

## Research Article

## Serum Level of Vascular Endothelial Growth Factor (VEGF) can be used to Assess Response of Radiation Therapy in Cervical Cancer

### Kadar Serum Vascular Endothelial Growth Factor (VEGF) dapat Digunakan untuk Menilai Respons Terapi Radiasi pada Kanker Serviks

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#### Abstract

**Objective:** To compare the sensitivity and specificity of Squamous Cell Carcinoma (SCC) and Vascular Endothelial Growth Factor (VEGF) levels to assess the response of radiation therapy.

**Method:** The study was conducted by the method of analytic observational cohort study in 24 patients with cervical cancer stage II-B and III-B in RSCM that met inclusion criteria. Examination of VEGF and SCC in serum samples was performed in the Prodia Laboratory Jakarta. The examination was conducted twice before and after radiation therapy. The subjects were treated by radiation therapy/chemoradiation according to standard procedures. After the completion of radiation was declared, the response of radiation therapy was conducted by clinical assessment.

**Result:** Of the 24 subjects, we obtained a mean level of SCC pre-radiation was  $23.43 \pm 5.84$  ng/ml and post-radiation was  $2.19 \pm 0.68$  ng/ml. The mean VEGF pre-radiation was  $790.41 \pm 111.06$  pg/ml and post-radiation was  $497.47 \pm 79.26$  pg/ml. ROC curves of each tumor marker obtained SCC pre-radiation AUC 40%,  $p$  0.53 (CI 0.18-0.68) and SCC post-radiation AUC 48.1%,  $p$  0.91 (CI 0.21-0.75) can not be used as a diagnostic and prognostic factors of response to radiation therapy. VEGF pre-radiation produced an AUC of 17.5%,  $p$  0.04 (CI 0.00-0.36), thus cannot be used as a prognostic factor for response to radiation therapy. VEGF after radiation produced an AUC of 92.5%,  $p$  0.01 (CI 0.81-1.00), thus can be a diagnostic factor for response to radiation therapy. VEGF post-radiation with cut-off point 614.75 pg/ml had a sensitivity 80%, specificity 75%, NDP 94.12%, NDN 42.86%; RKP 3.2; RKN 0.26 and accuracy 79.16%. There is a significant correlation between the decrease of serum VEGF level post-radiation and a positive response of radiation therapy ( $p$  0.01, CI 1.00-3.23).

**Conclusion:** Examination of VEGF levels can be used to assess the response of radiation therapy with a sensitivity of 80% and specificity of 75%.

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**Keywords:** cervical cancer, SCC, therapeutic response, VEGF

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#### Abstrak

**Tujuan:** Penelitian ini bertujuan membandingkan sensitivitas dan spesifisitas kadar SCC dengan VEGF untuk menilai respons terapi radiasi.

**Metode:** Penelitian dengan metode studi kohort analitik observasional terhadap 24 subjek penelitian yaitu penderita kanker serviks stadium II-B dan III-B di RSCM yang memenuhi kriteria inklusi. Pemeriksaan VEGF dan SCC pada sampel serum dilakukan di Lab. Prodia Pusat Jakarta, sebanyak dua kali pemeriksaan yaitu sebelum dan sesudah terapi radiasi. Subjek ditatalaksana dengan terapi radiasi/kemoradiasi sesuai prosedur standar. Setelah terapi dinyatakan selesai, kemudian dilakukan penilaian respons terapi secara klinis.

**Hasil:** Dari 24 subjek penelitian didapatkan rerata kadar petanda tumor SCC praradiasi  $23,43 \pm 5,84$  ng/ml dan pascaradiasi  $2,19 \pm 0,68$  ng/ml. Rerata VEGF praradiasi  $790,41 \pm 111,06$  pg/ml dan pascaradiasi  $497,47 \pm 79,26$  pg/ml. Dari kurva ROC masing-masing petanda tumor didapatkan SCC praradiasi AUC 40%;  $p$  0,53 (IK 0,18-0,68) dan SCC pascaradiasi AUC 48,1%;  $p$  0,91 (IK 0,21-0,75) tidak dapat dipakai sebagai faktor prognostik maupun diagnostik terhadap respons terapi radiasi pada kanker serviks. VEGF praradiasi AUC 17,5%;  $p$  0,04 (IK 0,00-0,36) tidak dapat dipakai sebagai faktor prognostik terhadap respons terapi radiasi pada kanker serviks. VEGF pascaradiasi AUC 92,5%;  $p$  0,01 (IK 0,81-1,00) dapat menjadi faktor diagnostik terhadap respons terapi radiasi pada kanker serviks. VEGF pascaradiasi dengan titik potong (cut-off point) 614,75 pg/ml memiliki sensitivitas 80%; spesifisitas 75%; NDP 94,12%; NDN 42,86%; RKP 3,2; RKN 0,26 dan akurasi 79,16%. Terdapat korelasi bermakna antara penurunan kadar serum VEGF pascaterapi dengan respons radiasi positif ( $p$  0,01; IK 1,00-3,23).

