

A cross-sectional study: The correlation of pro-angiogenic factors expression level and epithelial ovarian cancer staging



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ABSTRACT

Background: Cancer is caused by uncontrolled cell growth and angiogenesis as a vital component in tumor growth and metastasis. Angiogenesis provides nutrients needed by cancer cells in order to grow. Angiogenesis is triggered by pro-angiogenic mediators and overexpression of these mediators have been associated with malignant phenotype of ovarian cancer. It is thought that the more advanced tumors have higher tumor cell survival, proliferation, invasion, and angiogenesis, so that it will show higher expression of pro-angiogenesis mediators.

Objective: To examine the differences in nerve growth factor (NGF), tyrosin kinase receptor A (TrkA), and vascular endothelial growth factor (VEGF) expression level in different stages of epithelial ovarian cancer.

Patients and methods: This is a retrospective cross-sectional study conducted from June to August 2019 in the Department of Obstetrics

and Gynecology in association with the Department of Anatomic Pathology of Dr. Moewardi General Hospital, Surakarta, Indonesia. The staging of the epithelial ovarian cancer was determined using the FIGO 2014 staging system. NGF, TrkA, and VEGF expression level were determined using immunohistochemical examination.

Results: The samples were 53 formalin-fixed, paraffin-embedded ovary, its adnexa, uterus, and lymph nodes tissue blocks of epithelial ovarian cancer patients. There are statistically significant differences in the proportion of NGF, TrkA, and VEGF level of expression in different stages of epithelial ovarian cancer ($p < 0.05$).

Conclusion: There are statistically significant differences in NGF, TrkA, and VEGF level of expression in different stages of epithelial ovarian cancer.

Keywords: NGF, TrkA, VEGF, staging, ovarian cancer

Cite This Article: Prakosa, T., Mudigdo, A., Purwanto, B., Wasita, B., Cilmiaty, R., Widianingsih, V. 2020. A cross-sectional study: The correlation of pro-angiogenic factors expression level and epithelial ovarian cancer staging. *Bali Medical Journal* 9(1): 125-128. DOI:10.15562/bmj.v9i1.1605

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Received: 2019-09-16

Accepted: 2019-10-15

Published: 2020-04-01

INTRODUCTION

Ovarian cancer is the most lethal gynecologic malignancy and is the sixth most common malignancy among women in the world.^{1,2} Epithelial ovarian cancer arise from epithelial cells of the ovary and comprise around 90% of all ovarian cancers.³ Most ovarian cancer cases are diagnosed in its advanced stages with five-year survival rate of only 45%.^{4,5}

Cancer is caused by uncontrolled cell growth as a result of imbalance between cell growth and programmed cell death.⁶ Nerve growth factor (NGF) through its receptor, Tyrosine kinase A receptor (TrkA), may alter cell death and survival, proliferation, invasion, metastasis, and angiogenesis.^{3,7} NGF has a role in tumor growth and progression by overriding normal cell growth regulation.⁷ Overexpression of TrkA has been associated with malignant phenotype of various carcinomas, including ovarian cancer.⁸

Angiogenesis is a vital component in tumor growth and metastasis.^{9,10} Vascular endothelial growth factor (VEGF) is a pro-angiogenic factor for the vascular endothelium and one of the most important mediators in ovarian angiogenesis.¹¹

VEGF is expressed in endothelial cells and is involved in tumor cell proliferation and migration.¹² NGF also plays a role in ovarian angiogenesis by increasing VEGF expression through its receptor.¹ In ovarian cancer, VEGF causes tumor-induced ascites due to its ability to increase the vascular permeability.¹³ Angiogenesis is crucial because diffusion alone cannot provide the nutrients required for tumors beyond 2 cm.¹⁰

The FIGO staging system shows ovarian tumor progression, based on its involvement, size, and metastasis ability.¹⁴ It is thought that the more advanced tumors have higher tumor cell survival, proliferation, invasion, and angiogenesis, so that it will have higher expression level of pro-carcinogenesis and pro-angiogenesis mediators. Therefore, the objective of this study is to examine whether there is a difference of NGF, TrkA, and VEGF expression level in different stages of epithelial ovarian cancer. Furthermore, our interest is also to evaluate the correlation of NGF, TrkA, and VEGF level of expression with tumor progressivity which is shown by the stage of cancer.

