The blood samples were taken, the serums were separated, VEGF-C serums from 33 patients in Oncology clinic, Dr. Cipto Mangunkusumo Hospital, Indonesia. The subjects were all of the early stage cervical cancer patients. The purpose of this research was nested case control and consecutive sampling. The subjects were all of the early stage cervical cancer patients. Endostatin serums were examined by ELISA method, and endostatin serums were examined by Immunoassay method. Patients went through radical hysterectomy surgeries, and then pathology anatomy examinations.

**Method:** This research was done in Oncology Division, Department of Obstetrics and Gynecology Faculty of Medicine University of Indonesia, Dr. Cipto Mangunkusumo Hospital, Jakarta. The design of this research was nested case control and consecutive sampling. The subjects were all of the early stage cervical cancer patients in Oncology clinic, Dr. Cipto Mangunkusumo hospital that had qualified according to inclusion criteria and exclusion criteria. The bloods were taken, the serum samples were separated, VEGF-C serum samples were examined by ELISA method, and endostatin serum samples were examined by Immunoassay method. Patients went through radical hysterectomy surgeries, and then pathology anatomy examinations.

**Result:** The samples were 47 patient, consisted of 33 patients (70.21%) without lymph nodes metastasis and 14 patients (29.79%) with lymph nodes metastasis. By using ROC cut off point method, it was obtained cut off point of VEGF-C level was 10,066.9 pg/ml, with sensitivity 78.57%, and specificity 96.97%. It was obtained the increasing risk of lymph nodes metastasis in patients with VEGF-C level > 10,066.9 pg/ml compare to patients with VEGF-C level ≤ 10,066.9 pg/ml, with OR 80, 95% CI (7.99; 800.71), and p < 0.001. Based on cut off point, 184.5ng/ml, with sensitivity 64.3%, and specificity 63.4%, the endostatin level was divided into two groups, which were the < 184.5ng/ml group and the ≥ 184.5ng/ml group. Patients in the ≥ 184.5ng/ml group had the risk of lymph nodes metastasis 3.15 times more compare to patients in < 184.5 ng/ml group, but not statistically significant (p=0.075, 95% CI (0.856;11.595)).

**Conclusion:** VEGF-C serum levels can be used as prognosis factor or predictor of lymph node metastasis in early stage cervical cancer patients. Endostatin serum levels as the predictor of lymph node metastasis still need further research by adding sample quantity.

**Keywords:** endostatin, VEGF-C, lymph node metastasis, cervical cancer

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**INTRODUCTION**

Cervical cancer is the most common cancer that occurs in developing countries. There are estimated around 500,000 newly diagnosed cases every year in the world, with 250,000 cases resulted in death. In USA, cervical cancer ranked as the third gynecology malignancy with estimated 12,800 new cases in year 2000 with 4600 death.1,2 Whereas in different part of the world, Indonesia, cervical cancer is reported to be the number one female malignancy (17.8%). In one of Indonesian government hospitals, named Dr. Cipto Mangunkusomo Hospital, the statistic of year 1989-1992 showed that cervical cancer took a high percentage of total cancer cases, which was 76.2% out of 1,717 gynecology cancer cases with life expectancy in 5 years between 56.7% - 72%.3
In patient with early-stage cervical cancer that has undergone radical hysterectomy and lymphadenectomy, adjuvant therapy is given based on the evaluation of various prognostic factors. Clinical-pathological factors (stage, lesion size, histological type, degree of differentiation, deep cervical stromal invasion, lymphovascular invasion, lymph node metastasis), cell adhesion molecules (E-cadherin, katenin), the enzyme proteinase matrix metalloproteinase (MMP), kaptensin D, Heparanase), DNA index, tumor suppressor genes p53 and HPV type are the prognostic factors used in the therapy of cervical cancer, and new prognostic factors still needed.4-7

Tumor development, neovascularization, and metastasis depend on the ability of cancer cells to invade tissue that consist of extracellular matrix degradation and membrane structures basal.8,9 Angiogenesis, the formation of new blood vessels, is suspected to take role in the development and growth of primary tumor or metastases.10

Endostatin which is a 20-kDa proteolytic fragment of collagen XVIII, has been found to be a potent inhibitor of angiogenesis process, and can inhibit tumor growth and metastatic process in animal subject.11 In addition, endostatin therapy that repeated in several cycles of extension of time shows the effect of tumor dormancy without any resistance to endostatin itself.12 At the cellular level, endostatin inhibits endothelial cell proliferation and migration and induces cell endothelial apoptosis.13-14