**Kesimpulan:** Pemeriksaan kadar VEGF dapat digunakan untuk menilai respons terapi radiasi dengan sensitivitas 80% dan spesifisitas 75%.

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**Kata kunci:** kanker serviks, respons terapi, SCC, VEGF

#### INTRODUCTION

Cervical cancer is the primary cancer of the cervix (cervical canal and/or portio). Cervical cancer

ranks first in overall cancer incidence in Indonesian women.<sup>1</sup> Approximately 70% of cervical cancer patients came at advanced stage (> II-B). Re-

search by Sarika (2006) found from the 465 new cases of cervical cancer, most cases were diagnosed as stage III-B with the most common histopathology was squamous cell carcinoma.<sup>2</sup>

Primary therapy of advanced cervical cancer is radiation. But radiation therapy is known to be able to cause complications, such as fibrosis that causes stiffness in the radiated tissue. Advanced cervical cancer generally have a large tumor size and extension. Radiation therapy may not kill cancer cells in the central region. Assessment of clinical response is based solely on the findings of the response at the surface, so it will face obstacles in the face of treatment response. One method to assess treatment response is the examination of tumor markers.

SCC antigen is a tumor marker for the presence of squamous cells that produce serine protease inhibitor. Some studies found that serum SCC can be used to monitor the onset of cervical squamous cell carcinoma after primary therapy. SCC serum levels were still high and an increase after treatment showed a tumor or progressive disease. Using a cut-off value of SCC 3.0 ng/ml Strauss HG (2002) et al detect levels of serum SCC antigen pre-operative as an independent prognostic factor in squamous cell cervical cancer, both of recurrence-free and overall survival. From the analysis concluded that SCC antigen as tumor markers correlate with prognosis in operability of cervical cancer, tumor size, status of pelvic nodes, cervical stromal infiltration, and the degree of spread of the tumor into the parametrium.<sup>3</sup>

Hong JH (1998) et al who examined the levels of squamous cell carcinoma (SCC) found that levels of SCC antigen > 10 ng/ml is an independent predictor of poor prognosis in cervical cancer and can be used as a prognostic factor in selecting patients for intensive therapy. SCC levels were still high after radiation therapy is a strong predictor of treatment failure.<sup>4</sup>

Vascular endothelial growth factor (VEGF) plays an important role in tumor angiogenesis by increasing vascular permeability, endothelial cell growth, proliferation, migration and differentiation. VEGF can also facilitate the extravasation of tumor cells and then metastasize by way of destroying the tumor extracellular matrix wall by activating the proteolytic enzymes.<sup>5</sup>

Although a lot of evidence to suggest that VEGF

plays a role in microinvasion at an early stage, there has been no extensive studies to assess VEGF expression as a prognostic factor in locally advanced cervical cancer, including radiation therapy outcomes.<sup>6,7</sup>

Evaluation of the expression levels of VEGF are also useful in assessing response to therapy, such as post-radical hysterectomy, chemoradiation, and even monitor the results of treatment with anti-angiogenesis drugs. This research was undertaken to assess whether serum VEGF levels have prognostic value and can be used in the treatment of cervical cancer patient.<sup>8,9</sup>

As tumor markers both VEGF and SCC need to be explored to determine which one is more sensitive and specific for assessing response to radiation therapy in cervical cancer.

## METHODS

This study was a cohort study, which examined the serum levels of VEGF and SCC antigen in patients with cervical cancer stage IIB - IIIB before and after radiation therapy. The objective was to determine the relationship between the serum level of VEGF and SCC with radiation therapy response and then compare the sensitivity and specificity of VEGF and SCC to the response of radiation therapy.

