no correlation was identified between the pathological grading and the VEGF expression ( $r=0.178$ ,  $P=0.284$ ). All patients were categorized as complete response (CR) or partial response (PR) and the ADC values were increased significantly following radiotherapy. The mean ADC values in the CR group were higher than the PR group prior to radiotherapy ( $t=5.156$ ,  $P=0.0004$ ). Therefore, we concluded that the DWI with ADC value measurement may represent the grade of tumor histologic differentiation and the degree of VEGF expression, and may also serve as a useful marker to predict radiotherapy and anti-VEGF response in ESCC. ADC value may be a substitution for assessing tumor

angiogenesis and novel prognostic factor and contribute to the treatment of ESCC.

## Introduction

Esophageal squamous cell carcinoma (ESCC) is a frequently occurring malignant tumor of the digestive system (1). Radiotherapy is the principal treatment for advanced ESCC (2). Clinically, enhancement computed tomography (CT), contrast enhanced magnetic resonance imaging (MRI) and barium meal is the main methods for evaluating the lesion and therapeutic effects (3). However, these examinations are judged by anatomical morphological changes, and lack the ability to display the microscopic pathology and physiological status of the tumor (4).

Diffusion-weighted magnetic resonance imaging (DW-MRI) can noninvasively observe the movement of water molecules in living tissues and provide pathological and physiological changes *in vivo*, which can be used to diagnose and evaluate the therapeutic effect at the cellular level in the early stage of disease (5,6). Tumor cells have been demonstrated to proliferate vigorously and be arranged tightly; therefore, the movement of water molecules decreases sharply inside and outside cells (7). Consequently, the apparent diffusion coefficient (ADC) value of tumor tissue was lower than that of normal tissue, and the signal of DW-MRI was markedly higher than that of normal tissue, and the diffusion was limited (8). DW-MRI is a useful supplement to conventional imaging evaluation, and has recently been used in radiotherapy target delineation and efficacy evaluation of esophageal cancer (9).

Tumor growth, metastasis and invasion require numerous new blood vessels to provide nutrients and discharge metabolic waste (10). Vascular endothelial growth factor (VEGF) can promote the proliferation and migration of vascular endothelial cells, and is one of the factors that affect the angiogenesis of tumors (11). Jiang *et al* (11) demonstrated that VEGF protein expression was significantly greater in esophageal cancer than normal epithelial tissue. In addition, VEGF protein expression of the high microvessel density (MVD) group was significantly lower than in the low MVD group with relation to clinical pathological

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staging, differentiation and lymph node metastasis (12). VEGF might make a valuable index of recurrence and treatment of tumor in clinic, and also a useful marker to predict anti-VEGF treatment response, VEGF is a leading factor of tumor angiogenesis, the anti-angiogenesis therapy aimed at VEGF has probably provided a new chance to malignant tumor treatment (13).

In the present retrospective study, samples collected from 52 patients with pathologically proven ESCC from the First Affiliated Hospital of China Medical University were analyzed. DW-MRI was used to measure the ADC value of tumors, and the correlations between ADC value and VEGF expression and pathological grade of ESCC were observed. To explore the value of DW-MRI in the metabolism, proliferation, efficacy evaluation and prognosis evaluation of ESCC, so as to guide clinical treatment.

## Patients and methods

**Patient selection.** From March 2014 to January 2016, a total of 52 patients with pathologically proven ESCC from the First Affiliated Hospital of China Medical University were collected. The set of standard for: Random selection, cases perfect follow-up, all patients were at an advanced stage of ESCC, which was confirmed by pathology and clinically, No radiotherapy, chemotherapy and interventional therapy were performed prior MRI examination, No contraindications for MRI examination. The patients were aged between 47-80 years, with a median age of 65 years, and the cohort comprised of 32 males and 20 females. There were 10 well-differentiated cancers, 28 moderately differentiated ones and 14 poorly differentiated ones (14). All patients received standard intensity modulated radiation therapy. The above cases were confirmed by the ethics committee of the First Affiliated Hospital of China Medical University who also provided study approval and patients provided written, informed consent.