MATERIALS AND METHODS

We conducted a retrospective cross-sectional study from June to August 2019 in the Department of Obstetrics and Gynecology, in association with the Department of Anatomical Pathology of Dr. Moewardi General Hospital, Surakarta, Indonesia. This study was approved by the Health Research Ethics Committee of Dr. Moewardi General Hospital (739/V/HREC/2019).

Eligible patients were epithelial ovarian cancer patients diagnosed in January 2016 to December 2018, whose formalin-fixed, paraffin-embedded ovary, its adnexa, uterus, and lymph nodes tissue blocks were stored at the Department of Pathology. The tissue were taken during oophorectomy and uterine biopsy and were made into paraffin blocks in the same day of the surgery. We traced the medical record of the eligible patients. We mined the recorded data required to determine the stage of cancer using the FIGO 2014 staging system. The ovarian cancer stages were simplified into: stage I (FIGO stage IA, IB, IC); stage II (FIGO stage IIA, IIB); stage III (FIGO stage IIIA, IIIB, IIIC); and stage IV (FIGO stage IVA, IVB). Eligible patients with comorbidities such as diabetes, cardiovascular diseases, liver failure, and kidney failure were excluded from the study, resulting a limited number of study subjects. The patients were consecutively included in our study to reach the minimum sample size.

The study subjects' formalin-fixed, paraffin-embedded tissue blocks were obtained from the Department of Anatomical Pathology's sample archive storage. The study subjects' tissue blocks were stained for NGF, TrkA, and VEGF. The staining for NGF was performed with rabbit polyclonal antibody selective for proNGF and mature NGF. TrkA staining was done using selective anti-TrkA antibody. Meanwhile, VEGF staining was performed using polyclonal antibodies against VEGF. The pretreatment consisted of microwave

oven antigen retrieval for 4 times 5 minutes in citrate buffer.

The immunohistochemical analysis and evaluation was performed by a certified anatomical pathologist. Cytoplasm and immunoreactivity was scored for NGF, whereas membrane and cytoplasm immunoreactivity was evaluated for TrkA staining. The scoring for VEGF was done by evaluating the intensity of cytoplasmic staining. The intensity of the staining was divided into four categories: 0 = absent; 1 = stained 0-5% of tumor cells; 2 = 6-25% of tumor cells; 3 = stained 26-75% of tumor cells; and 4 = stained 76-100% of tumor cells. The pathologist examining the samples was blinded to the patients' clinical data. Based on the scores, we categorized the expression level as absent (score 0), weak (score 1-2), moderate (score 3), and strong (score 4).

Statistical analysis

The Fisher's exact test was used to determine the differences in the proportion of different level of NGF, TrkA, and VEGF expression in different stages of epithelial ovarian cancer. P-value <0.05 was assumed as statistically significant. All statistical analysis was conducted using SPSS version 21.0 for Windows.

RESULT

Our study investigated a total of 53 tissue samples from the selected epithelial ovarian cancer patients. The patients were classified into three age groups, under 40 years (15.10%), 41 to 60 years (64.15%), and over 60 years old (20.75%). The staging showed that the majority of patients were at stage IV (44%), followed by stage III (32%), stage II (13%), stage I (11%), see [Table 1](#).

The intensity of staining ranged from absent to strong in each case. The number of patients with variable immunohistochemical (IHC) results categorized based on the clinical stages are shown in [Table 2](#). The Fisher's exact tests showed a significant difference in NGF ($p < 0.001$), TrkA ($p = 0.001$), and VEGF ($p = 0.016$) level of expression in different stages of epithelial ovarian cancer.