The study population was all cervical cancer patients who came to RSUPN Dr. Cipto Mangunkusumo Jakarta with the following inclusion criteria, clinical stage IIB-IIIB (according to FIGO) with histopathology results squamous cell carcinoma; underwent chemoradiation/radiation; does not have any other systemic diseases that affect the levels of serum SCC antigen and VEGF and has not received treatment for cancer.

Patients were managed by chemoradiation or radiation in accordance with the study protocol. After chemoradiation or radiation has completed, another venous blood sampling was taken again about 2 (two) weeks after radiation therapy completed, to examine VEGF and SCC antigen serum levels and do clinical examination to assess the response of chemoradiation therapy.

Chemoradiation or radiation therapy response is a clinical response obtained from clinical examination 1 month after chemoradiation or radiation therapy is complete, consisting of complete response, partial response, no response/stable and

progressive. In this study, interpretation was divided into two groups of responses, positive response = complete response and negative response = response beside complete response.

RESULTS

The study subjects was 24 patients with advanced cervical cancer, which comprised of 9 patients stage II-B and 15 patients stage III-B. The tumor markers, Squamous Cell Carcinoma Antigen (SCC antigen) and Vascular Endothelial Growth Factor (VEGF), was measured twice, before and after radiation. The mean level of SCC pre-radiation was  $23.43 \pm 5.84$  ng/ml and post-radiation was  $2.19 \pm 0.68$  ng/ml, and the mean level of VEGF pre-radiation was  $790.41 \pm 111.06$  pg/ml and post-radiation was  $497.47 \pm 79.26$  pg/ml.

Measurement of tumor markers pre-radiation was performed to assess whether the SCC and VEGF could be used as a prognostic factor of response to radiation therapy. While the post-radiation was examined to assess whether the SCC and VEGF have diagnostic value to replace the clinical judgement that had been done before. Based on measurements using ROC curves, we obtained respectively SCC tumor markers and VEGF for therapeutic response. (Table 1)

**Table 1.** Results of ROC Curves of Tumor Markers and Response of Radiation Therapy

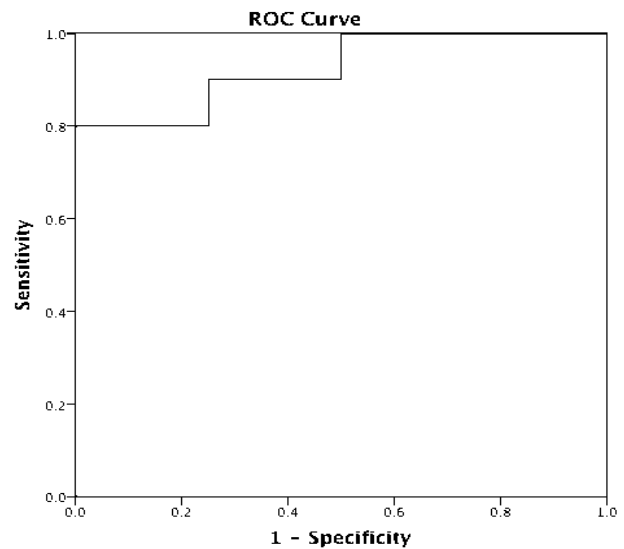
Tumor markers	AUC (%)	p	CI 95%
SCC:			
Pre-radiation	40.0	0.53	0.18 - 0.68
Post-radiation	48.1	0.91	0.21 - 0.75
VEGF:			
Pre-radiation	17.5	0.04	0.00 - 0.36
Post-radiation	92.5	0.01	0.81 - 1.00

AUC: area under curve

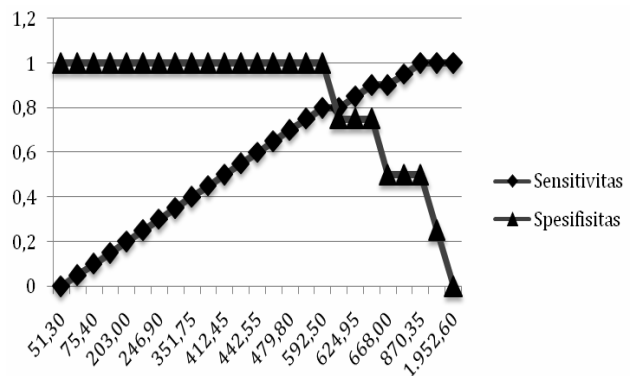
VEGF post-radiation with AUC 92.5% results, show that VEGF levels are excellent (AUC > 90%) to be used as a diagnostic factor in diagnosing response of radiation therapy in cervical cancer with p: 0.01 (CI: 0.81 - 1.00). (Fig. I)

