Serum VEGF levels as a predictor of recurrence in patients with advanced-stage esophageal squamous cell carcinoma following curative esophagectomy followed by chemotherapy or concurrent radiotherapy

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Received March 21, 2023; Accepted August 14, 2023

DOI: 10.3892/mco.2023.2682

Abstract. The present study evaluated serum levels of vascular endothelial growth factor (VEGF) as a predictor of recurrence in patients with advanced-stage esophageal squamous cell carcinoma (ESCC) following curative esophagectomy followed by chemotherapy or concurrent radiotherapy. Patients with locally advanced resectable ESCC underwent R0 esophagectomy followed by chemotherapy or concurrent radiotherapy as an adjuvant. Serum VEGF levels in 173 patients, including 57 patients with recurrent disease, and 183 healthy controls were determined using a Luminex assay. The results demonstrated that the serum VEGF levels were significantly higher in 57 patients with locally advanced resectable ESCC at recurrence compared with the levels at pre-treatment (P<0.001). The patients with recurrence exhibited significantly higher serum VEGF levels during chemotherapy or concurrent radiotherapy than patients with no recurrence (P<0.05). Patients with low serum VEGF levels had a significantly longer survival time than those with high serum VEGF levels prior to treatment (P<0.01). The median survival times were 70 and 25 months in patients with locally advanced resectable ESCC with serum VEGF levels <161.75 and ≥161.75 pg/ml following treatment, respectively (P<0.01). Compared with patients with VEGF levels <147 pg/ml following treatment, patients with locally advanced resectable ESCC with VEGF levels ≥147 pg/ml had a significantly higher risk of recurrence (P<0.01). Patients with low serum VEGF levels (<147 pg/ml) had significantly higher recurrence-free survival rates than those with high serum VEGF levels (≥147 pg/ml) following treatment (P<0.01). The findings of the present study demonstrate that serum VEGF levels are a potential predictor of recurrence and of the treatment outcomes of chemotherapy or concurrent radiotherapy in patients with locally advanced resectable ESCC.

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Key words: vascular endothelial growth factor, esophageal squamous cell carcinoma, recurrence, predictor, survival, prognosis

Introduction

Esophageal cancer (EC) is the seventh most prevalent type of cancer and the sixth leading cause of cancer-related mortality worldwide (1,2). This type of cancer has two major histological subtypes, squamous cell carcinoma (SCC) and adenocarcinoma (AC). SCC is the primary pathological subtype in East Asia, eastern and southern Africa, and Southern Europe, while AC is predominant in North America and other parts of Europe (3). Esophageal squamous cell carcinoma (ESCC) develops from precancerous lesions, gradually followed by the progression of hyperplasia, dysplasia and SCC (4). Patients with locally advanced cancer frequently develop recurrent disease after surgery alone, and either chemotherapy or chemoradiotherapy is recommended as an adjunct to surgery for such patients (5). The 5-year survival rate of patients with ESCC is ~18%, which reflects late diagnosis, the aggressiveness of the disease and a lack of effective treatment strategies (6,7). The poor prognosis of patients with ESCC may be due to tumor invasion and metastasis (8). In patients with advanced-stage or metastatic ESCC, combination chemotherapy regimens extend survival; however, the median survival rate remains <1 year (9). Therefore, therapeutic efficacy needs to be evaluated through biomarkers to predict the prognosis and treatment response of patients with locally advanced resectable ESCC.

Vascular endothelial growth factor (VEGF) is a critical and effective factor that stimulates angiogenesis and partici-

pates in tumor invasion and metastasis (10). Serum VEGF levels are of diagnostic and prognostic value in certain types of cancer, including EC (11,12). Greater invasion depth and higher histological grade are associated with a higher risk of recurrence (13-16). The high incidence of synchronous and metachronous metastases are the main reasons for the poor prognosis of patients with superficial ESCC. The recurrence of superficial ESCC following esophagectomy normally involves regional lymph node or distant metastases (17). Although a high serum VEGF level is a prognostic factor for patients with locally advanced ESCC (12), whether the serum VEGF levels can predict recurrence is yet to be elucidated.

In the present study, association between serum VEGF levels and, the curative effects and prognostic values were investigated in patients with advanced-stage ESCC following curative esophagectomy followed by chemotherapy or concurrent radiotherapy, particularly in patients with recurrent ESCC. The significance of the serum VEGF levels, as a predictor of recurrence, was also evaluated in patients with locally advanced resectable ESCC.

Patients and methods

Patients and treatment modality. Between January, 2012 and June, 2016, a total of 173 patients with a mean age of 60.9±8.1 years, included 147 males and 26 females, with locally advanced resectable ESCC confirmed by histopathological analysis were enrolled at Jiangsu Cancer Hospital (Nanjing, China). These patients were diagnosed with stage III or stage IV ESCC using to the latest TNM staging by the International Union Against Cancer (2009). The patients were classified as having locally advanced resectable ESCC and underwent R0 resection. Metastasis was confirmed by imaging, including lymphatic metastasis and distant metastasis. A total of 183 healthy controls included 142 males and 41 females were enrolled between January, 2013 and December, 2013 at Nanjing Yian physical examination center (Nanjing), with a mean age of 48.3±13.7 years. The present study was approved by the Biomedical Research Ethics Committee of Jiangsu Cancer Hospital (approval no. 2010ke-052). All of the participants provided written informed consent. The experiments were performed in accordance with the Declaration of Helsinki. All patients were treated with chemotherapy, with a chemotherapy cycle once every 3-4 weeks. Among them, 57 patients were treated with concurrent radiotherapy at four different intervals with the course of radiotherapy being 4-6 weeks. Initially, the patients did not receive any treatment (pre-treatment group). The patients then underwent chemotherapy (Chemo group) or concurrent radiotherapy (Chemo + Radio group) after R0 resection. In Chemo group, patients received at least four cycles of chemotherapy with different chemotherapeutic drugs. Data for the first four cycles were analyzed. Chemotherapy regimens included the TP regimen, which was taxane (PTX, TAX, TXT or DOC) combined with platinum, the PF regimen which was 5-fluorouracil and its derivatives (5-FU, FT207 or CAPE) combined with platinum, and the GP regimen which was gemcitabine (GEM) combined with platinum. The platinum was one of DDP, LBP, CBP and NDP (18). The patients received concurrent radiotherapy for 30-45 days at the first course of chemotherapy, with radiotherapy schemes, such as GTV (Gross Tumor Volume) 60-65 Gy/28-33 fractions (f), CTV (Clinical Target Volume) 50-55 Gy/28-33 f and PTV (Planning Target Volume) 50-66 Gy/28-33 f. A total of 5 months after the end of chemotherapy or concurrent radiotherapy, 57 patients exhibited recurrence at the original lesion or metastasis, including lymph node metastasis and distal metastasis; these patients were then classified as patients with recurrent disease (recurrent patient group). Patients with recurrence then received further treatments, including chemotherapy or concurrent radiotherapy. In the course of further treatment, the chemotherapy regimens or radiotherapy regimens were the same as those aforementioned.

