ORIGINAL RESEARCH PAPER

Obstetrics & Gynaecology

EXPRESSION OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) ON BENIGN AND MALIGNANT EPITHELIAL OVARIAN TUMOR TISSUE

KEY WORDS: Ovarian Tumor, Malignant Ovarian Cancer, NGAL Expression, ALLRED.

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ABSTRACT

Ovarian tumors are neoplasms derived from ovarian tissue To date, malignant ovarian tumors are treated with adjuvant chemotherapy. Several studies have reported glicoprotein such as Neutrophil gelatinase-associated lipocalin (NGAL) have important roles as a marker for clinical response to chemotherapy and disease prognosis.

This is a comparative analytic study using samples from ovarian tissue obtained from April 2018 to September 2018. Data were analyzed by calculating kappa values and relationships between variables using the Chi-square statistical test. P values less than 0.05 were considered significant with 95% CIs.

NGAL expression increased significantly in grade I epithelial ovarian tumors (15 patients ;78,9%). The majority of patients with ALLRED score (+) were found in malignant epithelial ovarian tumors (19 patients; 73,1%) and ALLRED score (-) were most common in benign epithelial ovarian tumors.

INTRODUCTION

Ovarian tumors are neoplasms that originate from ovarian tissue which based on their consistency can be solid or cystic. If based on its histopathology, ovarian tumors can be benign or malignant. These tumors are divided into three groups based on the anatomical structure from which the tumor originates from the ovarian epithelium, germ cells, sex cord - stromal.[1] For malignant ovarian cancer itself consists of 90 - 95% ovarian epithelial cancer, and the rest 5 - 10% consists of germ cell tumors and cord-stroma sex tumors.[2,3] By occupying its position as the leading cause of malignant ovarian cancer, it can be said that epithelial ovarian cancer is one of the reproductive cases which is the main cause of morbidity and mortality in women.[4] Based on the World Health Organization (WHO) report in 2002, stated that ovarian cancer in Indonesia ranks fourth for new cases with an incidence of 15 per 100,000 after breast, uterine, and colorectal cancer.

Several studies have reported the function of a glycoprotein in the form of Neutrophil gelatinase-associated lipocalin (NGAL) in predicting the prognosis / response to therapy. NGAL is a 24 kDa protein that is stored in specific neutrophils of human. In addition, lipocalin can bind to certain cell surface receptors and form macromolecular complexes. NGAL forms a covalent complex with gelatinase B but exists mainly in the monomeric and homodimeric form. NGAL is not found in normal ovaries, but is regulated in ovarian cancer cells. [14-17]

Triebel et al identified an association between NGAL and MMP-9 gelatinolytic enzymes (matrix metalloproteinase 9 or gelatinase B) which are known to degrade several components of the basement membrane including type I gelatin and type I, IV, V, XI, NGAL; that role is one part of ovarian cancer carcinogenesis. Further evidence indicates that NGAL has an important role in inflammation, metabolic diseases, growth regulation and cell adhesion to normal tissue and tumor cells. [18, 19, 20] According to Lim et al (2007), NGAL expression has increased regulation of ovarian cancer cells. Immunoreactive NGAL (irNGAL) expression in ovarian tumors increases according to the grade and stage of the disease. NGAL expression has decreased regulation on cancer cell lines undergoing epithelio-mesenchymal transition (EMT) induced by epidermal growth factor (EGF). Cho & Kim (2009) reported an increase in NGAL expression and its relationship with tumor differentiation in ovarian cancer. Therefore, based on these studies it can be concluded that NGAL is a good marker for monitoring changes from benign ovarian tumors to premalignant, and these malignancies and molecules may be involved in the development of epithelial ovarian cancer.[2,4,19]

Based on the description above, this study is interested in conducting research so that they can find out the differences in NGAL expression from benign epithelial ovarian tumor tissue and patients with malignant ovarian tumors in H. Adam Malik General Hospital Medan and Dr. Pirngadi Medan in 2018.

METHOD

This is a comparative analytic study using samples from ovarian tissue obtained from April 2018 to September 2018. Immunohistochemistry analysis was subsequently performed at the Pathology Anatomy laboratory. The samples of this research were 26 women with malignant epithelial ovarian tumor and 26 women with benign epithelial ovarian tumor.

RESULT

The following are the results of the characteristics of the research subjects based on age, parity, and menopausal status.

Table 1. Research Subject Criteria

Characteristics	Malignant Epithelial Ovarian Tumor		Benign Epithelial Ovarian Tumor	
	N	%	N	%
Age (year)				
• <20	0	0	1	3,8
• 20-50	14	53,8	17	65,4
• >50	12	46,2	8	30,8
Parity				
• Virgo	1	3,8	0	0
• Nullipara	2	7,7	6	23,1
• Parity ≥1	23	88,5	20	76,9
Menopausal Status				
• Menopause	11	42,3	8	30,8
• Non Menopause	15	57,7	18	69,2

Based on table 1. the majority of patients were in the malignant epithelial ovarian tumor group having the age

range between 20-50 years (53.8%), as well as in the benign epithelial ovarian tumor group (65.4%). Based on parity, both groups had parity ≥ 1, around 88.5% from the malignant epithelial ovarian tumor group and 76.9% from the benign epithelial ovarian tumor group. Based on menopausal status, both groups were non menopause, 57.7% in the malignant epithelial ovarian tumor group and 69.2% in benign epithelial ovarian tumors.