To get the cut-off point of serum VEGF levels after radiation, we made a graph of VEGF sensitivity and specificity values. (Fig. II)

Post radiation serum VEGF levels at 614.75 pg/ml had a highest sensitivity and specificity values, with sensitivity 80% and specificity 75%. According to the 2x2 table with a value of 614.75 pg/ml as the cut-off point on the response of radiation therapy, we obtained the following results. (Table 2)



**Figure 1.** ROC Curve of VEGF Serum Levels in Post-radiation Patients.



**Figure 2.** Cut-off Value from Sensitivity and Specificity of VEGF Levels in Post-Radiation Patients.

**Table 2.** Diagnostic Value of VEGF (pg/ml) Post-Radiation and Response of Radiation Therapy.

VEGF value	Response of therapy				Total
	Positive	(%)	Negative	(%)	
≤614.75	16	94.1	1	5.9	17
>614.75	4	57.1	3	42.9	7
Total	20	83.3	4	16.7	24

From the table above, the value obtained sensitivity 80%, specificity 75%, positive predictive value (PPV) 94.12%, negative predictive value (NPV) 42.86%, positive likelihood ratio (PLR) 3.2; negative likelihood ratio (NLR) 0.26 and accuracy 79.16%.

Table 3 below shows the trend of up and down in both serum tumor markers SCC and VEGF for therapeutic response.

**Table 3.** Classification of Tumor Markers pre and Post Radiation and Therapeutic Response.

Tumor markers	Response of radiation therapy				RR	CI 95%	p
	Pos	(%)	Neg	(%)			
SCC:							
• Increase	18	85	3	14.3	1.29	0.57 - 2.92	0.44
• Decrease	2	66.6	1	33.3			
VEGF:							
• Increase	15	100	0	0	1.80	1.00 - 3.23	0.01
• Decrease	5	55.6	4	44.4			

It appears that the decline in serum levels of SCC from pre to post-radiation did not correlate with a positive response (p 0.44; CI 0.57 to 2.92). While the decline in serum levels of VEGF in pre and post-radiation therapy correlated with a positive response (p 0.01, CI 1.00 to 3.23).

## DISCUSSION

Based on the pathology report in 2002, cervical cancer ranks first out of 10 cases of most cancers in both men and women and cancer in women only, with a total of 2532 cases.<sup>10</sup> From the results of tumor markers measurements, it appears that the SCC tumor markers can not be used to assess response of radiation therapy. SCC pre-radiation levels reached only 40% AUC with p: 0.53 (CI: 0.18 to 0.68). These results indicate that the SCC pre-

radiation can not be used to assess the response to radiation therapy in cervical cancer. The SCC levels reached 48.1% AUC after radiation with p: 0.91 (CI: 0.21 to 0.75) indicating that the SCC also can not substitute the clinical assessment of therapeutic response.

VEGF as tumor markers was also measured before and after radiation. VEGF results obtained pre-radiation covers only AUC 17.5%, although the p: 0.04 (CI: 0.00 - 0.36), as same as SCC, can not be used for assessing response to radiation therapy in cervical cancer. But VEGF post-radiation with AUC 92.5% results, show that VEGF levels are excellent (AUC > 90%) to be used as a diagnostic factor in diagnosing response of radiation therapy in cervical cancer with p: 0.01 (CI: 0.81 - 1.00).