Serum samples and detection of serum VEGF levels. Blood samples were collected at the pre-treatment stage and at four intervals during chemotherapy (at 21-28 days after each of the 4 cycles of chemotherapy). At the beginning of the next cycle, samples were collected and tested for VEGF levels to monitor the previous treatment cycle. Samples from the recurrent patient group were collected at recurrence (re-0 cycle), and at day 21-28 after the completion of the course of further treatment (re-1 and re-2 cycle). The samples were stored at -80˚C following centrifugation for 10 min at 1,500 x g at 4˚C.

The serum levels of VEGF were measured using Luminex FLEXMAP 3D instruments and xPONENT™ software (Luminex Corporation) with Human cytokine/chemokine panels (cat. no. MPXHCYTO-60K-01; MilliporeSigma). The preparation of blood samples, the setting of detection parameters and the calculation of serum VEGF levels were performed according to the manufacturers' protocols (19).

Statistical analysis. Serum VEGF levels are presented as the mean ± SD. The differences between two groups were analyzed using an unpaired Student's t-test. The comparisons of ≥3 groups were performed using ANOVA, followed by pair-wise comparisons using the Bonferroni post hoc test. Patient characteristics for patients with recurrent and non-recurrent ESCC were analyzed using Pearson's χ^2 test or Fishers exact test (where the expected count in >20% of cells was <5). The follow-up period ended on April 25, 2020, and the overall survival (OS) was calculated. The cut-off values for the OS and recurrence-free survival (RFS) of patients with regard to serum VEGF levels were assessed using the receiver operating characteristic (ROC) and the area under curve (AUC), sensitivity and specificity were also calculated (Table SI). Multivariate analysis was performed using the Cox proportional hazards regression model. RFS rates were calculated from the date of surgery to the date of recurrence using the Kaplan-Meier method and the significance of comparisons between groups was measured using a log-rank test. When survival curves crossed over, these data were analyzed using the weighted, two-stage test. $P<0.05$ was considered to indicate a statistically significant difference. All the statistical analyses were performed using GraphPad Prism 5 (Dotmatics), except Pearson's χ^2 test and Fishers exact test which were performed using SPSS Statistics 21.0 (IBM).