In a study of endostatin levels in patients with thyroid cancer, result showed that the concentration of endostatin was significantly higher in patients with distant metastases than in patients with remission and healthy patients. During endogenous TSH stimulation, levels of endostatin significantly decreased.15

Vascular endothelial growth factor (VEGF), a high-potential mitogen on endothelial cells, is an important mediator of angiogenesis and also involves in endothelial cell differentiation and development vascular system.16-18 Therefore, VEGF is considered to be the most important angiogenesis factor closely related to the induction and maintenance of the neovascularature in tumors. VEGF shows its works by binding two receptor tyrosine kinases, KDR/Fk-1 and FLT-1, which expressed on the endotel cell.19-21

The goal of this study is to analyze endostatin serum and VEGF-C serum as the predictor of lymph node metastasis and lymph nodes metastasis in early stadium of cervical cancers.

METHODS

A nested case-control study was conducted at the nursery and surgery room in Department of Gynecology Oncology of Dr. Cipto Mangunkusumo Hospital. Target populations of this research were all patients with cervical cancer who came to Dr. Cipto Mangunkusumo Hospital and met the inclusion and exclusion criteria. The inclusion criteria were: patient who had another tumor during surgery which was not cervical cancer, but carcinoma and condylomata acuminata. Thus, the total number of cases which can be assessed was as many as 47 cases.

Patients who came to the polyclinic of gynecologic oncology had to go through a clinical examination, cervical lesions measurement, radiology, rectoscopy, cystoscopy, thorax x-ray, and BNO-IVP to determine the cancer stage.

Patients with cervical cancer stage IB/IIA who met the criteria of inclusion and exclusion were examined, given informed consent, and 5cc of blood sample was obtained from the patients to check the serum levels of endostatin and VEGF-C serum for surgery preparation. Serum test was conducted by the Prodia Laboratory using immunoassay technique. All blood was evaluated in the same laboratory and with same technique. However, duplo was not done in the laboratory due to the limited amount of blood. After the results of the test have been obtained, the results were categorized into two groups using the ROC method. Cut off point selected was the optimal result between sensitivity and specificity.

Histopathologic test was performed to assess the lymph node metastasis, histological type, degree of differentiation, lymphovascular infiltration, invasion of parametrium, and vaginal incision margin. It was conducted in the anatomic pathology of Dr. Cipto Mangunkusumo Hospital using the same standard method. Faults that might occured include error in test procedure, inaccurate reading, and the number of slides. However, they were all minimized through the same inspection standards. The test was done by competent personnel who examined and read all tissues and the lymph node samples.

Important note on this research is that 2 out of 47 cases (4.25%) had histological type and degree of differentiation which were different from the results of anatomic pathology and tissue biopsy. Likewise, there were 7 cases that did not get a description of the lymphovascular invasion on biopsy results. Therefore, as the basic data study, the results of histopathological test were used.
RESULT

In this case-control study, 14 were the case group with positive metastasis, and 33 were the control group with negative metastases. In accordance with the problem formulation, which is metastatic nodes in cervical cancer is one of the strong prognostic determinants, the study aimed to identify factors that can predict lymph node metastasis. Once these factors can be identified, arranged or designed scoring systems to predict whether patients with these factors have a risk of lymph node metastasis. The probability of occurrence of metastases can also be calculated. The risk of lymphatic metastases is influenced by clinical pathology factors (stage, lesion size, histological type, degree of differentiation), changes in serum levels of endostatin, and VEGF-C serum levels. To prove the hypothesis, bivariate analysis was performed between categories of each variable, and then the ratio of equilibrium metastasis and its p value were calculated. Patients with stage IB2 (lesion size > 40 mm) had OR 12.5 occurrence of lymph node metastasis OR 12.5 than patients with stage IB1 (lesion size ≤ 40 mm) (95% CI: 1.60, 97.64, p = 0.016). Patients with lesion size > 40 mm had occurrence of metastasis OR times more than patients with a lesion size ≤ 40 mm (95% CI: 2.05, 48.69, p = 0.004). Patients with poorly differentiated metastases had occurrence of lymph node metastasis OR 2.76 compared to patients with well differentiated (95% CI: 1.03, 7.42, p = 0.043). Patients with positive vaginal incision had OR 5.55 lymphatic metastases occurrence compared to patients with negative vaginal incision line (95% CI: 1.10, 27.89, p = 0.037). Patients with positive lymphatic invasion had OR 20 lymphatic metastases occurrence compared to patients with negative lymphatic invasion (95% CI: 2.32; 171.77, p = 0.006). Patients with positive lymphatic vascular invasion had OR 20 lymph node metastases occurrence compared to patients with negative lymphatic invasion (95% CI: 2.32; 171.77, p = 0.006). Patients with levels of VEGF-C > 10066.90 had OR 80 metastases occurrence compared to patients with levels of VEGF-C ≤ 10066.90 (95% CI: 7.99; 800.71, p < 0.001).