Hong JH (1998) et al examined the levels of squamous cell carcinoma (SCC) and found that le-

vels of SCC antigen > 10 ng/ml was an independent predictor of poor prognosis in cervical cancer, and could be used as a prognostic factor in selecting patients for intensive therapy. High levels of SCC antigen after radiation therapy was a strong predictor of treatment failure.<sup>4</sup>

Some studies find that serum SCC antigen can be used to monitor the incidence of cervical squamous cell carcinoma after primary therapy. SCC antigen serum levels which remains high or even increase after treatment showed a tumor or progressive disease.<sup>3,11,12</sup>

Radiation therapy is the primary treatment modality in invasive cervical cancer and can achieve satisfactory results in patients with early stage. While in patients with advanced stage therapeutic modalities are still many failures. Preliminary re-

search conducted by Hong JH et al (1998) showed that there is a failure rate of 30% in patients with squamous cell carcinoma of the cervix with large lesions (bulky) stage IB-IIA and IIB after definitive radiation therapy, and will increase to 50% in patients with stage III.<sup>3,4,7</sup>

Evaluation of the expression levels of VEGF are also useful in assessing therapeutic response, such as post-radical hysterectomy, chemoradiation, and even monitor the results of treatment with anti-angiogenesis drugs.<sup>7,8</sup>

Serum levels of VEGF post-radiation with AUC 92.5% results, show that VEGF levels are excellent (AUC > 90%) to be used as a diagnostic factor in diagnosing response of radiation therapy in cervical cancer with  $p$ : 0.01 (CI: 0.81 - 1.00).

Cheng WF get a mean (median) intra-tumoral VEGF protein in patients with cervical cancer was 180 pg/mg, whereas in normal tissue 0 pg/mg. Large lesions > 4 cm than < 4cm (1030 compared to 118). Limfo-vascular invasion than not (568 compared to 118) and patients with lymph-nodes metastasis than without metastasis (795.5 compared to 121 pg/mg). Over-expression of VEGF obtained from immuno-histochemical examination of 10/20 (50%) in cases with metastatic nodes, while the nodes without metastasis at 16/84 with  $p$  = 0.002.<sup>13</sup>

In this study, analysis of ROC curve and sensitivity and specificity graphs showed that VEGF levels of 614.75 pg/ml had a highest sensitivity (80%) and specificity (75%). The results suggest that serum VEGF levels after radiation with a cut-off 614.75 pg/ml can be used to diagnose the response of radiation therapy in cervical cancer, as mention from the values of sensitivity, specificity, PPV, NPV and sufficient accuracy, although the value of PLR 3.2 (<10) and NLR 0.26 (> 0.1) is still inadequate.

Hansgen et al which examined serum VEGF in 42 patients with locally advanced (FIGO II-IV) squamous cell cervical cancer who were treated with radiation, found that the concentration of VEGF did not correlate with tumor stage. Comparison of VEGF levels with clinical outcome after 6 months of therapy with patients obtained a complete response occurred significantly decreased levels of VEGF (304 pg/ml  $\pm$  188) compared to patients with symptoms of tumor (892 pg/ml  $\pm$  756,  $p$  < 0.0005). Thus concluded that high serum VEGF levels before therapy associated with poor res-

ponse to radiation therapy in locally advanced cervical cancer.<sup>14-16</sup>

This study also found that the reduction in VEGF serum levels pre and post-radiation were associated with a positive treatment response ( $p$  0.01, CI 1.00 to 3.23). Thus if serum VEGF decrease after radiation therapy, the therapeutic response will be positive.

Loncaster et al examined 100 patients with locally advanced cervical cancer (IB large lesions up to IIB), which consisted of 94 squamous cell carcinomas, 5 adenocarcinomas and 1 adenosquamous carcinoma, found that there is no correlation between VEGF expression with disease stage, tumor differentiation, patient age and tumor radio-sensitivity. In survival analysis, VEGF expression was an independent prognostic factor of the most significant ( $p$  = 0.001).<sup>6,13-17</sup>

This study suggests that serum VEGF levels post radiation can be used to be a diagnostic factor for response of radiation therapy in advanced cervical cancer with AUC 92.5%,  $p$  0.01 (CI: 0.81 to 1.00). VEGF serum levels with cut-off 614.75 pg/ml had a sensitivity of 80%, specificity 75%, PPV 94.12%, NPV 42.86%, PLR 3.2, NLR 0.26 and accuracy 79.16%. And there is a significant correlation between the decrease in serum VEGF levels after radiation therapy with a positive response of radiation therapy ( $p$  0.01, CI 1.00 to 3.23).

## CONCLUSION

Examination of VEGF levels can be used to assess the response of radiation therapy with a cut off point of 614.75 pg/ml, with high sensitivity (80%) and specificity (75%). There is also a significant correlation between the decrease in serum VEGF levels after radiation therapy with a positive response of radiation therapy.

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