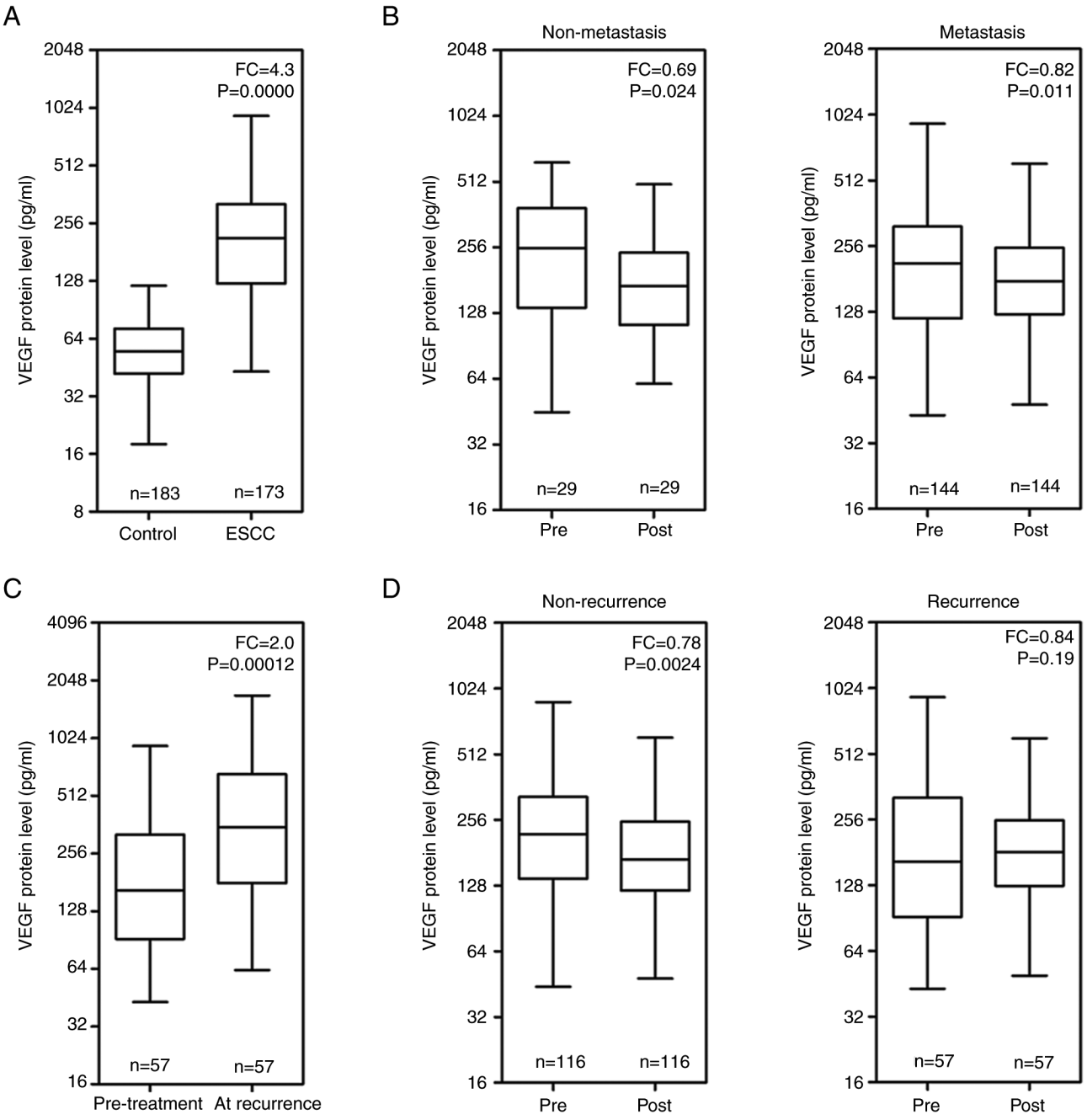

Figure 1. Boxplots of serum VEGF levels in patients with locally advanced resectable ESCC at the pre-treatment stage and following chemotherapy or concurrent radiotherapy. Median values of (A) VEGF levels in the healthy controls, and patients with stage III and stage IV ESCC, (B) VEGF levels in 57 patients with recurrent disease at the pre-treatment stage and at recurrence, (C) VEGF levels in patients with locally advanced resectable ESCC with non-metastasis and metastasis at the pre-treatment stage and following treatment, (D) VEGF levels in patients with locally advanced resectable ESCC with no recurrence and recurrence at the pre-treatment stage and following treatment. Pre, pre-treatment stage; Post, following chemotherapy or concurrent radiotherapy; FC, fold change; VEGF, vascular endothelial growth factor.

Results

Serum VEGF levels are increased in patients with locally advanced resectable ESCC and are maintained at high levels in patients with recurrent disease following therapy. Compared with the healthy controls, patients with stage III and stage IV ESCC had significantly higher serum VEGF levels (P<0.001), with the mean serum VEGF level in the patients being 4.3-fold that of the controls (Fig. 1A). Compared with the pre-treatment stage, the serum VEGF levels significantly decreased in both non-metastatic and metastatic patients with locally advanced resectable ESCC following treatment (P<0.05; Fig. 1B). The serum VEGF levels in 57 patients with locally advanced resectable ESCC with recurrent disease were significantly higher at the time of recurrence compared with the levels in the same patients at the pre-treatment stage (P<0.001; Fig. 1C). The serum VEGF level was significantly lower in patients with no disease recurrence following treatment compared with the levels at the pre-treatment stage (P<0.01). However, the difference in the serum VEGF levels between the pre-treatment

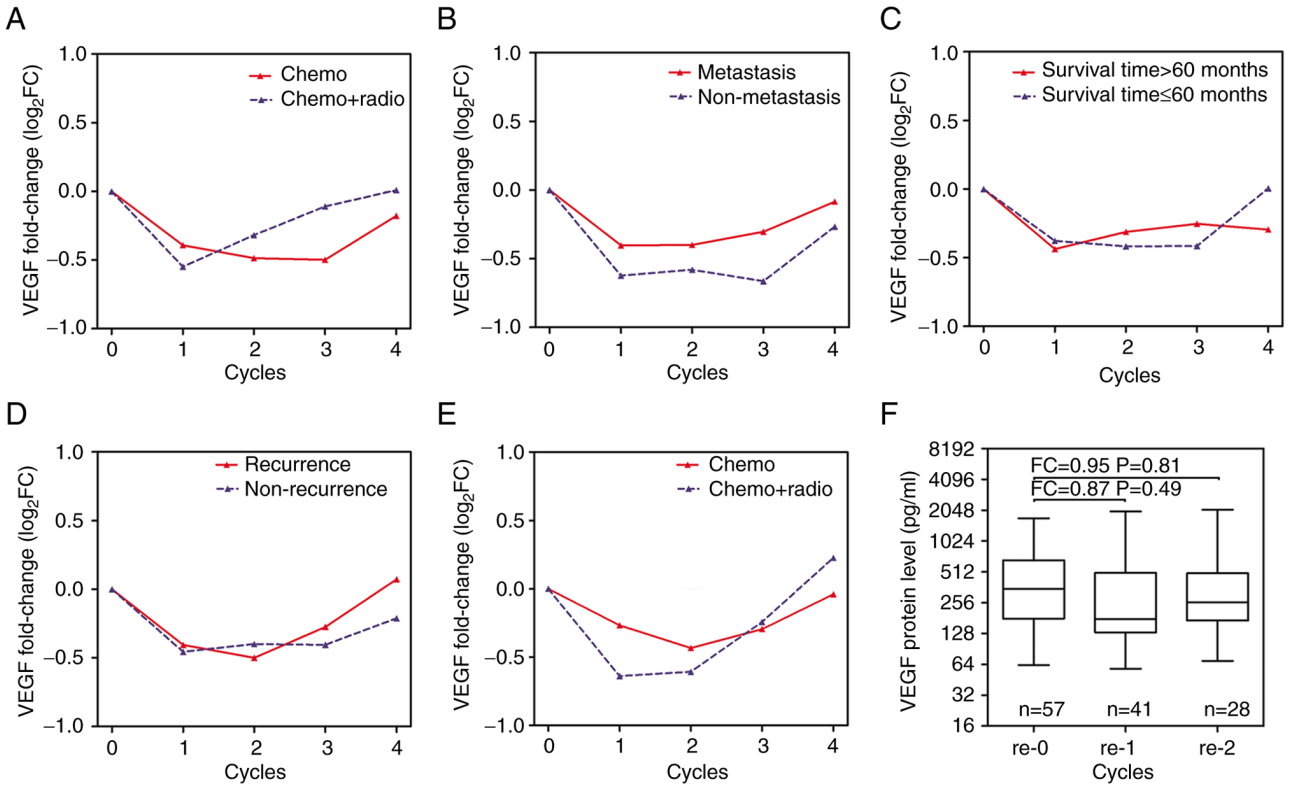

Figure 2. Changes in the serum VEGF level in patients with locally advanced resectable ESCC following treatment. Mean changes in serum VEGF levels for (A) treatment modality, (B) metastasis, (C) survival time and (D) recurrence relative to the pre-treatment stage in patients with locally advanced resectable ESCC. (E) Mean changes in serum VEGF levels for treatment modality relative to the pre-treatment stage in 57 patients with recurrent disease. (F) Median values of VEGF levels in 57 patients with recurrent disease following further treatment. The levels were assessed at recurrence (re-0 cycle) and at the 1st (re-1 cycle), 2nd cycle (re-2 cycle) following further treatments in 57 patients with recurrent disease. Chemo, patients received at least four cycles of chemotherapy; Chemo + Radio, patients received concurrent radiotherapy at the 1st cycle of chemotherapy; FC, fold change which is the ratio of the mean of VEGF levels at each cycle to the mean value of VEGF levels at 0 cycle or re-0 cycle; VEGF, vascular endothelial growth factor; log_2FC, log value of the fold change; chemo, chemotherapy; radio, radiotherapy.

and post-treatment stage was not significant in patients with recurrence (P>0.05; Fig. 1D).

