RESEARCH ARTICLE

Influence of Serum VEGF Levels on Therapeutic Outcome and Diagnosis/Prognostic Value in Patients with Cervical Cancer

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Abstract

Objective: To explore the influence of serum vascular endothelial growth factor (VEGF) level on therapeutic outcome and diagnosis/prognostic value in patients with cervical cancer. Materials and Methods: A total of 37 patients diagnosed with cervical cancer by biopsy were selected and treated with concurrent chemoradiotherapy. Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was adopted before treatment to assess VEGF levels, and its relationships with clinicopathological features and short-term therapeutic effects were analyzed. Results: The median VEGF level in 37 patients before treatment was 647.15 (393.35~1125.16) pg/mL. Serum VEGF levels in patients aged <50 years, in International Federation of Gynecology and Obstetrics (FIGO) stage IIIa~IVa, with lymph node metastasis and tumor size >4 cm were significantly increased ($P<0.05$). The complete remission (CR) rate was 48.7% (18/37), partial remission (PR) rate was 35.1% (13/37), stable disease (SD) rate was 13.5% (5/37) and progressive disease (PD) rate was 2.70% (1/37), so the objective remission rate (ORR) after treatment was 83.8% (31/37). Logistic regression analysis showed that tumor size and serum VEGF level before treatment were independent risk factors affecting the therapeutic outcome, and the higher the level of serum VEGF, the worse the prognosis when tumor size>4 cm. Some 56.8% of patients manifested with myelosuppression, 37.8% with leucopenia, 24.3% with thrombocytopenia, 5.41% with diarrhea, 46.0% with nausea and vomiting, 21.6% with hair loss and 8.11% with hepatic and renal injury during the treatment. Conclusions: Serum VEGF level may reflect the degree of malignancy of cervical cancer and predict therapeutic effect, which is of great importance to cancer diagnosis and prognosis.

Keywords: Cervical cancer - vascular endothelial growth factor - prognosis evaluation - auxiliary diagnosis

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Introduction

Cervical cancer is one of the most common gynecological malignant tumors. The data published by International Agency for Research on Cancer (IARC) shows that the estimated global annual number of new cervical cancer cases reaches up to 529 800 and 275 100 cases die from cervical cancer every year with the incidence ranking the third and mortality the fourth in female malignant tumors (Jemal et al., 2011).

Cervical cancer patients have no obvious symptoms and signs in the early stage and fail to receive treatment as early as possible due to shyness and other reasons, therefore, most patients have lost the opportunity of surgical treatment when seeing the doctor and concurrent chemoradiotherapy becomes the only option. Moreover, although the short-term effect of cervical cancer is significant, the rate of local recurrence and distant metastasis after treatment are higher, which leads to a large refractory (Peirson et al., 2013). Though different cervical cancer patients with the same International Federation of Gynaecology and Obstetrics (FIGO) tumor stage, histopathological type, pathological grade etc., choose the same treatment, there are big differences in the remission rate and long-term survival rate after treatment. Studies have shown that vascular endothelial growth factor (VEGF) signaling pathway in cervical cancer plays an important role in tumor progression and prognosis (Stepan et al., 2012; Barbu et al., 2013; Salomon-Perzyńska et al., 2014), but specific data to prove the diagnosis value of serum VEGF level in middle and advanced cervical cancer and its impact on therapeutic effect and prognosis is lacked. This study examined the serum VEGF levels of cervical cancer patients before and after treatment and followed them up combined with clinicopathological features, aiming to explore the clinical features and significance of serum VEGF level changes of patients with middle and advanced cervical cancer.

Materials and Methods

General data

A total of 37 patients initially visited in Xiangyang Central Hospital in Hubei Province from January 2013

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to January 2014 and diagnosed as cervical cancer by cervical biopsy served as study objects who were aged 23~62 years with the average age being (43.6±9.3) years; 56.76% (21/37) with menopause; 19 in stage IIb, 8 in stage IIIa, 9 in stage IIIb and 1 in stage IVa according to FIGO staging criteria; 30 with squamous carcinoma and 7 with adenocarcinoma, adenosquamous carcinoma or small cell carcinoma according to WHO histopathological grading; 10 in high differentiation and 27 in moderate and low differentiation; 21 with maximum neoplastci foci diameter ≤4 cm and 16 >4 cm; 13 with lymph node metastasis at the first visit and 24 without lymph node metastasis. Homochronous females with qualified physical examination, aged 24~67 years, with the average age being (45.1±7.2) years, were selected as control group. The research was approved by the Ethics Committee of the hospital, and all patients and their families signed the Informed Consent Form.

Methods

Concurrent chemoradiotherapy was used, three weeks a course, a total of two cycles. Radiotherapy: 6MV-X linear accelerator was used for pelvic external radiotherapy, with the total dose of pelvic center being 45 Gy. 192 Ir high dose rate was used for after-loading therapy, with the total dose at A point being 36~42 Gy.