Based on histopathological distribution of patients in this study, in malignant epithelial ovarian tumors, the histopathological features found in this study were adenocarcinoma musinosum, serenum adenocarcinoma and endometriod carsinoma in 12 (46.2%), 11 (42.3%) and 3 (11.5%). In benign epithelial ovarian tumors, the histopathological features found in this study were cystadenoma musinosum, serous cystadenoma and endometriosis cysts as many as 11 (42.3%), 8 (30.8%) and 7 (26.9%).

Table 2. Proportion of NGAL Expression in Malignant Epithelial Ovarian Tumor Based on Tumor Grade and ALLRED Score

Grade	ALLRED				P Value
	Negative		Positive		
	N	%	N	%	
Grade I	1	14,3%	15	78,9%	0.002
Grade II	1	14,3%	3	15,8%	
Grade III	5	71,4%	1	5,3%	

Chi Square Test

Based on table 2 above, it was found that the majority of samples with positive ALLRED score were Grade I with 15 samples (78,9%). On the other hand, majority of samples with negative ALLRED score were Grade III with 5 samples (71,4%). From the analysis, a P value of 0.002 was found, indicating that NGAL expression increased significantly in grade I epithelial ovarian tumors.

Table 3. Perbedaan Ekspresi NGAL pada Tumor Ovarium Epitel Ganas dan Jinak Berdasarkan Skor Allred

ALLRED Score NGAL Expression	Research Sample				p-value*
	Malignant Epithelial Ovarian Tumor		Benign Epithelial Ovarian Tumor		
	%		%		
Negatif	7	26,9	17	65,4	0.005
Positif	19	73,1	9	34,6	
Jumlah	26	100	26	100	

Chi Square Test

Based on table 3 above, it was found that the majority of patients who had positive ALLRED score were found in malignant epithelial ovarian tumors in as many as 19 samples (73.1%) while the negative ALLRED score were mostly found in benign epithelial ovarian tumors in as many as 17 samples (65.4%). based on analysis using the Chi Square test found significant results (P = 0.005).

DISCUSSION

Based on the inclusion and exclusion criteria, the samples of this research were 26 women with malignant epithelial ovarian tumor and 26 women with benign epithelial ovarian tumor. The majority of patients were in the malignant epithelial ovarian tumor group having the age range between 20-50 years (53.8%), as well as in the benign epithelial ovarian tumor group (65.4%). Based on parity, both groups had parity ≥ 1, around 88.5% from the malignant epithelial ovarian tumor group and 76.9% from the benign epithelial ovarian tumor group. Based on menopausal status, both groups were non menopause, 57.7% in the malignant epithelial ovarian tumor group and 69.2% in benign epithelial ovarian tumors.

According to Lim et al, NGAL expression is upregulated in ovarian cancer. NGAL Immunoreactivity in ovarian tumor were altered according to each tumor Grade. The majority of samples with positive ALLRED score were Grade I with 15 samples (78,9%).

Wu stated that NGAL expression were higher in malignant epithel ovarian tumor when compared to benign epithel ovarian tumor. Furthermore, this expression increased according to an increase in clinical stage ($p < 0,05$). The majority of patients who had positive ALLRED score were found in malignant epithelial ovarian tumors in as many as 19 samples (73.1%) while the negative ALLRED score were mostly found in benign epithelial ovarian tumors in as many as 17 samples (65.4%). based on analysis using the Chi Square test found significant results (P = 0.005).

Cho & Kim (2009) in his study found that the immunoreactivity of LCN2 was significantly related to tumor differentiation. About 98.3% of ovarian cancers are positively colored with LCN2, whereas in benign ovarian tumors it is only positively colored around 72.7%. Well-differentiated tumors show the highest LCN2 expression. This staining expression may indicate the amount of epithelial differentiation. LCN2 expression is associated with phenotypic epithelial ovarian tumors and disappears as cancer progresses and the epithelial tumor is poorly differentiated. As is well known, LCN2 is said to play a role in the process of response to inflammation and recently chronic inflammation is known as a risk factor for epithelial malignancies. We can therefore see an increase in the regulation of LCN2 expression in ovarian premalignans and early ovarian malignancies when the inflammatory process is increased.[19,32]

Thus, it can be concluded that NGAL has an important role as a marker for clinical monitoring of responses to chemotherapy and disease prognosis. NGAL is widely explored in serum and ovarian tissue and is studied as a urinary biomarker in kidney injury and as a determinant of the prognosis and response to chemotherapy. This is caused by NGAL undergoing upregulation in various tumors.[19,33]

CONCLUSION

The majority of the study sample was 20-50 years old, had one children and had not yet reached menopause, both in benign and malignant ovarian tumor groups. The most common histopathological picture found in benign ovarian tumors is cystadenoma musinosum, whereas in malignant ovarian tumors is adenocarcinoma musinosum. NGAL expression in malignant epithelial ovarian tumors is the most (+) with the majority (+) values found in grade I. The majority of study samples that have ALLRED (+) values are found in malignant epithelial ovarian tumors while the ALLRED (-) values are mostly found in Benign epithelial ovarian tumor.[34]

Increased expression of NGAL in ovarian cancer gives us a new understanding, where NGAL increases in grade I and II ovarian cancer, where we know grade I and II ovarian cancer has a better response to radiation and chemotherapy compared to grade III and grade ovarian cancer IV. Grade itself is not in line with the clinical stage of ovarian cancer, so this gives us a new understanding of the function of NGAL as a prognostic factor in ovarian cancer, where ovarian cancer patients with advanced stages, but with a picture of PA showing borderline or well-differentiated results will provide an outcome which is better for the therapy we provide. This can be input for further research to prove the role of NGAL in advanced ovarian cancer on the therapeutic response of ovarian cancer, using a larger method and sample size.

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