**MRI device and scanning sequence and parameters.** All MRIs were performed by a 3.0T system (GE Medical System, Signa Excite HD) with 8-channel phased-array coil and GE ADW4.4 workstation. MRI examinations were performed prior to and following radiotherapy for 4-6 weeks. The MRI protocols included the T1-weighted (T1W), T2-weighted (T2W), and DWI. All patients were imaged in supine headfirst position. Sagittal T2W SE sequence: Repetition time (TR): 1750 ms, echo time (TE): 98.0 ms, field of view (FOV): 340 mm section thickness: 4 mm, space: 1 mm, matrix: 512x512 were performed. Axial T2W sequences with fat suppression: TR: 7500 ms, TE: 102 ms, FOV: 380 mm, section thickness: 4 mm, space: 1 mm, matrix: 512x384 were performed. Axial T1W GR sequences: TR: 230 ms, TE: 3.15 ms, FOV: 400 mm, section thickness: 4 mm, space: 1 mm, matrix: 512x512 were performed. Axial DWI was performed with single-shot SE-EPI sequences and b values, 0 and 800 s/mm<sup>2</sup>, TR: 7500 ms, TE: 65.0 ms, FOV: 380 mm, section thickness: 4 mm, space: 1 mm were performed. ADC maps were generated with commercially available software GE ADW4.4 workstation system.

**MRI image analysis and processing.** Two radiologists with 5 years of abdominal MRI experience reviewed the MRI images independently. The ADC value was automatically

calculated by a computer program included in the GE ADW4.4 workstation Functool software (GE Healthcare). The region with the most clearly displayed lesion was set as the region of interest (ROIs). Liquefaction, cystic change and necrosis of tumors were avoided when the ROIs were established. The ADC values of each ROI were measured 3 times, and the mean ADC value was set as the final value.

**VEGF expression was evaluated by immunohistochemical staining.** All the slices underwent conventional dewaxing and hydration processes at room temperature using xylene 0.5 h, 100% alcohol for 0.5 h, 100% alcohol for 1.5 h, 95% alcohol for 1.5 h, 90% alcohol for 1 h 80% alcohol for 1 h and finally 70% alcohol for 0.5 h; and were treated with 3% H<sub>2</sub>O<sub>2</sub> solution at room temperature for 10 min. After sealing solution, rabbit anti-rat VEGF polyclonal antibody (cat. no. ab1316; 1:100, Abcam, Cambridge, MA, USA) were added. Then all the slices were placed at 4°C overnight and were incubated with goat anti-rabbit IgG (ab187910, 1:1,000, Abcam, Cambridge, UK) secondary antibody at 37°C for 30 min. Visualization was performed by DAB solution (30 min at room temperature) and re-dyeing was finished with hematoxylin (30 min at room temperature). The negative control group used PBS buffer instead of the primary antibody. A total of 5 fields of view under high power light microscopy (at magnification, x100) were randomly selected from each sample and the cells in which the cytoplasm was stained brown or yellow were considered as expression-positive cells. The percentage of positive cells in total cells was then calculated. Percentage of positive cells=(number of positive cells/total cells) x100%. VEGF is principally expressed in cytoplasm or cell membrane of tumor cells, and weakly positive in tumor vascular endothelial cells and hepatic sinusoidal endothelial cells. Criteria: Negative (-), staining cell number <5%, weak positive (+): Between 5 and 25%, positive (++) : Between 26 and 50%, strong positive (+++) : >50%.

**The changes of ADC value prior to and following radiotherapy of ESCC.** MRI examinations were performed prior to and following radiotherapy for 4-6 weeks, and the treatment response was evaluated. The mean ADC values were measured separately. The correlation between ADC value and VEGF expression and pathological grade of ESCC was observed, and the change of ADC value of esophageal lesions prior to and following radiotherapy were compared.