The clinical characteristics of patients at the pre-treatment stage were presented in Table SII. The serum VEGF levels in the patients with recurrent disease were significantly higher compared with those in patients with no recurrence (P<0.001). Serum VEGF levels in patients with lymph node metastasis were significantly higher compared with those in patients with distal metastasis (P<0.05). The clinical characteristics of patients with recurrence or no recurrence were presented in Table SIII.

Serum VEGF levels changed in patients with locally advanced resectable ESCC following chemotherapy or concurrent radiotherapy. Serum VEGF levels markedly decreased at the 1st treatment cycle compared with pre-treatment, then increased gradually after the 1st treatment cycle in patients treated with concurrent radiotherapy and after the 3rd treatment cycle in patients treated with chemotherapy alone (Fig. 2A). Compared with patients with metastatic locally advanced resectable ESCC, patients with non-metastatic locally advanced resectable ESCC exhibited a greater degree of decline in serum VEGF levels following treatment with the four treatment cycles (Fig. 2B). Serum VEGF levels tended to increase in patients with a survival time ≤60 months after the 3rd treatment cycle (Fig. 2C). Serum VEGF levels in patients with recurrent disease markedly increased, in a gradual manner, at the 3rd and 4th treatment cycles compared with those in patients with no recurrence (Fig. 2D). Serum VEGF levels in patients with recurrent disease treated with concurrent radiotherapy were notably lower at the 1st and 2nd treatment cycles, and then notably higher at the 4th treatment cycle compared with those in patients with recurrent disease treated with chemotherapy alone (Fig. 2E). Following recurrence, patients continued to receive further treatments, including chemotherapy or concurrent radiotherapy. However, the serum VEGF levels in the patients with recurrent disease demonstrated no significant differences after two cycles of further treatments (P>0.05; Fig. 2F).

Serum VEGF levels remain high in patients with recurrent disease at the fourth treatment cycle. No significant difference was demonstrated in serum VEGF levels between the patients who underwent chemotherapy and concurrent radiotherapy, for patients with either non-metastatic or metastatic disease (P>0.05; Fig. 3A). Similarly, no significant difference was demonstrated in serum VEGF levels between the patients who underwent chemotherapy and concurrent radiotherapy, for those with no recurrence and those with recurrent disease (P>0.05; Fig. 3B). Although serum VEGF levels fluctuated during the four treatment cycles, the median values of the serum VEGF levels following chemotherapy were always lower

