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The Relationship between Progesterone Receptor Expression with Cell Differentiation and the Stage of Endometrial Carcinoma

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ABSTRACT

Introduction: Endometrial carcinoma is a primary malignant tumour in the endometrial epithelium and the second gynecologic malignancy after cervical carcinoma in the world. The incidence of endometrial carcinoma increased as the increasing of life expectancy and obesity epidemic. Surgery is the definitive therapy, but some patients with surgical pathology risk are necessary to perform adjuvant therapy. Assessment of hormone receptor, in this case, is progesterone receptor, could be applicable in evaluating risk factors patients whereby it will affect the prognosis.
Method: This is a cross-sectional study. Sixty-five sample consisting of block paraffin tissue of the endometrium from endometrial carcinoma cancer type I who underwent surgery in Adam Malik General Hospital Medan. Immunohistochemistry assay to determine progesterone receptor expression and statistical analysis performed to assess the relationship between progesterone receptor expression with cell differentiation and the stage of endometrial carcinoma-Chi-square analysis with confidence of interval 95%, $p<0.05$ is significant.
Result: The result of the study, of Sixty-five endometrial carcinoma patients, found the majority of age is $\geq$ 50 years (67.7%), with BMI overweight (44.6%), well-differentiated cell (44.6%) and stage III (41.5%). Positive progesterone receptor expression found in 30 patients (46.2%) and negative in 35 patients (53.8%). In this study found there is a significant relationship between cell differentiation and the stage of endometrial carcinoma *(p<0.05).* The decreasing of progesterone receptor expression means the poorer of cell differentiation (r = -0.659) and increasing the stage of endometrial carcinoma (r = -0.428).
Conclusion: There is a significant relationship between progesterone receptor expression with cell differentiation and the stage of endometrial carcinoma *(p<0.05).* The decreasing of progesterone receptor expression means more inadequate cell differentiation (r = -0.659) and increasing the stage of endometrial carcinoma (r = -0.428).

Keywords: *Endometrial Carcinoma, Progesterone Receptor, Cell Differentiation, Stage.*

INTRODUCTION

Endometrial carcinoma ranks second after cervical carcinoma and its incidence increases with increasing life expectancy and the obesity epidemic.[1,2] Based on the Surveillance, Epidemiology, and End Results Program (SEER) data, it is estimated that 60,050 new cases of endometrial carcinoma in 2016 in the United States with 10,470 deaths.[3,4] Clinically, histologically and biologically, endometrial carcinoma is divided into two categories, namely, type I and type II with the most number of which is a type I which is associated with unopposed estrogen conditions, a low grade with histologic types generally endometrioid and hormone receptor-positive. [5,6]

Endometrial carcinogenesis is upregulated by estrogen (proliferative effect) and down-regulated by progesterone.

Progesterone, through its receptors, acts against the effects of estrogen by inducing differentiation, inducing apoptosis and inhibiting invasion.[7]

The primary treatment of endometrial carcinoma is surgery, but some patients at risk of surgery may require adjuvant therapy. The question is how to assess patients who need adjuvant therapy. Several factors determine these risk factors such as histology type, cell differentiation, Lymphovascular Space Involvement (LVSI), and lymph node involvement. Besides, several biological molecules are prognostic factors, one of which is the hormone receptor, namely the progesterone receptor (PR).[8] Previous investigations have linked PR to cell differentiation, histology, adnexal spread, and recurrence. PR expression decreased with increasing cell grade, repetition and reduced survival rates.[9,10]

This study was a descriptive cross-sectional study. The study sample was a paraffin block preparation of endometrial tissue from type 1 endometrial carcinoma patients who underwent surgery (laparotomy surgical staging) at the Department of Obstetrics and Gynecology, H. Adam Malik Hospital Medan, Indonesia. We obtained sixty-five paraffin blocks which were then carried out by immunohistochemical examinations at the Laboratory of Pathology Anatomy, Faculty of Medicine, University of North Sumatra (FK USU). We obtained patient clinicopathological data from medical records which included age, BMI, cell differentiation, and stage. The inclusion criteria were paraffin block of endometrial adenocarcinoma type I tissue, and the patient had complete medical data; Exclusion criteria were non-labelable paraffin block preparations (no paraffin block found), and inability to conduct immunohistochemical tests for progesterone receptor expression. The study took place from May 2016 to January 2017. This research protocol was approved by the Ethics Commission of the Faculty of Medicine, University of North Sumatra (June 2016).

