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Correlation of VEGF-C tissue expression and cervical lesion diameter on cervical cancer patients given neoadjuvant therapy

Heru Priyanto[1,2*], Ambar Mudigdo[3], Andrijono[4], Bhisma Murti[5]

ABSTRACT

Background: Management of cervical cancer is still debated. The diameter of cervical lesions are predictors of lymph node metastases, lymphovascular invasion, survival rates and are related to cell hypoxia. VEGF-C plays a role in the process of angiogenesis and lymphangiogenesis which are important for metastasis.

Objective: To describe the correlation between VEGF-C tissue expression and the diameter of the cervical cancer lesion before and after being given neoadjuvant chemotherapy.

Methods: We conducted an observational study using consecutive sampling in the Obstetrics Gynecology and Anatomy Pathology Department of Dr. Moewardi Hospital, the teaching hospital of the Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia. A total of 30 tissue biopsies of IB2 and IIA2 cervical cancer patients before and after undergoing Paxus-Carboplatin chemotherapy were examined for immunohistological expression of VEGF-C. The diameter of the largest cervical lesions of each patient was recorded.

Result: The mean of the largest diameter of the cervical lesion prior to neoadjuvant chemotherapy was bigger than after neoadjuvant chemotherapy (5.62 vs. 3.50, p<0.001). A decrease in VEGF-C tissue expression was significantly related to the decrease in the diameter of the largest cervical lesion after neoadjuvant chemotherapy administration (p=0.008).

Conclusion: There is a significant negative correlation between VEGF-C tissue expression with the diameter of the cervical lesions given neoadjuvant chemotherapy.

Keywords: cervical cancer, tissue VEGF-C, cervical lesion diameter, neoadjuvant chemotherapy
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[1]Doctorate Program in Medical Sciences, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
[2]Department of Obstetrics and Gynaecology, Faculty of Medicine, Sebelas Maret University/ Dr. Moewardi Hospital, Surakarta, Indonesia
[3]Department of Pathology Anatomy, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi Hospital, Surakarta, Indonesia
[4]Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Indonesia, Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia
[5]Magister Program of Public Health, Faculty of Medicine, Sebelas Maret University, Indonesia

*Corresponding to:
Heru Priyanto Samadi,
Gyne-oncologist
Oncology Division - Obstetry Gynecology Department Dr. Moewardi Hospital, Sebelas Maret University
drherupriyanto@yahoo.com

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INTRODUCTION

Cervical cancer, the fifth most common cancer in women worldwide, has mortality to incidence ratio of 55%.[1,2] In Indonesia, the cancer is the most commonly found malignancy in women (17.8%). In Dr. Cipto Mangunkusumo General Hospital, Indonesia, cervical cancer was accounted for 76.2% of 1,717 gynecological cancers from 1989-1992 with an overall 5-year survival rate ranging from 56.7% to 72%.[1,2]

Vascular Endothelial Growth Factor (VEGF) is a key signal used by hypoxia cells to trigger blood vessel growth.[3,4] When a tumor has been greater than 1 millimeter, it causes the cells to be deprived of oxygen and energy unless new blood vessels are formed. The larger the size of tumor cells, the more hypoxic the cells will be. Thus, the expression of VEGF-C tissue is also increasing. Various factors trigger the synthesis of VEGF. The main stimulating factor is hypoxic tissues or cells. The other factors are various cytokines (PDGF, EGF, IGF, and many more), COX-2 and various oncogenes.[3,4]

Vascular endothelial growth factor-C (VEGF-C) is one of the VEGF derivatives. In addition to its role in vasculogenesis, it also plays a role in the lymphangiogenesis process, the formation process of new lymphatic channels that play an essential role for metastasis. Vascular endothelial growth factor-C (VEGF-C) may work both in blood vessels and lymph vessels through activation of the VEGF R2 receptor. VEGF-C / VEGF R3 induction signal is known to play an important role in the process of lymphangiogenesis and metastasis in sentinel nodes.[5]