**Statistical analysis.** SPSS 17.0 statistics software (SPSS, Inc., Chicago, IL, USA) was employed to perform statistical analysis. Experiments were performed three times and data are expressed as mean ± SD. If multiple sets of variables were consistent with homogeneity of variance, one-way analysis of variance (ANOVA) was for multi-group comparisons, followed by LSD post-hoc test. The mean ADC values were tested by homogeneity of variance. LSD post-hoc test was used to compare between VEGF expression groups and pathological grade groups. Homogeneity test of variance was used to compare ADC values and VEGF expression. Kruskal-Wallis H test was used to compare VEGF expression and pathological grading. The Pearson's correlation test was used to correlate between ADC values and VEGF expression and pathological grading. The degree of correlation was classified as a direct

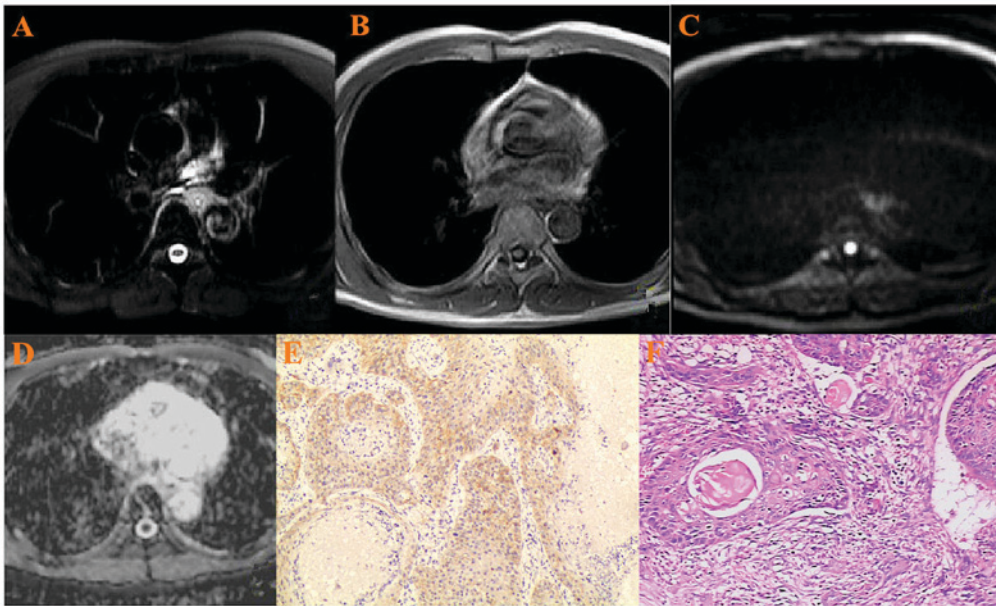


Figure 1. A 75-year-old patient with ESCC in the mid-thoracic portion. (A) ESCC was hyperintense on T2-weighted image, (B) hypointense on T1-weighted image, (C) hyperintense on the DW-MRI ( $b=800 \text{ s/mm}^2$ ) (D) hypointense on ADC map. ADC value:  $1.62 \pm 0.35 \times 10^{-3} \text{ mm}^2/\text{s}$ . (E) Low VEGF staining in cytoplasm of ESCC: Weak positive (+) (x200 magnification), (F) Histopathology: Well differentiated squamous cell carcinoma (x200 magnification). ESCC, esophageal squamous cell carcinoma; DW-MRI, diffusion weighted-magnetic resonance imaging; ADC, apparent diffusion coefficient; VEGF, vascular endothelial growth factor.