Chemotherapy

Chemotherapy was conducted at the first day of radiotherapy, and TP scheme combined with chemotherapy was used. Paclitaxel 135 mg/m2 was intravenously dripped on day 1, and cisplatin 50 mg/m2 was intravenously injected on day 2~3.

Sample collection and detection

Sample collection: All patients were extracted about 5 mL venous blood at fasting state in the morning, serum was got at 4 °C after centrifugation for 15 min at a speed of 3500 r/min, and the supernatant was stored at -80 °C to be tested.

Sample detection: Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was adopted to detect VEGF levels. Human serum VEGF detection kits were purchased from Wuhan Cusabio Biotech Co., Ltd and operated in strict accordance with the steps in the kit instructions. BIO-RAD xMark Microplate Spectrophptometer enzyme-linked instrument was used for analysis.

Observational indexes

Pathological features of patients were recorded, including menopausal status, tumor differentiation degree, FIGO staging. Serum VEGF levels as well as clinical effects and adverse reactions were observed.

Evaluation criteria

Evaluation of target lesions refers to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1): ①Complete response (CR): All target lesions disappeares; ②Partial response (PR): The total maximum diameter of baseline lesions decreases by more than 30%; ③Progressive disease (PD): The total maximum diameter of baseline lesions increased by more than 20% or new lesions occurrs; ④Stable disease (SD): The total maximum diameter of baseline lesions decreased to a level, lower than PR or increased to a degree lower than PD. Evaluation of non-target lesions: ① CR: All non-target lesions disappeares or tumor marker levels are normal; ②SD: One or multiple non-target lesions and/or tumor marker levels, existing in a stable status, are higher than normal; ③PD: One or multiple new lesions occurrs and/or non-target progressive lesions. CR and PR must be rechecked and

Table 1. The Relationship Between Serum VEGF Levels and Clinicopathological Features of Cervical Cancer Patients

Clinicopathological feature	Case number n	VEGF/ (pg/mL) M (P_{25}~P_{75})	*Z* value	*P* value
Age				
<50	16	924.00 (538.30~1466.56)	2.1613	0.0307
≥50	21	599.12 (293.77~893.91)		
Menopausal status				
Premenopause	16	785.21 (592.94~1256.04)	1.7321	0.0833
Postmenopause	21	517.26 (343.67~1031.75)		
FIGO staging				
Stage IIb	19	393.35 (231.08~1200.55)	2.5677	0.0102
Stage IIIa~IVa	18	847.50 (626.54~1125.16)		
Histological type				
Squamous carcinoma	30	619.12 (387.97~950.14)	1.6481	0.0993
Adenocarcinoma/ adenosquamous carcinoma/small cell carcinoma	7	1046.91 (626.54~1843.27)		
Differentiated degree				
High differentiation	10	546.54 (196.76~801.09)	−1.5219	0.1280
Medium and low differentiation	27	665.77 (431.19~1360.87)		
Lymphnodes status at the first visit				
Positive	13	1046.91 (769.33~1460.33)	3.2291	0.0012
Negative	24	494.57 (288.37~779.84)		
Tumor size /cm				
≤4	21	471.87 (282.96~1031.75)	2.2533	0.0242
>4	16	785.21 (636.85~1343.01)		

confirmed at least 4 weeks after the first evaluation, and SD is confirmed at least 6 ~ 8 weeks after treatment with SD at least once in lesion measurement. The objective remission rate (ORR) = (CR+PR) /total cases × 100%.

Statistical data analysis

SAS 9.3 statistical package was used. Serum VEGF levels were skewness distribution and expressed by median and quartile [M (P_{25}~P_{75})], and rank-sum test was used to compare different clinical features. Enumeration data was expressed as a percentage using χ^2 test. The therapeutic effect related factors were analyzed by Logitic regression model. The difference was statistically significant when P<0.05.

Results

Serum VEGF level and its relationship with clinicopathological features of cervical cancer

Compared with the median VEGF level in 37 patients before treatment being 647.15 (393.35~1125.16) pg/mL, and serum VEGF level in patients aged <50 years, in FIGO stage IIIa~IVa, with lymph node metastasis and tumor size >4 cm was relatively higher, with the difference being statistically significant (P<0.05) (Table 1).

Analysis of serum VEGF levels and the short-term effect

CR rate was 48.65% (18/37), PR rate was 35.14% (13/37), SD rate was 13.51% (5/37) and PD rate was 2.70% (1/37), so ORR after treatment was 83.78% (31/37). Logistic regression analysis of factors affecting the therapeutic effect showed that tumor size and serum VEGF level before treatment were independent risk factors affecting the therapeutic effect, and the higher the level of serum VEGF, the worse the therapeutic effect when tumor size>4 cm (Table 2).

Adverse reactions in concurrent chemoradiotherapy for cervical cancer

Early complications of concurrent chemoradiotherapy were mainly myelosuppression and gastrointestinal reactions in period I ~ II, which could be tolerated and significantly improved after symptomatic treatment. In addition, some patients manifested with hepatic and renal injury, hair loss, thrombocytopenia and other adverse reactions (Table 3).