Second hypothesis: clinical pathology factors (stage, lesion size, histological type, degree of differentiation), endostatin serum levels, and VEGF-C serum levels are predicting factors of lymph node metastasis. To prove the above hypothesis, multivariate logistic regression analysis was performed with the lymphatic metastases being determined as the dependent variable, while the factors that were tested as independent variables. From the above results of bivariate analysis, factors that had a high OR with the value of p ≤ 0.25 was chosen for subsequent inclusion into the multivariate analysis model. The results of multivariate analysis are as follows. Endostatin levels ≥ 184.5 was a risk factor, OR 2.086 (95% CI: 0.402, 10.821, p = 0.381). Parametrium invasion risk lymphatic metastases with OR 2.534 (95% CI: 0.406, 15.829, p = 0.320). Positive vaginal incision margin had OR 3.492 (95% CI: 0.424, 28.764, p = 0.245). Stage IIA had OR 2.146 (95% CI: 0.231, 19.912, p = 0.502). Parity > 4 had OR 3.539 (95% CI: 0.602, 20.792, p = 0.162). Poor differentiation had OR 1.822 (95% CI: 0.340, 9.768, p = 0.484). Size of primary lesion > 40 mm had OR 5.550 (95% CI: 0.678, 45.457, p = 0.110). Based on this analysis, model to predict the occurrence of lymphatic metastasis can be made. The probability of occurrence of lymphatic metastasis = -3.471 + 0.735 (endostatin levels ≥ 184.5) + 0.93 (positive parametrium invasion) + 1.25 (positive vaginal incision margin) + 0.764 (stage IIA) + 1.264 (parity > 4) + 0.600 (poor differentiation) + 1.714 (primary lesion size > 40 mm). The equation had a good calibration, and robust quality based on the parameters of discrimination.

Third hypothesis: The risk of lymphovascular invasion is influenced by clinical pathology factors (stage, lesion size, histological type, degree of differentiation), endostatin serum levels, and VEGF-C serum levels. To prove the hypothesis, bivariate analysis was performed between categories of each variable, and then the ratio of metastases equilibrium and its p value were calculated. From these calculations, there were several variables that significantly arised, which were: Patients with primary lesion size > 40 mm had 10 times lymphovascular invasion risk compared to patients with primary lesion size ≤ 40 mm (95% CI: 1.21, 92.25, p = 0.033). Patients with medium differentiation had OR 13.5 lymphovascular invasion occurrence more than patients with well differentiation (95% CI: 1.42; 128.25, p = 0.023). Patients with poor differentiation had OR 29.25 lymphovascular invasion occurrence more than patients with well differentiation (95% CI: 2.78; 306.80, p = 0.005). Patients with positive parametrium invasion had OR 4.4 lymphovascular invasion occurrence more than patients with negative parametrium invasion (95% CI: 1.03, 18.73, p = 0.045). Patients with high levels of VEGF-C > 10066.9 had OR 12.5 lymphovascular invasion occurrence more than patients with levels of VEGF-C ≤ 10066.90 (95% CI: 1.44; 108.18, p = 0.022).