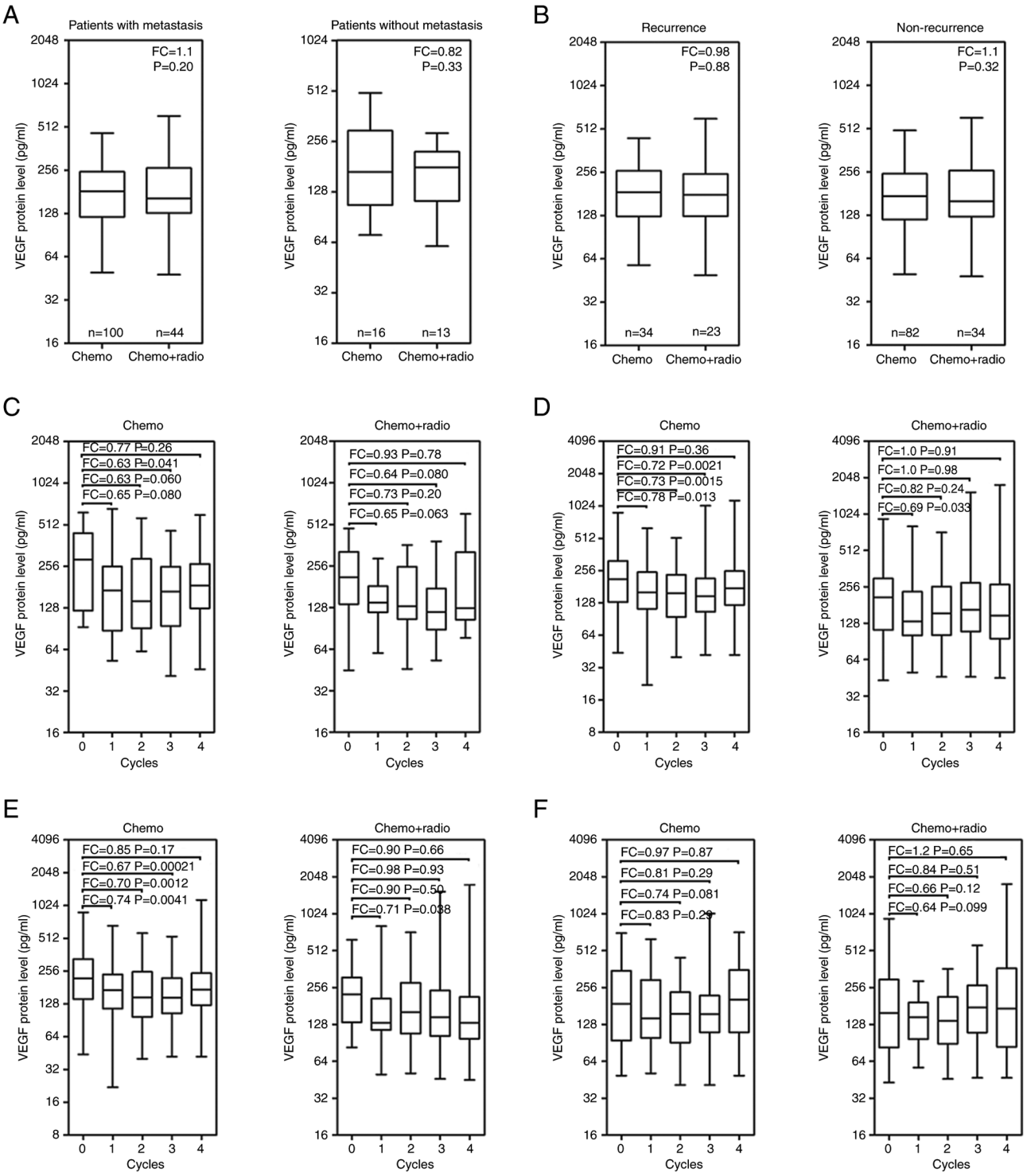

Figure 3. Boxplots of serum VEGF levels in patients with locally advanced resectable ESCC following the treatments. Median VEGF levels in (A) metastatic and non-metastatic patients, and (B) patients with recurrence and no recurrence treated with Chemo and Chemo + Radio, respectively. Median VEGF levels from pre-treatment (0 cycles) to the 1st, 2nd, 3rd and 4th cycles of treatment in (C) non-metastatic patients, (D) metastatic patients, (E) non-recurrent patients and (F) recurrent patients. Chemo, patients received at least four times of chemotherapy; Chemo + Radio, patients received concurrent radiotherapy at the 1st cycle of chemotherapy; FC, fold change; VEGF, vascular endothelial growth factor. In C-F, FC was the ratio of the mean of VEGF levels at each cycle to the mean value of VEGF levels at 0 cycle.

than those in the non-metastatic patients at the pre-treatment stage (P>0.05; Fig. 3C). Patients with metastatic disease who received chemotherapy or concurrent radiotherapy, median serum VEGF levels at the 1st treatment were significantly decreased compared with those at pre-treatment (P<0.05; Fig. 3D). Serum VEGF levels significantly decreased in patients with no recurrence treated with chemotherapy alone, at the 1st, 2nd and 3rd cycles, compared with the levels at the pre-treatment stage (P<0.01; Fig. 3E). A significant decrease in serum VEGF levels was demonstrated in patients with no recurrence treated with concurrent radiotherapy, at the 1st treatment cycle, compared with the levels at the pre-treatment

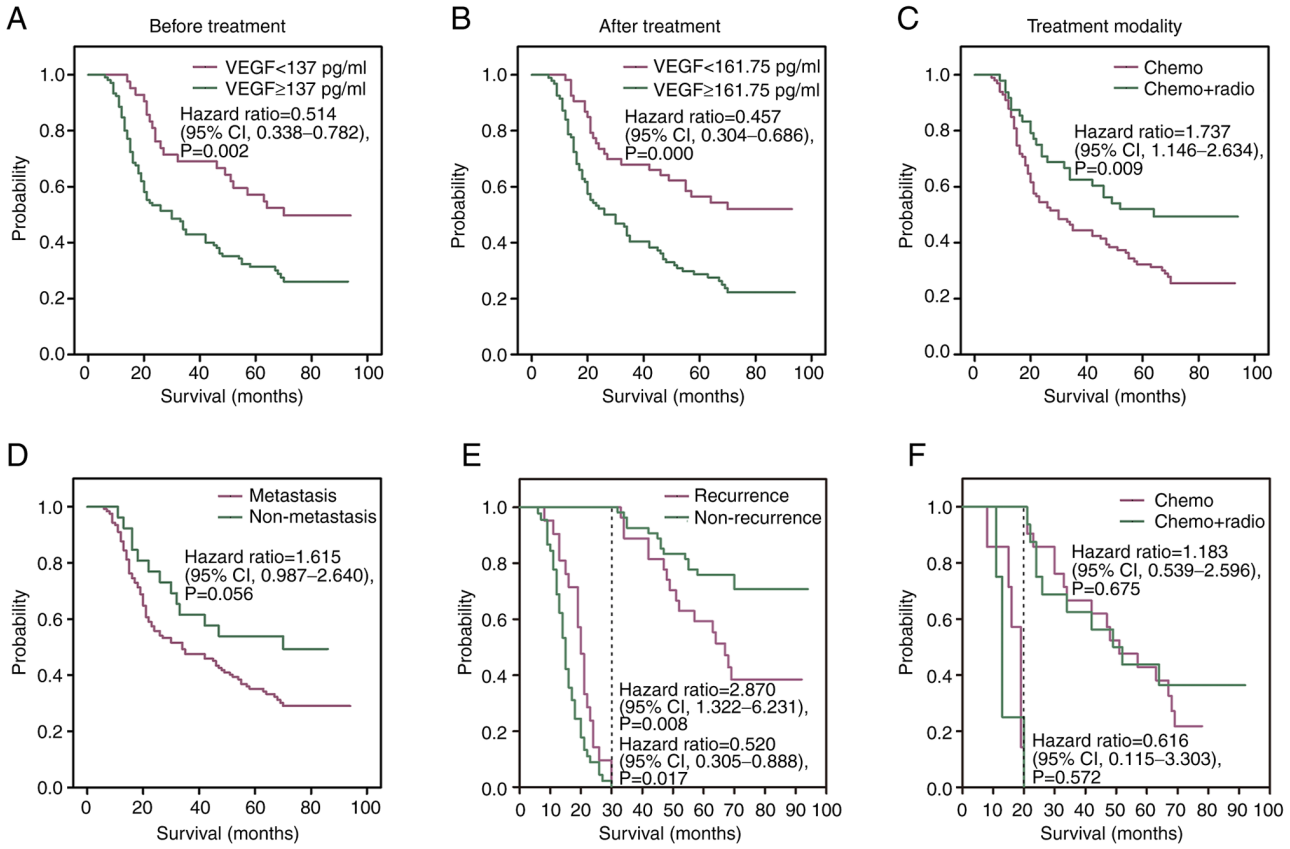

Figure 4. Prognostic values of serum VEGF levels in patients with locally advanced resectable ESCC. (A) OS of patients with high (≥137 pg/ml) and low (<137 pg/ml) VEGF levels at the pre-treatment stage in 173 patients with ESCC. (B) OS of patients with high (≥161.75 pg/ml) and low (<161.75 pg/ml) VEGF levels following chemotherapy or concurrent radiotherapy in 173 patients with ESCC. (C) OS of 173 patients with ESCC in the Chemo group and Chemo + Radio group. (D) OS of 173 patients with ESCC with metastasis and no metastasis. (E) OS of 173 patients with ESCC with recurrence and no recurrence. 30 months is a node, represented by the vertical dashed line. (F) OS of 57 patients with recurrent disease in the Chemo group and Chemo + Radio group in. 20 months is a node, represented by the vertical dashed line. OS, overall survival; VEGF, vascular endothelial growth factor.

stage (P<0.05). Although no significant differences were demonstrated in the serum VEGF levels at the 2nd, 3rd and 4th treatment cycles, the median values of the serum VEGF levels trended to decrease gradually in patients with no recurrence that underwent concurrent radiotherapy (Fig. 3E). Serum VEGF levels did not demonstrate a significant change in patients with recurrence during either treatment compared with the levels at the pre-treatment stage (P>0.05; Fig. 3F).