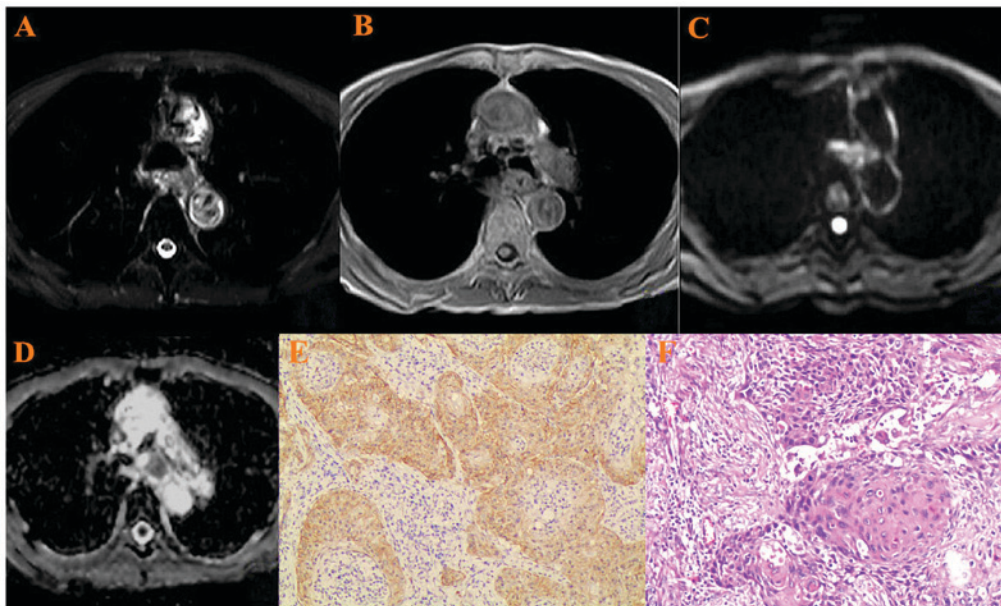


Figure 2. A 68-year-old patient with ESCC in the upper thoracic portion. (A) ESCC was hyperintense on T2-weighted image, (B) hypointense on T1-weighted image, (C) hyperintense on the DW-MRI ( $b=800 \text{ s/mm}^2$ ) (D) hypointense on ADC map. ADC value:  $1.48 \pm 0.16 \times 10^{-3} \text{ mm}^2/\text{s}$ . (E) Moderate VEGF staining in cytoplasm of ESCC: Positive (++) (x200 magnification), (F) Histopathology: Moderately differentiated squamous cell carcinoma (x200 magnification). ESCC, esophageal squamous cell carcinoma; DW-MRI, diffusion weighted-magnetic resonance imaging; ADC, apparent diffusion coefficient; VEGF, vascular endothelial growth factor.

correlation if  $r$  was a positive value and an inverse correlation if  $r$  was a negative value.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

*Correlation of VEGF expression level with MRI features and pathology characters.* All 52 esophageal cancer showed

hyperintense on T2WI (Figs. 1-3A), hypointense on T1WI (Figs. 1-3B), hyperintense on the DWIs (Figs. 1-3C), hypointense on ADC map (Figs. 1-3D). Two experienced pathologists from The First Affiliated Hospital of China Medical University reviewed the pathological grading independently. There were 10 well-differentiated cancers, 28 moderately differentiated ones and 14 poorly differentiated ones (Figs. 1-3E). VEGF was highly expressed in ESCC with a positive rate of 90.3%,