Fourth hypothesis: clinical pathology factors (stage, lesion size, histological type, degree of differentiation), endostatin serum levels, and VEGF-C serum levels were predicting factors of lymphovascular invasion. To prove the above hypothesis, multivariate logistic regression analysis was performed with lymphatic metastases being determined as the dependent variable, while the factors that were tested as independent variables. From the results of bivariate analysis above, factors that have a high OR with the value of p ≤ 0.25 were selected for subsequent inclusion into the multivariate analysis model. The results of multivariate analysis were as follows. Endostatin levels ≥ 184.5 ng/ml was a protective factor of lymphovascular invasion, OR 0.565 (95% CI: 0.082, 3.897, p = 0.562). Stage IIA was a risk factor lymphovascular invasion, OR 5.927 (95% CI: 0.636, 55.232, p = 0.118). Stage IIA had OR 1.299 (95% CI: 0.171, 9.855, p = 0.800). Stage IB2 had OR 1.967 (95% CI: 0.088, 44.052, p = 0.670). The size of lesions > 40 mm had OR 6.266 (95% CI: 0.363;
108.211, p = 0.207). Poor differentiation had OR 28.404 (95% CI: 1.487; 542.740, p = 0.026). Medium differentiation had OR 11.940 (95% CI: 0.838; 170.104, p = 0.067). Positive vaginal incision margin had OR 1.716 (95% CI: 0.124, 23.667, p = 0.687). Positive parametrium invasion had OR 6.662 (95% CI: 0.611, 72.641, p = 0.120). Based on this analysis, a model to predict the occurrence of lymphovascular invasion can be made as follows. The probability of occurrence of lymphovascular invasion = -3.281 + -0.571 (endostatin levels ≥ 184.5) + 1.779 (parity > 4) + 0.262 (stage IIA) + 0.677 (stage IB2) + 1.835 (primary lesion size > 40 mm) + 3.347 (poor differentiation) + 2.480 (differentiation medium) + 0.540 (limit of positive vaginal incision) + 1.896 (positive parametrium invasion). The equation had a good calibration, and robust quality based on the parameters of discrimination.

Fifth hypothesis: clinical pathology factors (stage, lesion size, histological type, degree of differentiation), endostatin serum levels. To prove the hypothesis, bivariate analysis between categories of each variable was performed, and then ratio of metastases equilibrium and its p value were calculated. From these calculations, there are several variables that significantly arised, which were: Patients with primary lesion size > 40 mm had the risk of VEGF-C levels > 10066.90 pg/ml of 5.16 times compared to patients with primary lesions ≤ 40 mm (95% CI: 1.13, 23.54, p = 0.034).

Seventh hypothesis: endostatin serum levels are associated with higher levels of VEGF-C serum. To test this hypothesis, bivariate analysis between the two variables was performed. Therefore, the obtained results were as follows. The risk for having VEGF-C levels > 10066.90 was 9 times higher in patients with endostatin levels ≥ 184.5 than in patients with higher levels of endostatin < 184.5 (95% CI: 1.675, 48.367, p = 0.006).

## DISCUSSION

Up until now, there are not any satisfactory theories to explain the role of endostatin in lymphatic metastasis, and as anti-angiogenesis therapy. Clinically, endostatin increases the risk of lymphatic metastasis in bivariate analysis (OR = 3.15, p = 0.075), as well as multivariate analysis (OR = 2.086, p = 0.381), but not statistically significant. Endostatin is produced by the body as a reaction to the presence of a malignant tumor through basement membrane degradation. With

### Table 1. Prediction of Lymphatic Metastases Occurrence in Patients with Early Stage Cervical Cancer.

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Coef</th>
<th>SI</th>
<th>(95% CI RI)</th>
<th>OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endostatin level ≥ 184.5</td>
<td>0.735</td>
<td>0.840</td>
<td>0.402; 10.821</td>
<td>2.086</td>
<td>0.381</td>
</tr>
<tr>
<td>Positive parametrium invasion</td>
<td>0.930</td>
<td>0.935</td>
<td>0.406; 15.829</td>
<td>2.534</td>
<td>0.320</td>
</tr>
<tr>
<td>Positive vaginal incision margin</td>
<td>1.250</td>
<td>1.076</td>
<td>0.424; 28.764</td>
<td>3.492</td>
<td>0.245</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>0.764</td>
<td>1.137</td>
<td>0.231; 19.912</td>
<td>2.146</td>
<td>0.502</td>
</tr>
<tr>
<td>Parity &gt; 4</td>
<td>1.264</td>
<td>0.903</td>
<td>0.602; 20.792</td>
<td>3.539</td>
<td>0.162</td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>0.600</td>
<td>0.857</td>
<td>0.340; 9.768</td>
<td>1.822</td>
<td>0.484</td>
</tr>
<tr>
<td>Primer lesion size &gt; 40mm</td>
<td>1.714</td>
<td>1.073</td>
<td>0.678; 45.457</td>
<td>5.550</td>
<td>0.110</td>
</tr>
<tr>
<td>Constanta</td>
<td>-3.471</td>
<td>1.179</td>
<td>0.031</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Prediction of Lymphovascular Invasion Occurrence in Patients with Early Stage Cervical Cancer.