Table 2. Logistic Regression Analysis on Therapeutic Effect Related Factors

Relevant factors	B	Wald value	*P* value	OR	95%CI
Age	-0.825	0.616	0.433	0.438	0.056~3.438
Menopausal status	-1.169	0.840	0.360	0.311	0.026~3.786
FIGO staging	0.471	0.186	0.667	1.601	0.188~13.636
Histological type	0.638	0.191	0.662	1.892	0.108~33.138
Differentiated degree	-1.798	1.317	0.251	0.166	0.008~3.573
Lymph nodes at the first visit	-2.625	2.219	0.136	0.072	0.002~2.292
Tumor size	0.178	4.436	0.035	1.195	0.182~7.868
VEGF level before treatment	-0.005	4.819	0.028	0.995	0.991~0.999

Discussion

Cervical cancer, one of the malignant tumors badly threatening female health, is considered an important public health issue. Although some effective screening methods have been used for early diagnosis and screening for cervical cancer, most patients are already at an advanced stage when seeing the doctor or manifest with larger mass in the early stage due to the limitation of public education, cultural factors, etc., therefore, concurrent chemoradiotherapy becomes the most common option. Clinical observations show that radiosensitivities of different cervical cancer patients differ greatly, therefore, an effective molecular marker is desperately needed to be found to predict tumor invasion and metastasis potentials and help select treatment options and judge prognosis so as to change or amend treatment timely.

VEGF, also known as vascular permeability factor, has specific roles in vascular endothelial cells and is a multifunctional cytokine. Its main functions include promoting endothelial cell proliferation, improving vascular permeability, increasing the degree of hemangiectasis, promoting the movement of endothelial cells and eventually leading to angiogenesis. VEGF, one of the most directly functional and powerful growth factors currently found, is an indispensable part for tumor growth, invasion and metastasis. A study showed that VEGF could induce plasma protein extravasation which provided the matrix for tumor cell growth and establishment of a new capillary network by enhancing vascular permeability (Gong et al., 2014). So far, there are many studies dedicated to the relationship between serum VEGF levels and tumor behaviors of cervical cancer patients, but the results are different. Srivastava et al examined serum VEGF level changes of 110 cervical cancer patients and 50 healthy subjects before and after treatment and analyzed their correlation with clinicopathological features as well as other predictors, whose results showed that serum VEGF levels of cervical cancer patients were significantly higher than those of healthy subjects and closely related to clinical stage, tumor size, etc. (Srivastava et al., 2009). Another study of Zusterzeel et al, which got a similar conclusion, detected serum VEGF levels in 167 cervical cancer patients and found that serum VEGF levels, which were significantly correlated with tumor stage, tumor size and vascular compartment infiltration, had a significant impact on disease free survivals and overall survivals of the patients, and further multivariate COX regression analysis showed that serum VEGF levels were prognostic factors of cervical cancer (Zusterzeel et al., 2009). However, the study of Katanyoo et al showed that median serum VEGF level before treatment was 611.3 pg/mL, which was irrelevant to tumor stage, histological type, tumor size etc. (Katanyoo et al., 2011). The differences of serum VEGF levels in CR and RD patients after treatment were not statistically significant. Nagy et al also held

Table 3. Adverse Reactions in Concurrent Chemoradiotherapy for Cervical Cancer

Adverse reactions	Hepatic and renal injury	Hair loss	Nausea and vomiting	Diarrhea	Thrombocytopenia	Leukopenia	Myelosuppression
Occurrence rate [n (%)]	3 (8.11)	8 (21.62)	17 (45.95)	2 (5.41)	9 (24.32)	14 (37.84)	21 (56.76)

that VEGF wasn't a predictive factor of cervical cancer prognosis (Nagy et al., 2011). Univariate analysis showed that tumor size, expression levels of vascular endothelial growth factor receptor 2 (VEGFR2) and mRNA as well as age could affect the radiosensitivity and chemosensitivity of cervical cancer patients, while multivariate logistic regression model showed that VEGFR2 was the only predictive factor of tumor prognosis.

This study examined the serum VEGF levels of cervical cancer patients before and after treatment, followed them up combined with clinicopathological features, and found that serum VEGF levels were closely related to age, FIGO staging, lymph node metastasis and tumor size, while Logistic regression analysis showed that tumor size and serum VEGF level before treatment were independent risk factors affecting the therapeutic effect, which was consistent with part of the research results of Kim et al (Belfort-Mattos et al., 2010; Kim et al., 2011; Liu et al., 2013).

To sum up, the results of this study showed that increased serum VEGF levels before treatment might indicate a higher risk of poor therapeutic effect in middle and advanced cancer patients receiving concurrent chemoradiotherapy. Therefore, serum VEGF detection in patients with middle and advanced cervical cancer provides an important reference for prognosis and therapeutic effect evaluation, a preliminary basis for anti-angiogenesis treatment trials and ideas for individualized treatments for cervical cancer. However, due to the limited sample size of this study and undone analysis of overall survival rate, more large-scale and long-term clinical researches are needed to confirm the results of the present study.

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