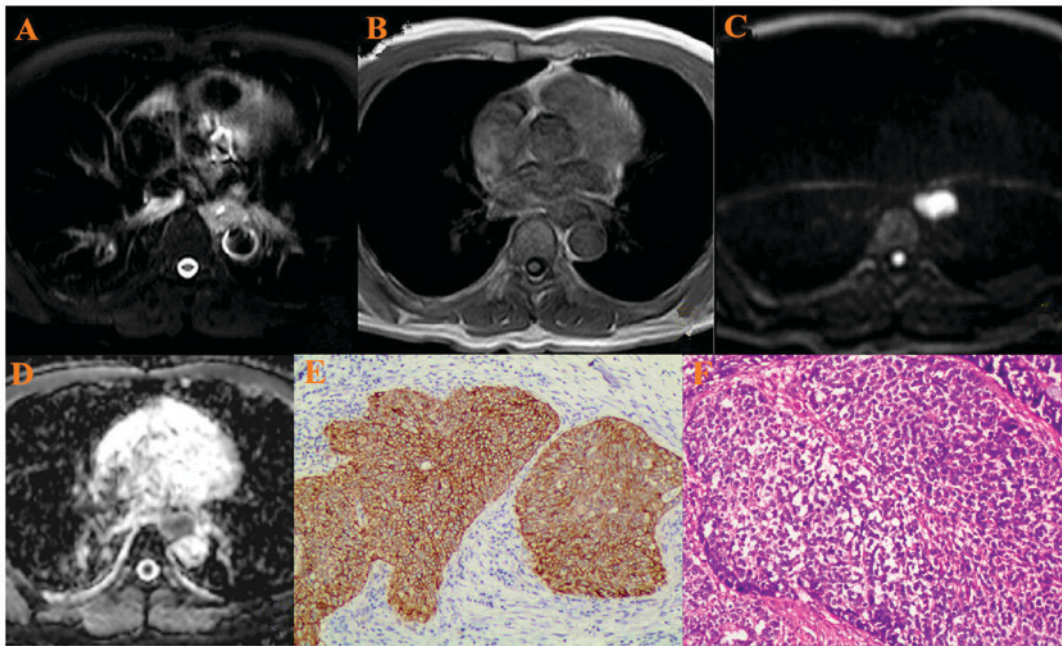


Figure 3. A 72-year-old patient with ESCC in the mid-thoracic portion. (A) ESCC was hyperintense on T2-weighted image, (B) hypointense on T1-weighted image, (C) hyperintense on the DW-MRI ( $b=800 \text{ s/mm}^2$ ) (D) hypointense on ADC map. ADC value:  $1.31 \pm 0.25 \times 10^{-3} \text{ mm}^2/\text{s}$ . (E) High VEGF staining in cytoplasm of ESCC: Strong positive (+++) ( $\times 200$  magnification), (F) Histopathology: Poorly differentiated squamous cell carcinoma ( $\times 200$  magnification). ESCC, esophageal squamous cell carcinoma; DW-MRI, diffusion weighted-magnetic resonance imaging; ADC, apparent diffusion coefficient; VEGF, vascular endothelial growth factor.

and weak positive in 5, positive in 15 and strong positive in 32 samples (Figs. 1-3F). When  $b$  was  $800 \text{ s/mm}^2$ , the mean ADC value in all esophageal cancer was  $1.44 \pm 0.32 \times 10^{-3} \text{ mm}^2/\text{s}$ .

*Pathological grading was positively correlated with ADC values in ESCC.* When  $b$  was  $800 \text{ s/mm}^2$ , the mean ADC value of the esophageal cancer with different differentiating degree (well-moderately-poorly differentiated) were  $1.65 \pm 0.25 \times 10^{-3}$ ,  $1.48 \pm 0.31 \times 10^{-3}$  and  $1.32 \pm 0.18 \times 10^{-3} \text{ mm}^2/\text{s}$ , respectively. Variance analysis demonstrated that the difference of ADC value were statistically significant between the three groups ( $F=6.285$ ,  $P=0.006$ ). LSD post-hoc test demonstrated that there was significant difference between well differentiation and moderately differentiation and poor differentiation; however, no significant difference between moderately differentiation and poor differentiation ( $P=0.005$ ,  $P=0.035$  and  $P=0.158$  respectively) was identified. Spearman correlation analysis indicated that the pathological grading of ESCC was positively correlated with ADC values ( $r=0.635$ ,  $P=0.0007$ ) (Fig. 4). Therefore, the ADC values may represent the tumor histological differentiation grade.

*VEGF expression was inversely correlated with the ADC values in ESCC.* When  $b$  was  $800 \text{ s/mm}^2$ , the mean ADC value of the esophageal cancer with VEGF grading (Weakly positive, positive, strongly positive) were  $1.45 \pm 0.37 \times 10^{-3}$ ,  $1.61 \pm 0.28 \times 10^{-3}$  and  $1.27 \pm 0.21 \times 10^{-3} \text{ mm}^2/\text{s}$ , respectively. Variance analysis demonstrated that the difference of ADC values were statistically significant between the three groups ( $F=7.125$ ,  $P=0.005$ ). An analysis of variance demonstrated that there were no significant differences between VEGF weak and strong positive groups and weak and positive groups ( $P=0.185$  and  $P=0.054$ ).