Prognostic value of the serum VEGF level in patients with locally advanced resectable ESCC. Patient characteristics, including age, sex, stage, metastasis, treatment modality, treatment evaluation and serum VEGF levels were analyzed with regard OS using univariate analysis. The OS of patients with ESCC differed significantly between patients with serum VEGF levels <137 and ≥137 pg/ml at the pre-treatment stage (P=0.002; Fig. 4A). The median OS rates were 70 and 25 months in patients with locally advanced resectable ESCC with serum VEGF levels <161.75 and ≥161.75 pg/ml following treatment, respectively (P=0.0002; Fig. 4B). Furthermore, patients treated with concurrent radiotherapy had a significantly longer OS (51.4±27.8 months), compared with the OS of patients treated with chemotherapy (40.9±27.9 months) (P=0.009; Fig. 4C). Although metastasis was associated with a poor prognosis, no significant differences were demonstrated in the OS of patients with non-metastatic and metastatic disease (P=0.056; Fig. 4D). The two-stage test demonstrated the OS between patients with recurrence and patients without recurrence, with 30 months as a node. Compared with patients with recurrence, patients without recurrence had a significantly longer OS among patients with who survived >30 months (P=0.008; Fig. 4E). Among patients who survived <30 months, patients with recurrent had a significantly greater survival time compared with patients without-recurrence (P=0.017; Fig. 4E). The two-stage test also demonstrated the OS between patients with recurrence treated with concurrent radiotherapy and chemotherapy, with 20 months as a node. However, post-operative concurrent radiotherapy for patients with recurrence did not significantly prolong OS compared with chemotherapy only among patients with survival <20 months or survival ≥20 months, (P=0.572 and P=0.675, respectively; Fig. 4F).

Serum VEGF level is a predictor of recurrence in patients with locally advanced resectable ESCC. Age, sex, stage, metastasis, treatment modality, treatment evaluation and serum VEGF level, were indicated to be significant univariate factors, which predicted post-operative recurrence. The serum VEGF level following treatment was the only independent predictor of recurrence in patients with

Table I. Univariate and multivariate analyses to predict postoperative recurrence.

	Univariate			Multivariate[3]	
Group	Total, n	Recurrence, n (%)	P-value	Hazard ratio (95% CI)	P-value
Gender			0.699		
Male	147	51 (34.6)			
Female	26	6 (23.1)			
Age, years			0.205		
≤60	88	35 (39.8)			
60-70	62	19 (30.6)			
≥70	23	3 (13.0)			
TNM staging			0.308		
III	62	23 (37.1)			
IV	111	34 (30.6)			
Metastasis			0.741		
Yes	144	48 (33.3)			
No	29	9 (31.0)			
Metastasis mode			0.135[4]		
Lymph node metastasis	84	29 (34.5)			
Distal metastasis	13	4 (30.8)			
Both	47	15 (31.9)			
Treatment[1]			0.290		
Chemo	116	34 (29.3)			
Chemo + Radio	57	23 (40.4)			
Evaluation[2]			0.968		
Improved	127	44 (34.6)			
No healed	9	5 (55.6)			
Missing	37				
Serum VEGF			<0.001	1.747 (0.780-3.909)	0.175
≥147	112	42 (37.5)			
<147	61	15 (24.6)			

[1]Chemo, patients received at least four cycles of chemotherapy; Chemo + Radio, patients received concurrent radiotherapy at the first cycle of chemotherapy. [2]According to the clinical treatment effect. [3]Multivariate analysis was performed when there were differences in the univariate analysis. [4]Comparison between lymph node metastasis and distal metastasis. VEGF, vascular endothelial growth factor.

locally advanced resectable ESCC following treatment, as demonstrated by multivariate Cox logistic regression analysis (Table I). No significant difference in the recurrence-free survival rates was demonstrated between patients with high serum VEGF levels (≥102 pg/ml) and low serum VEGF levels (<102 pg/ml) at the pre-treatment stage (P=0.097; Fig. 5A). However, patients with low serum VEGF levels (<147 pg/ml) had significantly higher RFS rates compared with those with high serum VEGF levels (≥147 pg/ml) following treatment (P=0.004; Fig. 5B). The two-stage test demonstrated that the time from the post-operative stage to recurrence when compared between serum VEGF levels <232 and ≥232 pg/ml in 57 patients with recurrent disease following chemotherapy or concurrent radiotherapy, had 17 months as a node. Among patients who survived ≥17 months, serum VEGF levels (232 pg/ml) demonstrated no significant correlation with the time from the post-operative stage to recurrence (P=0.665; Fig. 5C). However, patients who survived <17 months, with serum VEGF levels <232 pg/ml had a significantly shorter time period from the post-operative stage to recurrence than those with serum VEGF ≥232 pg/ml (P=0.002; Fig. 5C). Furthermore, serum VEGF levels (992 pg/ml) at recurrence in 57 recurrent patients were not associated with the time from the post-operative stage to recurrence (P=0.092; Fig. 5D). The survival from recurrence to the end of follow-up was also calculated. Patients with recurrent disease with high serum VEGF levels (≥992 pg/ml) and low serum VEGF levels (<992 pg/ml) at recurrence had similar survival times (P=0.544; Fig. 5E).

All patients with recurrent disease received further treatments, including chemotherapy or concurrent radiotherapy. A low serum VEGF level following further treatment was beneficial for the survival of patients with recurrent disease. Patients with recurrent disease with low serum VEGF levels

Table II. The individual changes in serum VEGF levels before and after treatment in patients with recurrent disease.