One of the options for early treatment of cervical cancer is the administration of neoadjuvant chemotherapy. Neoadjuvant chemotherapy is given in an early-stage cervical cancer before radical hysterectomy is performed, especially in patients with lesions greater than 4 cm which is called bulky lesions. One of the objectives of giving neoadjuvant chemotherapy is to shrink large lesions in the cervix or invasion of the vaginal wall, thereby increasing the radicality of the operation.[6,7] A shrinking large lesions in the cervix reduces the need for oxygen in cancer cells. Therefore, HIF 1-α expression will decrease, followed by decreased transcription of the VEGF-C gene as well. As a result, the synthesis of

m-RNA VEGF-C also decreases. It is desirable that the production of VEGF-C expressed in the tissue also decreases, followed by a decrease in VEGF-C secreted into the circulation.[6,7]

Assessment of the chemotherapy response can be done based on the Response Evaluation Criteria in Solid Tumors (RECIST) by looking at the diameter reduction of cervical lesions.[6,8,9] the evaluation of the lesion size can be done using various methods, including a clinical examination, imaging with ultrasound, CT scan and MRI. However, advanced cervical cancer was difficult to evaluate because of the invasion of the parametrium. Some argue for increasing the accuracy of the assessment of therapeutic response using RECIST; additional new biomarkers are needed.[4,8,10] Biomolecular markers indicative of the ability of cancer cells to develop and to metastasize are expected to be used as a mean to evaluate the cancer response to neoadjuvant chemotherapy.

Our study aims to explore the correlation between VEGF-C tissue expression and the diameter of cervical cancer lesion before and after being given neoadjuvant chemotherapy.

SUBJECTS AND METHODS

The observational study was done from January to April 2017. Patients who visited the oncology gynecology clinic underwent a clinical examination and a clinical measurement of cervical lesions using transabdominal ultrasonography. The samples were recruited based on the inclusion and exclusion criteria. The inclusion criteria were: patients with stage IB2 or IIA2 cervical cancer who have never received any medical treatment for cancer, the biopsy showed cervical carcinoma, and there is no contraindication to chemotherapy administration. The exclusion criteria were: any other tumor or cancer other than cervical cancer is found, or the patient refuses to participate in the study. Any of these following situations cause a dropout: the patient did not finish all three rounds of chemotherapy, the disease becomes progressive after only two rounds of chemotherapy, or the patient refuses to participate anymore in the study.

The demographic data were collected from their medical record. The patients were planned for neoadjuvant therapy. The neoadjuvant chemotherapy consists of Paclitaxel 175 mg per square meter of body-surface area and Carboplatin which dose was calculated as follows: 6 (AUC) x (25 + GFR). The chemotherapy was given for three rounds. The interval between each round was three weeks.

Before and after the neoadjuvant chemotherapy, the patients underwent a clinical measurement of cervical lesions using transabdominal ultrasonography, and the cervical tissue was biopsied. The tissue obtained was immediately fixed in 10% neutral-buffered formalin for routine pathologic examination. Sections were rehydrated and embedded in paraffin wax. The sections were then dewaxed, incubated with VEGF-C antibody from Abcam Cambridge Science Park, stained with hematoxylin, and examined under an Olympus Cx 21 microscope with 400 times of magnification.

The VEGF-C tissue expression was evaluated based on a method reported by Birner *et al.*[11] The area colored by the reagent was given a score of 1 if 0-10% were colored, 2 for 11-50%, 3 for 51-80%, and 4 for >80%. The intensity of the reagent color was given a score of 1 when weak, 2 when moderate, and 3 when intense. Both scores were summed to obtain the VEGF-C tissue expression score.

RESULT

Our initial samples were 40. However, there were 10 dropouts so that we eventually analyzed 30. The characteristics of the sample are shown in Table 1. The largest proportion of research was 40 to 60 years old (73.3%), with median age 47. The youngest was 28 years old, and the oldest was 63. Based on the histological examination, the samples consist of 25 (83.3%) were squamous cell carcinomas, 4 (13%) were adenocarcinoma, and 1 (3%) was small cell carcinoma.

Table 2 shows the distribution of the before and after neoadjuvant chemotherapy cervical lesion diameter are normal ($p>0.05$). Table 3 shows the mean of the largest diameter of cervical lesions prior to neoadjuvant chemotherapy is significantly larger than after ($p<0.001$).

