RESEARCH COMMUNICATION

Pretreatment Levels of Serum Vascular Endothelial Growth Factor do not Correlate with Outcome in Patients with Locally Advanced Cervical Cancer

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Abstract

Objective: To evaluate pretreatment levels of serum VEGF in locally advanced cervical cancer patients, and assess any association with clinocopathological parameters and response to radiotherapy. Methods: Patients with histologically proven and diagnosed locally advanced cervical cancer or stages IIB-IVA were included in this study. Blood serum was obtained by peripheral venous puncture about 24 hours before the beginning of radiotherapy. All patients were followed up at one and three month intervals from the last day of the complete treatment for evaluating the responses to radiotherapy. Results: Mean age of the 40 patients was 52.8±11.1 years. Sixty percent were in stage IIB and 90% had squamous cell carcinoma. The median pretreatment level of serum VEGF was 611.3 pg/ml (0.00-4,067.20 pg/ml). The pretreatment levels of serum VEGF did not correlate with stage (p=0.75), tumor histology (p=0.91), tumor size (p=0.46) or tumor characteristics (p=0.49). Almost all patients received concurrent chemoradiation as a curative treatment, with a complete response found in 94.9%. Values for patients who were completed response was rather lower than patients with persistent disease, but without statistical significance (581.4 pg/ml vs 759.6 pg/ml, p=0.37). Conclusion: Pretreatment levels of serum VEGF do not correlate with clinicopathological factors or response to radiation therapy.

Keywords: Locally advanced cervical cancer - serum VEGF - clinicopathological parameters - radiotherapy

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Introduction

Cervical cancer is the second most common cancer in women worldwide (Parkin et al., 2002), including in Thailand (Sriplung et al., 2006). Standard treatment of locally advanced stage of cervical cancer (stage IIB-IVA) is concurrent chemoradiation (CCRT). In 2010, Chemoradiotherapy for Cervical Cancer Meta-analysis Collaboration (CCCMAC) analyzed that CCRT can increase local control, but the results from chemotherapy is less effect to decrease distant metastatis. Moreover, increasing stage conduct decreasing affect on chemotherapy when given at the time of concurrent with radiotherapy (CCCMAC, 2010). Therefore, new approaches to systemic therapy are needed. Recently, several studies have suggested that molecular targeted therapeutics may produce a solution to the current outcomes in gynecologic cancer treatment (Burger, 2010).

Angiogenesis is an important process for tumor proliferation and metastasis of solid tumor. Vascular endothelial growth factor (VEGF) is an important factor in normal and pathologic angiogenesis (Ferrara and Henzel, 1989; Senger et al., 1990; Folkman, 1992). Serum VEGF has been recognized as a surrogate marker of tumor angiogenesis (Ugurel et al., 2001). The rising of serum VEGF was correlated with progressive tumor, lymph node involvement and poor prognosis including breast, ovarian and cervical cancer (Kudelka et al., 1997; 1998; Bachtiary et al., 2002). However, the relationship between serum VEGF and tumor behavior in cervical cancer has been reported of limited number of studies with disagreement of results (Gadducci et al., 2007; Zusterzeel et al., 2009).

The purpose of this study was to evaluate pretreatment levels of serum VEGF in locally advanced cervical cancer patients and its association with clinocopathological parameters. Additionally, the aim of this study is to consider the relationship between serum (VEGF) levels and the response to radiotherapy.

Materials and Methods

Patients

An approval from the institutional Ethics Committee for Research Involving Human Subjects was obtained before conducting study. Forty patients with histologically proven and diagnosed as locally advanced stage (stage IIB-IVA) of cervical cancer were included in this study. All patients underwent radical radiotherapy with or without

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concurrent chemotherapy at the Radiation Oncology Unit, Faculty of Medicine, Vajira Hospital, University of Bangkok Metropolis between January 2009 and June 2010.

Tumor stage and histological diagnosis were defined according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). All patients were staged by clinical examination by radiation oncologist and gynecologist together. Informed consent was obtained from all patients before blood specimens were collected.