The immunohistochemical examination performed was an assessment of progesterone receptor expression. Tissue staining was carried out with primary antibodies, namely Monoclonal Mouse Anti-Human Progesterone Receptor, clone PgR 636. Paraffin-fixed tissue was cut to 4 µm; then slide deparaffinization was performed; followed by rehydration with washing under running water. The slide was inserted into the Dako Epitope Retrieval: set up preheat 65^0C, then at 98^0C for 15 minutes. The slides were inserted into Tris Buffered Saline pH 7.4. Block peroxidase was performed, then washed in Tris Buffered Saline pH 7.4. Next, we formed the blocks with Normal Horse Serum 3%, washed it back with Tris Buffered Saline pH 7.4. Incubation was done with the primary antibody at a dilution of 1: 200. Then the Tris Buffered Saline ph 7.4 was washed. Counterstain was done with Hematoxylin, then washed with running water. Interpretation of the progesterone receptor expression was made by two observers (pathologists) using the Allred score. The difference in the accuracy of the two observers was assessed by calculating the kappa value. The kappa value was 99%, which means that data analysis can be used data from just one observer's reading.

Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS®) version 24 (IBM, International Business Machines) with a chi-square test to assess the association of progesterone receptor expression with cell differentiation and endometrial carcinoma stage. Then the Spearman correlation test was performed to assess the correlation of progesterone receptor expression with cell differentiation and endometrial carcinoma stage. *p-value* <0.05 was considered significant.

From June 2016-January 2017, 65 paraffin blocks were obtained from patients with endometrial carcinoma who had undergone surgery.

Table 1: Frequency distribution based on the characteristics of research subjects

Characteristics	Endometrial Carcinoma	
	n	%
Age		
< 50 years	21	32.3
≥ 50 years	44	67.7
BMI		
Underweight	0	0
Normoweight	14	21.5
Overweight	29	44.6
Obesity	22	33.8
Cell differentiation		
Good	29	44.6
Moderate	16	24.6
Bad	20	30.8
Stage		
I	21	32.3
II	17	26.2
III	27	41.5
Total	**65**	**100**

Based on patient characteristics (Table 1), the majority of endometrial carcinoma patients were aged >50 years (n = 44, 67.7%), with overweight BMI (n = 29, 44.6%), well differentiation cell (29; 44.6%), and stage III carcinoma (27; 41.5%).

Table 2: Expression of progesterone receptors in endometrial carcinoma

Expression	Endometrial carcinoma	
	n	%
Positive	30	46.2
Negative	35	53.8
Total	**65**	**100.0**

From the table above, we observed that there are more people in the endometrial carcinoma group with negative progesterone receptor expression than the endometrial carcinoma group with positive progesterone receptor expression (53.8%), but the percentage does not differ much.

Table 3: Relationship between Progesterone Receptor Expression and Cell Differentiation in Endometrial Carcinoma

Expression	Cell differentiation								p-value
	Good		Moderate		Bad		Total		
	n	%	n	%	n	%	n	%	
Positive	22	73.3%	8	26.7%	0	.0%	30	100.0%	0.000
Negative	7	20.0%	8	22.9%	20	57.1%	35	100.0%	

**Fischer Exact Test*

Table 3 explains that the majority of endometrial carcinomas with positive progesterone receptor expression had well differentiation cell (73.3%), and conversely, most endometrial carcinomas with negative progesterone receptor expression had poor differentiation cell (57.1%). The relationship between the assessment of progesterone receptor expression and differentiation of endometrial carcinoma cells was assessed statistically by the Fischer-exact test. We obtained p-value of 0.000 ($p < 0.05$), which means that there is a significant relationship between progesterone receptor expression and cell differentiation in endometrial carcinoma.

Table 4: Correlation of progesterone receptor expression with cell differentiation

Variable	r	p
Cell differentiation	-0.659	0.000

**Spearman Test*

The correlation between progesterone receptors expression and the endometrial carcinoma cells differentiation was assessed statistically by using the Spearman test, the value of r = - 0.659 was obtained, which means that there is an inverse correlation with a strong correlation. We conclude that the weaker the expression of progesterone receptors, the higher the degree of cell histology (poor differentiation/G3) in endometrial carcinoma.