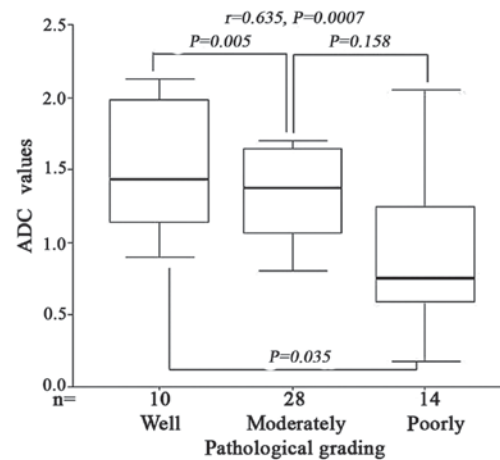


Figure 4. Pathological grading was positively correlated with ADC values in ESCC. There was significant difference between well and moderately differentiation and well and poor differentiation; however no significant difference between moderate and poorly differentiation was identified ( $P=0.158$ ). Spearman's correlation analysis indicated that the pathological grading of ESCC was positively correlated with ADC values ( $r=0.635$ ,  $P=0.0007$ ). ADC, apparent diffusion coefficient; ESCC, esophageal squamous cell carcinoma.

However a significant difference between positive and strong positive groups was identified ( $P=0.003$ ). Spearman correlation analysis indicated that VEGF expression was inversely correlated with the ADC values in ESCC ( $r=-0.321$ ,  $P=0.008$ ) (Fig. 5). Therefore, the ADC values may represent the degree of VEGF expression, and may be an alternative for assessing tumor angiogenesis to predict anti-VEGF response.

*Correlation between the pathological grading and VEGF expression in ESCC.* Kruskal-Wallis H test demonstrated that

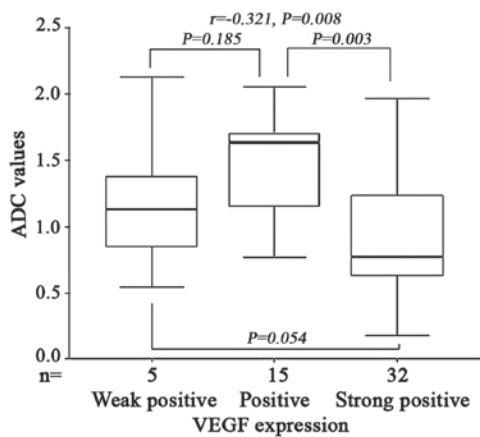


Figure 5. VEGF expression was inversely correlated with the ADC values in ESCC. There was no significant difference between VEGF weak positive and strong positive group, weak positive and positive groups ( $P=0.185$ ); however, statistically significant differences were detected between positive and strong positive groups, ( $P=0.054$  and  $P=0.003$ , respectively). Spearman correlation analysis indicated that VEGF expression were inversely correlated with the ADC values in ESCC ( $r=-0.321$ ,  $P=0.008$ ). VEGF, vascular endothelial growth factor; ADC, apparent diffusion coefficient.

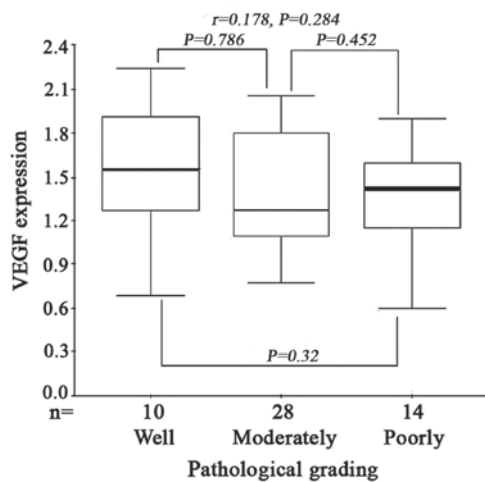


Figure 6. There was no significant difference VEGF expression between well differentiated and moderately differentiated, well differentiated and poorly differentiated, and moderately differentiated and poorly differentiated of VEGF expression ( $P=0.786$ ,  $P=0.329$ ,  $P=0.452$ ). Spearman correlation analysis indicated that there was no correlation between the pathological grading and the VEGF expression in ESCC ( $r=0.178$ ,  $P=0.284$ ). VEGF, vascular endothelial growth factor; ESCC, esophageal squamous cell carcinoma.