DISCUSSION

The risk of developing epithelial ovarian cancer is affected by some factors, including menopausal status. We divided the subjects into 3 groups based on their age with ranges related to women's common menopausal status, pre-menopause (<40 years), peri-menopause (40 – 60 years), and post-menopause (>60 years). This study showed that epithelial ovarian cancer is most prevalent in the peri-menopausal age group, followed by the

Table 1 The descriptive data of the study subjects

Characteristics	Study subjects (n=53)
Age in years	50.92 ± 11.12
under 40	8
41 to 60	34
over 60	11
Cancer stage	
I	6
II	7
III	17
IV	23

Table 2 Crosstabulation of NGF, TrkA, and VEGF expression based on the disease staging

Expression	Stage				Fisher's Exact (p-value)
	I (n=6)	II (n=7)	III (n=17)	IV (n=23)	
NGF					
Absent	1	0	0	0	< 0,001
Weak	4	3	0	0	
Moderate	0	1	12	7	
Strong	1	3	5	16	
TrkA					
Absent	0	0	0	0	0.001
Weak	2	0	0	0	
Moderate	2	0	2	0	
Strong	2	7	15	23	
VEGF					
Absent	0	0	0	0	0.016
Weak	3	0	1	0	
Moderate	1	2	2	3	
Strong	2	5	14	20	

NGF, nerve growth factor; TrkA, tyrosin kinase receptor A; VEGF, vascular endothelial growth factor.

post-menopausal age group. This result is coherent with previous study by Doubeni *et al*, which stated that epithelial ovarian cancer is more prevalent in the 55 to 64-year-old.⁵

The FIGO staging system is an indicator for ovarian tumor progression, based on its involvement, size, and metastasis ability.¹⁴ Angiogenesis is crucial for solid tumor growth, invasion, and metastasis after a short avascular phase, for primary tumors will not grow without angiogenesis.^{11,12,15} Angiogenesis provides nutrition needed for the growing tumors and allowing the tumor cells to establish continuity with the patient blood vessels.¹⁶ The transition of tumor avascular phase to vascular phase is thought to be induced by upregulation of factors stimulating vasculogenesis and down regulation of antiangiogenesis mediators.^{10,15} NGF and TrkA are involved in the angiogenic process of epithelial ovarian cancer via VEGF activation.³ The VEGF family and their receptors form an important signaling pathway of tumor angiogenesis.² Previous literatures showed a specific role of VEGF in tumor growth and neovascularization.⁹ High VEGF expression is associated with advanced stage carcinoma.¹⁵ Duncan *et al* revealed that cancer patients with higher level of VEGF have worse survival rates than those with medium, low, or no VEGF.⁹

Besides the importance of angiogenesis in tumor progression, cancer cell also shows a lack

of cell growth regulation control.¹¹ Overexpression of NGF and its high-affinity receptor, TrkA, can significantly promote abnormal proliferation of malignant tumor and cancer cells via activation and regulation of various pathways, such as PI3K/Akt, Ras/MAPK.^{7,17} Previous study by Molloy *et al* showed that NGF in carcinogenesis is involved in mitogenic stimulation, increasing the tumor metastatic and invasion ability, and inhibiting apoptosis.¹⁸ TrkA is suggested to be involved in tumorigenesis in non-neural tumors, and is commonly associated with tumor progression and poor outcome.⁶

CONCLUSION

There were differences in NGF, TrkA, and VEGF level of expression in different stages of epithelial ovarian cancer, indicating that the IHC may have a potential to be used for the diagnosis and management of ovarian cancer.

FUNDING DISCLOSURE

The study was self-funded by the authors.

CONFLICT OF INTEREST

The author reports no conflicts of interest in this work.

AUTHOR CONTRIBUTION

The authors gave equal contributions from designing and conducting the study, to the writing of the manuscript.

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