The treatment was composed of external beam radiotherapy to the pelvic region consisting of a total dose of 54-60 Gy applied in daily fractions of 1.8-2.0 Gy. Three to five fractions of intracavitary high dose-rate brachytherapy were applied on weekly fractions of 6.0-7.2 Gy each to point A, depending on tumor volume. Patients were seen in the first one and three month intervals after the last day of complete treatment to evaluate the response to radiotherapy. Persistent disease within the pelvis 3 months after completing of radiotherapy was defined as persistent disease.

Serum VEGF measurement

Blood serum was obtained by peripheral venous puncture about 24 hours before the beginning of radiotherapy. Blood samples were immediately stored on ice centrifuged at 2,500 g for 15 min at 4°C. The serum was frozen at -80°C until examination. For the measurement of serum VEGF, a commercially available enzyme-linked immunosorbent assay (ELISA) was used according to the manufacture's recommendations (RayBio® Human VEGF ELISA Kit Protocol). The results were calculated from the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points and expressed the results in picograms per milliliter (pg/ml) of serum. The sensitivity of less than 20 pg/ml was reported. This ELISA kit showed no cross-reaction with any of the cytokines tested. The intraassay and interassay variability are less than 10% and 12%, respectively.

Statistical analysis

Data were analyzed using SPSS statistical software, version 11.5 (SPSS Inc., Chicago, IL). Descriptive statistic was used to analyze clinicopathological data, which was summarized as number and percentage. Normal distribution of serum VEGF levels was tested using the Kolmogorov-Smirnov test. Due to the skewed distribution of serum VEGF, median and interquartile ranges were given. Comparisons between clinical subgroups of patients were tested using the Mann Whitney U test or the Kruskal Wallis test as appropriate. A two sided P-value <0.05 were considered statistically significance.

Results

Analysis of baseline characteristics of patients is based on data obtained from 40 patients, which included one patient who refused to get treatment. Mean age of patients was 52.8 ± 11.1 years (range, 27-76 years) More than half of all patients were in stage IIB and 90% of patients had squamous cell carcinoma as histologic cell type. These results were shown in Table 1.

The median pretreatment level of serum VEGF for 40 patients was 611.3 pg/ml (0.00-4,067.20 pg/ml). The lowest value of VEGF was found in patient with stage IIB, while patient who had the highest level was in stage IIIB. Both patients had tumor histology of squamous cell carcinoma and tumor size more than 4 cm. The pretreatment levels of serum VEGF did not correlate with stage, tumor histology, tumor size and tumor characteristic. Almost of patients got CCRT as treatment. Fourteen patients received cispaltin (40 mg/m^2 weekly), while 23 patients got carboplatin (2AUC weekly) as concurrent treatment with radiation.

Response to treatment was evaluated from 39 patients who got treatment completely. Thirty-seven patients (94.9%) showed complete response within 3 months after complete treatment. Two patients (5.1%) who had persistent disease got the salvage surgery including one patient who received radiation therapy alone, and another one got CCRT with weekly carboplatin. The pretreatment levels of serum VEGF of the patients who had a completed response was lower than the patients with persistent disease, but there was no statistical significance. All results

Table 1. Baseline Characteristics and Treatments of Patients (n = 40)

Characteristics		Number (%)
Stage	IIB	24 (60.0%)
	IIIB	16 (40.0%)
Histology	Squamous cell carcinoma	36 (90.0%)
	Adenocarcinoma	4 (10.0%)
Tumor size	< 4 cm	22 (55.0%)
	≥ 4 cm	18 (45.0%)
Tumor	Exophytic	17 (42.5%)
characteristic	Ulcero-infiltrative	23 (57.5%)
Treatment	Concurrent chemoradiation	37 (92.5%)
	Radiation therapy alone	2 (5.0%)
	No treatment	1 (2.5%)

Table 2. Pretreatment Levels of Serum VEGF in Different Clinicopathology and Treatment Outcomes

		N	Median VEGF (pg/ml)	Interquartile range	p-value
Stage					0.75
	IIB	24	629.9	406.0	
	IIIB	16	545.0	355.6	
Histology					0.91
	Squamous Cell Carcinoma	36	611.1	386.8	
	Adenocarcinoma	4	615.1	382.0	
Tumor size					0.46
	< 4 cm	22	575.5	397.7	
	≥ 4 cm	18	645.5	353.3	
Tumor characteristic					0.49
	Exophytic	17	612.7	372.4	
	Ulcero-Infiltrative	23	570.3	420.5	
Response to treatment (n=39)					0.37
	Complete Response	37	581.4	366.9	
	Persistent	2	759.6	-	

p-value is for non parametric Mann-Whitney U test

were shown in Table 2.