VEGF levels after treatment	Degree	Total (n=57)	Serum VEGF, mean ± SD pg/ml	P-value	Recurrence-free survival, mean ± SD months	P-value	Survival, mean ± SD months	P-value
Up	High (>100%)	10	242.05±80.41	0.462	18.89±6.60	0.337	51.67±23.37	0.712
	Low (≤100%)	17	206.28±137.39		23.14±11.81		47.71±25.48	
Down	High (>50%)	9	120.58±52.27	0.01	19.17±11.89	0.942	33.17±23.37	0.618
	Low (≤50%)	21	210.00±101.24	9	18.79±10.69		38.89±24.42	

Degree=(VEGF levels after treatment-VEGF levels before treatment)/VEGF levels before treatment x100. High, high degree group; Low, low degree group; VEGF, vascular endothelial growth factor; Up, VEGF levels increased after treatment; Down, VEGF levels decreased after treatment.

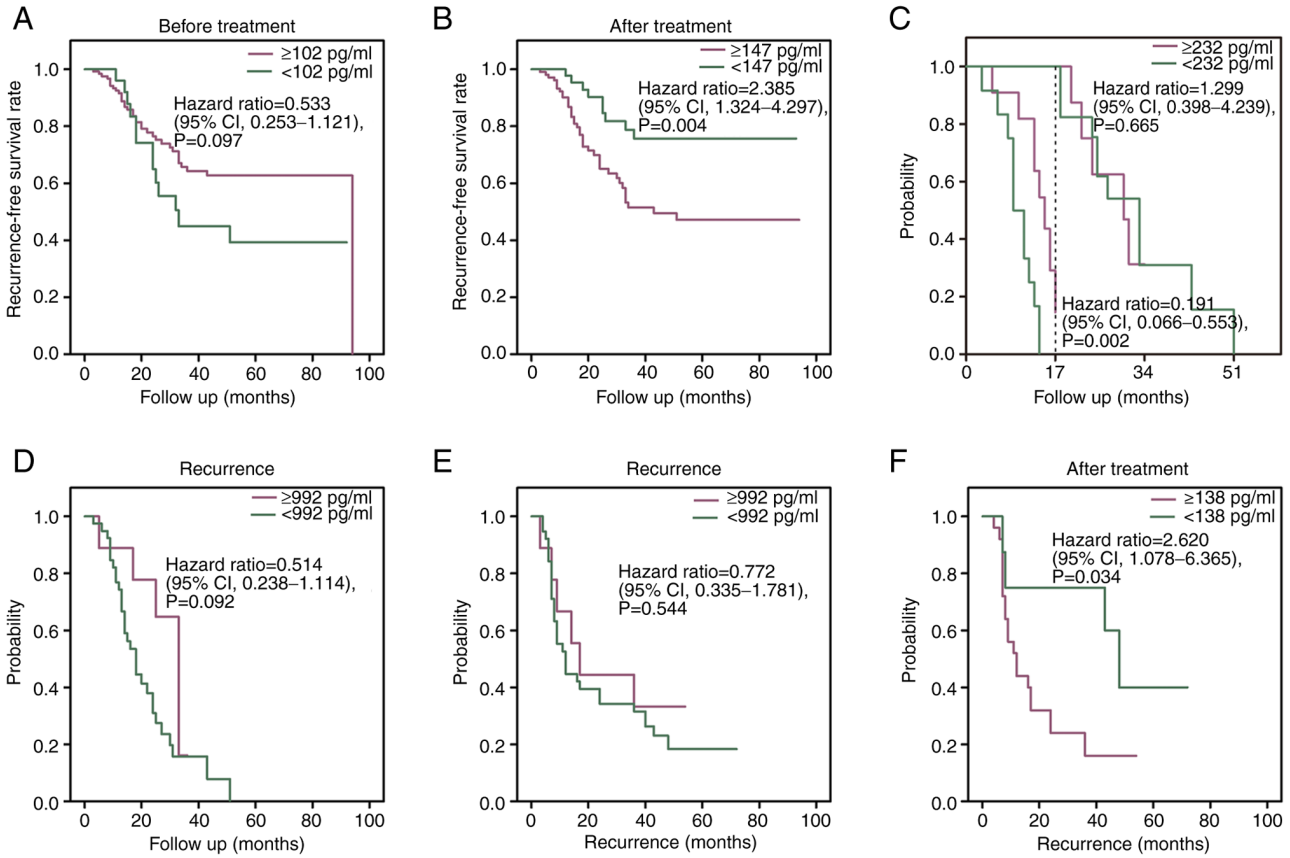

Figure 5. Serum VEGF levels as a predictor of recurrence in patients with locally advanced resectable ESCC. Kaplan-Meier analysis of recurrence-free survival rates with comparison between 173 patients with high and low serum VEGF levels at (A) the pre-treatment stage and (B) following chemotherapy or concurrent radiotherapy. Time duration from the post-operative stage to recurrence in patients with high and low serum VEGF levels (C) following chemotherapy or concurrent radiotherapy and (D) at recurrence in 57 patients with recurrence. Time duration from recurrence to end of follow-up in patients with high and low serum VEGF levels (E) at recurrence and (F) following further treatment in 57 patients with recurrence. VEGF, vascular endothelial growth factor.

(<138 pg/ml) following further treatment had a significantly longer survival time compared with those with high serum VEGF levels (≥138 pg/ml) (P=0.034; Fig. 5F). The changes in serum VEGF levels before and after treatment in individual patients with recurrent disease were examined. Compared with VEGF levels before treatment, the VEGF levels of 27 patients increased and 30 patients decreased after treatment. There were no differences in the concentrations of serum VEGF, RFS and OS between high degree (>100% increase) group and low degree (≤100% increase) group in patients who had higher VEGF levels after treatment than before treatment (P>0.05; Table II). However, compared with the low degree (≤50% decrease) group, serum VEGF levels in the high degree (>50% decrease) group were significantly lower in patients who had lower VEGF levels after treatment than before treatment (P<0.05; Table II).