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Coef</th>
<th>SI</th>
<th>(95% CI RI)</th>
<th>OR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endostatin level ≥ 184.5</td>
<td>-0.571</td>
<td>0.951</td>
<td>0.082; 3.897</td>
<td>0.565</td>
<td>0.562</td>
</tr>
<tr>
<td>Parity &gt; 4</td>
<td>1.779</td>
<td>1.139</td>
<td>0.636; 55.232</td>
<td>5.927</td>
<td>0.118</td>
</tr>
<tr>
<td>Stage IIA</td>
<td>0.262</td>
<td>1.034</td>
<td>0.171; 9.855</td>
<td>1.299</td>
<td>0.800</td>
</tr>
<tr>
<td>Stage IB2</td>
<td>0.677</td>
<td>1.586</td>
<td>0.088; 44.052</td>
<td>1.967</td>
<td>0.670</td>
</tr>
<tr>
<td>Lesion size &gt; 40mm</td>
<td>1.835</td>
<td>1.454</td>
<td>0.363; 108.211</td>
<td>6.266</td>
<td>0.207</td>
</tr>
<tr>
<td>Poor differentiation</td>
<td>3.347</td>
<td>1.505</td>
<td>1.487; 542.740</td>
<td>28.404</td>
<td>0.026</td>
</tr>
<tr>
<td>Medium differentiation</td>
<td>2.480</td>
<td>1.355</td>
<td>0.838; 170.104</td>
<td>11.940</td>
<td>0.067</td>
</tr>
<tr>
<td>Positive vaginal incision margin</td>
<td>0.540</td>
<td>1.339</td>
<td>0.124; 23.667</td>
<td>1.716</td>
<td>0.687</td>
</tr>
<tr>
<td>Positive parametrium invasion</td>
<td>1.896</td>
<td>1.219</td>
<td>0.611; 72.641</td>
<td>6.662</td>
<td>0.120</td>
</tr>
<tr>
<td>Constanta</td>
<td>-3.281</td>
<td>1.449</td>
<td>0.038</td>
<td>0.024</td>
<td></td>
</tr>
</tbody>
</table>
more advanced od tumors metastases, endostatin levels are higher. When metastasis occurs, there is also degradation of basal membrane, so that more endostatin is produced. In theory, endostatin on angiogenesis switch chart is one of the angiogenesis inhibitor. Endostatin is produced in line with the process of angiogenesis, which is described by an increase in VEGF-C. Thus, high levels of endostatin serum in this study in accordance with the high VEGF-C as proven by the significant difference between serum levels of VEGF-C and serum levels of endostatin, (OR = 9; and the value of p = 0.006).

In theory, it is said that the endostatin is angiogenesis inhibitor. The increasing levels of endostatin in tumors is a by product of the increasing VEGF-C. If removal of the primary tumor was done, it could accelerate the metastases more rapidly. Primary tumors have a role to suppress metastasis. This has accordance with the endostatin being produced by the primary tumor to slightly suppress angiogenesis. After the primary tumor is removed, endostatin production will decrease and VEGF-C will increase and then metastases will occur. If created a table based on levels of VEGF-C and endostatin levels of lymphatic nodes metastatic, could be seen the relationship of positive lymphatic nodes metastasis. High VEGF-C and low endostatin (lymphatic node metastasis 100%), High VEGF-C and high endostatin (lymphatic node metastasis 88.89%); Low VEGF-C and low endostatin (12.5% lymphatic node metastasis), low VEGF-C and high endostatin (lymphatic node metastasis 8.33%). Percentage view above explained that VEGF-C acted as stimulator of angiogenesis, and endostatin was functioning to inhibit VEGF-C also metastasis.

In this study, group obtained low VEGF-C and high endostatin is as much as 25.5% (12/47) with VEGF-C was proven as risk factors and predictors. The size of lesions > 40 mm, differentiation, invasion of parametrium, levels of VEGF-C > 10066.90 were risk factors. The value of p = 0.006.

COCLUSION

Stage, lesion size > 40 mm, differentiation, vaginal incision margin, lymphatic invasion, lymphovascular invasion, levels of VEGF-C > 10066.90 were risk factor for lymph node metastasis, and can be used as predictors. The size of lesions > 40 mm, differentiation, invasion of parametrium, levels of VEGF-C > 10066.90 were risk factor of lymphovascular invasion. Clinically, endostatin was increasing the risk of lymphatic metastatic, but not statistically proven. VEGF-C was proven as risk factors and predictors of lymphatic metastasis.

REFERENCES