there was no significant difference between VEGF expression and pathological grading in ESCC ( $H=1.875$ ,  $P=0.565$ ). No statistical differences were observed between VEGF expression and tumor differentiation ( $P=0.786$ ,  $P=0.329$ ,  $P=0.452$ , between well and moderate, well and poor, and poor and moderate differentiation respectively). Furthermore Spearman's correlation analysis indicated that there was no correlation between the pathological grading and VEGF expression in ESCC ( $r=0.178$ ,  $P=0.284$ ) (Fig. 6).

*Changes of ADC values in ESCC prior to and following radiotherapy.* All patients were evaluated as CR (30 cases) or PR (22 cases) by endoscopic examination 2 weeks

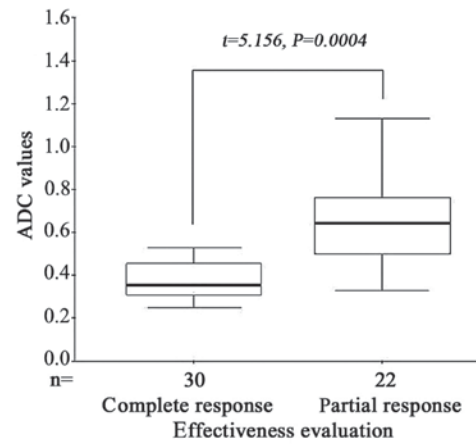


Figure 7. The mean ADC values of the CR group were significantly higher than PR group prior to radiotherapy ( $t=5.156$ ,  $P=0.0004$ ). ADC, apparent diffusion coefficient; CR, complete response; PR, partial response.

following radiotherapy. The mean ADC values of CR and PR groups prior to radiotherapy were  $1.63 \pm 0.21 \times 10^{-3}$  mm<sup>2</sup>/s and  $1.38 \pm 0.25 \times 10^{-3}$  mm<sup>2</sup>/s, respectively. The mean ADC value prior to radiotherapy of the CR group was significantly higher than the PR group ( $t=5.156$ ,  $P=0.0004$ ) (Fig. 7). The mean ADC values of CR and PR groups following radiotherapy rose to  $2.73 \pm 0.28 \times 10^{-3}$  and  $1.85 \pm 0.14 \times 10^{-3}$  mm<sup>2</sup>/s, respectively; however no statistical difference was detected between the two groups ( $t=0.886$ ,  $P=0.356$ ). Therefore, ADC values may be a useful marker to predict radiotherapy response. The higher the ADC values, the better the response to radiotherapy, which ultimately improves the therapeutic response and prognosis of the patient.

### Discussion

The development of MRI techniques and the clinical application of DW-MRI, staging and diagnosis have allowed the therapeutic evaluation of tumors to proceed to a higher level (15). The ADC values are obtained by setting the diffusion sensitivity coefficient ( $b$  value) (16). ADC value is a quantitative index that reflects the diffusion degree of water molecules (17). Therefore, DW-MRI can noninvasively observe the movement of water molecules in living tissues and provide pathological and physiological changes *in vivo* (18). Imaging techniques range from observing anatomical changes to functional states. Based on the selection of  $b$  value, Baur *et al* (19) reported that the larger the  $b$  value is, the larger the diffusion weight of the image, and the smaller the T2 penetration effect is, the reduced effect of blood perfusion, and the more accurate the ADC value. Previous studies have reported that, when  $b$  was 600 s/mm<sup>2</sup>, the DWI exhibited the lesion border with highest credibility compared with the other examined  $b$  value, and had the best consistency with pathology (20,21). Taking into account the measured ADC value to accurately reflect the level of water molecules dispersion in tissue, the present study selected  $b$  as 800 s/mm, and the mean ADC values of ESCC obtained by the present study was  $1.44 \pm 0.32 \times 10^{-3}$  mm<sup>2</sup>/s. The results are in line with the relevant literature, as several studies have reported that the ADC value is closely associated with the clinical stage, pathological grading, chemoradiotherapy response, and prognosis in gastric, lung, colorectal and liver

cancer and other solid tumors (22,23). In short, DW-MRI is a reliable noninvasive evaluation method that is being increasingly used in clinical practice.