Discussion

The majority of patients with EC are diagnosed at an advanced stage and the prognosis of metastatic patients is extremely poor, with a median OS of 4-6 months, due to the aggressiveness of the disease (20,21). Although surgical resection is a potential mainstay for curable EC, locoregional recurrence occurs in 23.8-58.0% of cases (22-26). In the present study, 32.9% (57/173) of the patients experienced recurrence, which suggested that the cohort of patients with advanced-stage disease in the present study was representative. However, the serum VEGF levels remained elevated in patients with recurrence following treatment compared with the levels at the pre-treatment stage, which tended to increase at the 4th treatment cycle; this indicated that the serum VEGF level may be a potential biomarker for recurrence in patients with locally advanced resectable ESCC.

Extensive local infiltration and regional lymph node metastasis render the complete removal of tumors difficult, which is one of the possible reasons for the failure of ESCC treatment (27). In the present study, the serum VEGF levels in 57 patients with recurrent ESCC were not only higher at recurrence compared with the pre-treatment stage, but also remained elevated following further treatment, which indicated that a high serum VEGF level was associated with recurrence in patients with locally advanced resectable ESCC. Moreover, the patients with serum VEGF levels ≥147 pg/ml had a significantly higher risk of developing recurrence following treatment. The mean serum VEGF levels in the patients with recurrence were ~2-fold higher than those in the patients with no recurrence at the pre-treatment stage (488.65 vs. 256.45 pg/ml), while the serum VEGF levels in the patients with no recurrence significantly decreased following treatment. These results indicated that the serum VEGF level was a predictor of recurrence in patients with locally advanced resectable ESCC, particularly in patients with high serum VEGF levels following chemotherapy or concurrent radiotherapy. Similarly, a previous study reported that, multivariate analysis indicated that the VEGF score was a significant parameter of the peritoneal recurrence of gastric cancer (28). VEGF has also been reported to be an independent predictor of tumor recurrence following orthotopic liver transplantation in hepatocellular carcinoma (29). Another study reported that compared with VEGF expression determined using immunohistochemistry (IHC), the pre-operative serum VEGF level was a useful predictor of post-operative recurrence in non-metastatic clear cell renal cell carcinoma, with tumors from only 26 patients (31.3%) demonstrating overexpression of VEGF using IHC (30). In the present study, the serum VEGF level at the pre-treatment stage was less effective predictor of recurrence compared with the serum VEGF level in patients with locally advanced resectable ESCC following chemotherapy or concurrent radiotherapy.

Low serum VEGF levels in patients with locally advanced resectable ESCC contribute to a longer median OS compared with high serum VEGF levels. However, no marked difference was demonstrated in the median OS between the patients with recurrence and those with no recurrence, which suggested that recurrence does not determine OS in patients with locally advanced resectable ESCC. Although the serum VEGF level at recurrence in patients with recurrence was not associated with survival from the time of recurrence to the end of follow-up, patients with low serum VEGF levels following further treatment had a longer survival from time of recurrence to the end of follow-up. These results indicated that the serum VEGF level also acted as a prognostic factor for patients with locally advanced resectable ESCC who exhibited recurrence. The serum VEGF level was associated with survival not only in patients with locally advanced resectable ESCC following treatment, but also in patients with recurrence following further treatments. A low serum VEGF level was correlated with longer survival time in patients with no recurrence and in those with recurrence following treatment. It has been previously reported that following surgery, certain patients develop locoregional recurrence and distant metastasis (31-33). VEGF is a prognostic factor for patients with locally advanced ESCC treated with concurrent chemoradiotherapy. Low VEGF levels following treatment and decreasing levels of VEGF during concurrent chemoradiotherapy have been previously reported to be significantly associated with improved clinical outcomes in patients with advanced-stage ESCC (12). Biologically, ESCC shares numerous characteristics with head and neck SCC (34). An association has also been previously reported between VEGF and the OS and metastasis-free survival of patients with head and neck SCC treated with radio-chemotherapy or radiotherapy (35). Furthermore, the serum VEGF level is a potential target for chemotherapeutic strategies in patients with oral carcinoma (36). The data in the present study suggested that serum VEGF levels may have predictive and prognostic potential in patients with locally advanced resectable ESCC treated with chemotherapy or concurrent radiotherapy.

The present study has certain limitations which should be noted. First, post-operative serum VEGF levels in patients with locally advanced resectable ESCC were used as measurements and, serum VEGF levels may be affected by R0 resection. Lai *et al* reported a significant decrease in serum VEGF levels from pre- to post-surgery in non-small cell lung cancer (37). Therefore, the VEGF levels in patients with locally advanced resectable ESCC may have decreased after R0 resection. Second, the standard deviation of serum VEGF levels was relatively large, particularly following chemotherapy or concurrent radiotherapy, while serum VEGF levels in certain patients were always low before and during treatments, which suggested that the individual differences in VEGF were relatively large.

In conclusion, the present study demonstrated that patients with ESCC with a tendency for recurrence have high serum VEGF levels during chemotherapy or concurrent radiotherapy and high serum VEGF levels at recurrence compared with the pre-treatment stage. The serum VEGF levels no longer decrease in patients with recurrence following further treatment. The serum VEGF levels following chemotherapy or concurrent radiotherapy were associated with the OS and RFS of patients with locally advanced resectable ESCC. The serum VEGF levels in patients with recurrent ESCC were not influenced by the time duration between the post-operative stage to recurrence; however, low serum VEGF levels were associated with longer survival time following further treatment. Serum VEGF levels are critical for the prognosis and prediction of recurrence in patients with locally advanced resectable ESCC.

Acknowledgements

Not applicable.

Funding

The present study was supported by The 333 Project of The Science and Technology Department of Jiangsu Province (grant no. BRA2020389).

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

JW and RM designed the experiments. HC, ZW and JZ performed the experiments. HC managed the project. CJ and MD collated and investigated data. CJ, MD and XX analyzed the data. HX determined the serum VEGF concentration using the ELISA and Luminex methods and wrote the manuscript. RM reviewed the manuscript. HX, HC, JZ, CJ, ZW, JW, MD, XX and RM confirm the authenticity of all of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed under approval of Biomedical Research Ethics Committee of Jiangsu Cancer Hospital (approval no. 2010ke-052). Written informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Written permission for publication was obtained from all patients who participated in the present study.

Competing interests

The authors declare that they have no competing interests.

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