Table 4 showed the analysis of the VEGF-C tissue expression before and after difference when compared to the lesion diameter difference. The analysis showed that there is a significant inverse correlation ($r=-0.474$. $p=0.008$) between the variables.

DISCUSSION

In this study, the samples are patients with cervical cancer stage IB2 and IIA2 that have no invasion of the parametrium. We assessed the reduction in primary tumor diameter in the cervix after neoadjuvant chemotherapy to evaluate the response to chemotherapy.

Based on age, 16.67% of the samples were less than 40 years old, 73.33% were 40-60 years old, and 10% were over 60 years old. This age-based incidence corresponds to data from Aziz F who conducted a study in 2002, showing that the most

Table 1. Sample characteristics

Characteristics	f	%
Age		
<40 years old	5	17%
40-60 years old	22	73%
>60 years old	3	10%
Education		
Elementary school	15	50%
Junior high school	4	13%
High school	8	27%
Diploma	1	3%
Graduate school	2	7%
Parity		
0-2	8	27%
2-4	21	70%
>4	1	3%
Cancer histology		
Squamous cell carcinoma	25	83%
Adenocarcinoma	4	13%
Small cell carcinoma	1	3%

Table 2. Normality test of the largest diameter of cervical lesions before and after neoadjuvant chemotherapy

Lesion diameter	Shapiro-Wilk
Before neoadjuvant chemotherapy	0.264
After neoadjuvant chemotherapy	0.765

Table 3. Paired T-test comparing the largest diameter of the cervical lesion before and after neoadjuvant chemotherapy administration

	N	Mean of the largest cervical lesion diameter	Median	SD	p
Before chemotherapy administration	30	5.62	5.45	0.91	<0.001
After chemotherapy administration	30	3.50	3.45	0.88	

Table 4. The Spearman's rank test to evaluate the correlation between the difference of VEGF-C expression and difference the largest diameter of the cervical lesion

	Largest cervical lesion diameter
VEGF-C tissue expressions	
r	-0.474
p	0.008

cervical cancer patients in Cipto Mangunkusumo Hospital Jakarta were in the 45-54 years age group.[6]

The histopathology examination shows 83.33% were squamous cell carcinoma, 13.33% were adenocarcinoma, and 3.33% were small cell carcinoma. The histopathology is related to HPV infection. Based on the meta-analysis of 12 countries, the prevalence of HPV infection in squamous carcinoma was 78.4-98.1%, whereas in the case of adenocarcinoma the prevalence was 85.7-100%.[12]

Our study showed that there is a significant reduction in lesion size after the administration of neoadjuvant chemotherapy. Our result is in line with the study by Franc *et al.* which concluded that VEGF-C expression increased by the increase in the stage of cervical cancer.[13]

Our analysis showed that there is a significant negative correlation between the difference in immunohistochemical VEGF-C tissue expression with the difference in the diameter of cervical lesions. The result means that the less the difference of the lesion diameter, the more the difference of the VECF-C expressed in the tissue after the chemotherapy. The shrinking of the lesions after chemotherapy is related to the principle of the healing process, which includes resorption and regeneration. However, it took longer for the lesion to shrink than the expression or secretion biomarkers to decrease. When cancer cells die due to exposure to chemotherapy, some biomarkers will be instantly less expressed in tissues or less secreted in serum. Thus, rather than evaluating the gross appearance, evaluating the correct biomarker enables a physician to assess the effectiveness of chemotherapy much earlier.[14] Because the RECIST method relies on an imaging evaluation of the lesions, the evaluation of the response to therapy using the RECIST method does not reflect the actual cell-level activity and biomolecular process.[15]

CONCLUSION

There was a negative correlation between VEGF-C tissue expression with a diameter of cervical lesions in cervical cancer which given neoadjuvant chemotherapy Paclitaxel-Carboplatin with statistically moderate and significant correlation (r= -0. 47, p=0.008). More research is needed to confirm whether VEGF-C tissue expression is the ideal biomarker to evaluate the therapeutic response of cervical cancer when exposed to neoadjuvant-chemotherapy.

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