The present study demonstrated that ADC values were positively correlated with the pathological grading of ESCC. It was also demonstrated that, prior to radiotherapy, the higher the ADC value, the better the radiotherapy response. Therefore, we postulate that ADC values may be used to quantify the pathological grade, radiotherapy response and prognostic indicators of esophageal cancer.

Tumor angiogenesis is a major factor in tumor growth and metastasis (24). Vascular endothelial growth factor (VEGF) serves an important role in the regulation of tumor angiogenesis and differentiation of vascular endothelial cells (17). VEGF can promote the proliferation and differentiation of vascular endothelial cells, and can induce the morphogenesis of capillaries through paracrine way, can stimulate the growth of endothelial progenitor cells (EPCs), which are characterized by high proliferative capacity and neovascularization (25). VEGF and its induced high blood vessel density can promote tumor circulatory metastasis, and VEGF can degrade vascular basement membrane, which creates a suitable microenvironment for circulatory metastasis. VEGF also can promote the formation of new lymphatic vessels in lymph nodes, which leads to an earlier lymph node metastasis (26). Studies have demonstrated that VEGF is overexpressed in gastric, lung, colorectal, breast, prostate, liver cancer and other solid tumors (27,28). **Recent studies have reported that the expression of VEGF in esophageal cancer is associated with lymph node metastasis, distant metastasis and clinical stage ( $P < 0.05$ ), but there is no significant correlation with pathological grading and depth of invasion (29,30). Additionally, overexpression of VEGF suggested poor prognosis of esophageal cancer, a meta-analysis reported that the risk of late stage (III and IV) increased in esophageal cancer patients with VEGF overexpression ( $HR = 1.55$ ), and the odds ratio (OR) was 2.14 (31). The results of the present study demonstrate that VEGF was highly expressed in ESCC with a positive rate of 90.3%, yet there was no correlation between the pathological grading and the VEGF expression, this result is in accordance with other reported values (32). VEGF has become an important target for anticancer treatments with humanized anti-VEGF monoclonal antibodies such as Bevacizumab being approved by the Food and Drug Administration (FDA), and have proven beneficial to cancer therapy in metastatic colorectal, metastatic breast, advanced non-small cell lung cancer and metastatic renal cell carcinoma (33). In addition, there are some evidences for its challenging efficacy in the treatment of other solid tumors, including hepatocellular carcinoma, gastric and esophageal cancer (34). However, there is still lack of definite biomarkers to predict the anti-VEGF response. VEGF may be useful prognostic biomarker and also a potential marker to predict anti-VEGF response in tumor therapy. However, the clinical application of VEGF is limited by the factors such as trauma, complications and the error of pathological sampling (35,36).**

The present retrospective study demonstrated that ADC values were significantly inversely correlated with VEGF expression. Therefore, we hypothesize that ADC values may be a viable alternative for assessing VEGF expression level to predict prognosis and anti-VEGF response.

To conclude, DWI with ADC values measurement may represent the grade of tumor histologic differentiation and the degree of VEGF expression, and also a useful biomarker to predict radiotherapy and anti-VEGF response in ESCC. ADC values may be a substitution for assessing tumor angiogenesis and also a novel prognostic factor and contribute to the treatment of ESCC.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Authors' contributions

QC and GL designed the experiment. QC was a major contributor in writing the manuscript. YW, SZ and HZ performed the experiments and analyzed the data. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital of China Medical University (Shenyang, China). All patients provided written informed consent.

#### Patient consent for publication

The study participants provided consent for the data and any associated images to be published.

#### Competing interests

The author's declare that they have no conflicts of interest.

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