Discussion

The tumor angiogenesis has been studied in considerably advances about the biological processes and anti-angiogenic agents for more than three decades. The VEGF level is the most influence amongst the numerous factors involved in angiogenesis (Frumovitz and Sood, 2007). The relationship between serum VEGF and clinicopathological factors in cervical cancer is still controversy (Gadducci et al., 2007). Our results show that the pretreatment levels of VEGF in locally advanced stage of cervical cancer patients do not correlate with stage, tumor histology, tumor size and tumor characteristic. From previous studies, the association between VEGF and stage was shown (Lebrecht et al., 2002; Mitsuhashi et al., 2005; Zusterzeel et al., 2009), but Mitsuhashi et al., (2005) analyzed this correlation only in squamous cell type. Nonetheless, some authors reported that no correlation was found in staging factor (Moon et al., 2000; Bachtiary et al., 2002; Srivastava et al., 2009). For tumor histology, some literature and our study showed no significant difference of serum VEGF between squamous cell carcinoma and adenocarcinoma (Bachtiary et al., 2002; Zusterzeel et al., 2009). Whereas others demonstrated that adenocarcinomas was more angiogenic (Fujimoto et al., 1999; Santin et al., 1999). Furthermore, the relationship with tumor size was found by some authors (Mitsuhashi et al., 2005; Srivastava et al., 2009; Zusterzeel et al., 2009), although this correlation was not shown by Bachtiary et al., (2002). There is no study which reported about tumor characteristic with VEGF level. Our study did not demonstrate the tumor grade because of variation in system of pathological report.

The range of serum VEGF levels is very wide in our study, varying from 0.0-4,067.2 pg/ml. The largest study by Zusterzeel et al., (2009), which included 167 cervical cancer patients, showed that range of serum VEGF fluctuated from 3.0-5,360.0 pg/ml. Therefore, using VEGF as a tumor marker is unpractical. Moreover, each study used difference cutoff level of VEGF. Bachitary et al., (2002) chose the median pretreatment serum VEGF level of 244 pg/ml for their cutoff value. They found that all 19 patients with complete response for radiation therapy, 11 patients had serum VEGF < 244 pg/ml with no statistically significance. However, all four patients who had serum VEGR > 244 pg/ml had treatment failure (p-value=0.03). On the other hand, 581.7 pg/ml was chosen as the cutoff level by Lebrecht et al., (2002), but they did not show any correlation between this level and their treatment outcomes. Our study did not choose any level to be the cutoff point, but we found that the patients with persistent tumor after complete treatment had a trend of higher level of serum VEGF than patients with complete response from radiation therapy. However, there was no statistical significance due to a relatively small number of patients which is the limitation of our study.

All those studies included patients from stage I to stage IV, so there were small numbers of patients in locally advanced stage (stage IIB-IVA) (Bachtiary et al., 2002; Lebrecht et al., 2002; Mitsuhashi et al., 2005; Srivastava et al., 2009; Zusterzeel et al., 2009) which was the targeted population in our study. As a consequence of varying stages between each study, the relationship between pretreatment serum VEGF levels which were presented as the median are considerably different between the previous studies and our study. Additionally, one reason that might be the explanation of these equivocal results from each study is that VEGF play an important role in tumor angiogenesis, but it is not the only determining factor. Moreover, angiogenesis in malignancies is a dynamic process. Therefore, serum VEGF level is supposed to be only a snapshot impression and very sensitive to change (Zusterzeel et al., 2009).

In summary, this is the pilot study of Asian-Thai locally advanced cervical cancer patients. We found that pretreatment level of serum VEGF do not correlate with clinicopathological factors and response to radiation therapy. However, the long term follow-up is needed to investigate the correlation between serum VEGF and other treatment outcomes including progression-free survival and overall survival.

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