Table 5: Relationship between Progesterone Receptor Expression and Endometrial Carcinoma Staging

Expression	Cell differentiation								p-value
	Stage I		Stage II		Stage III		Total		
	n	%	n	%	n	%	n	%	
Positive	12	40.0%	12	40.0%	6	20.0%	30	100.0%	0.003
Negative	9	25.7%	5	14.3%	21	60.0%	35	100.0%	

**Chi-square Test*

In table 5 we see that in the group of positive progesterone receptor expression, 12 subjects (40%) had stage I endometrial carcinoma, 12 subjects (40%) had stage II, and only six subjects had stage III (20%). In the group of negative progesterone receptor

expression, the majority had stage III endometrial carcinoma (60%). With the chi-square test, we obtained p-value = 0.003 (p <0.005), which means that there is a significant relationship between progesterone receptor expression and the staging of endometrial carcinoma.

Table 6: Correlation of Progesterone Receptor Expression by Stage

Variable	r	p
Stadium	-0.428	0.003

**Spearman Test*

The correlation between the expression of progesterone receptors and the staging of endometrial carcinoma was statistically assessed using the Spearman test, with the value of r = -0.428, which means that there is an inverse correlation with moderate strength. We conclude that the weaker the expression of progesterone receptors, the higher the staging of endometrial carcinoma.

As found in research by Tulumang et al., Holman et al. and Salom et al., the majority of endometrial carcinoma is found in the elderly (> 51 years).[1,11,12] According to Pike et al., endometrial carcinoma increases with age because, in perimenopausal women, there is a decrease in E_2 and P_4 levels. This condition, what is said to be unopposed estrogen, continually occurs. In addition, there is also the increasing use of estrogen therapy for menopausal symptoms. According to the study of Salom et al. and Chiang et al., the majority of overweight patients were due to excessive endogenous estrogen production due to aromatization of androgens to estradiol and conversion of androstenedione to estrone in peripheral adipose tissue. Additionally, obese premenopausal women are more likely to experience chronic anovulation.[12,13]

The majority had well-differentiated cells, in agreement with Binder et al. who postulated that most of the endometrioid endometrial carcinoma (type I) is well-moderately differentiated (G1-G2) because it is a progression of endometrial hyperplasia and has a better prognosis.[14] According to The Cancer Genome Atlas (TCGA), endometrial carcinoma type I is a tumour with a low copy number that is generally associated with a PTEN mutation and is associated with good cell differentiation.[15] Based on the staging, the results of this study contradict the results of studies by Holman et al. and Salom et al., which stated that most endometrial carcinomas were diagnosed at an early stage.[11,12] From the results of this study, it was found that most cases were found at an advanced stage, possibly due to the low level of patient knowledge in recognizing symptoms and the absence of standard screening in detecting endometrial carcinoma. However, further research is needed to conclude this.

From this research, we found that the majority have positive progesterone respondent expressions, in accordance to the study of Xie et al. and Yang S et al. which stated that the expression of progesterone receptors would decrease during endometrial carcinoma which causes the loss of growth inhibition regulated by progesterone. Loss of progesterone receptor expression can be caused by two things: the absence of the progesterone receptor or the downregulation of progesterone receptors. In endometrial carcinoma, there is phosphorylation and ubiquitination of progesterone receptors by proteasomes.[15,16]

In this study, we found that there was an inverse correlation between progesterone receptor expression with cell differentiation, and between progesterone receptor expression and endometrial carcinoma stage. The weaker the progesterone receptors expression, the higher the degree of cell histology (poor differentiation/G3) and the higher the stage of endometrial carcinoma.

Our research was in line with the research of Kreizman-Shefer et al. and Van der Horst et al. which states that PR in endometrial carcinoma cells is correlated to cell differentiation, histology, adnexal spread, and recurrence. PR expression decreases to negative in endometrial

carcinoma. PR expression decreased with increasing degree of cell histology and is inversely correlated with myometrial invasion, due to a decrease in E-cadherin expression and an increase in EMT.[10,17]

The study by Clarke et al. also stated that almost all low-grade endometrioid carcinomas show positive PR expression, which is generally a strong expression. Likewise, high-grade endometrioid carcinoma shows a low PR expression.[18]

A study by Huvila et al. assessed the expression of progesterone receptors in recurrent endometrial carcinoma patients, which found that the loss of progesterone receptor expression was associated with recurrence in stage I and II endometrial carcinoma patients. This study also postulated that Progesterone receptor status is a more significant predictor when compared to LVSI or tumour size so that patients with early-stage endometrial carcinoma with negative progesterone receptors are strongly recommended for adjuvant therapy, namely radiation.[19]

As a conclusion in this study, there are almost as many groups of positive and negative progesterone receptor expression groups in endometrial carcinoma. There is a significant relationship between progesterone receptor expression with cell differentiation and stage in endometrial carcinoma. The weaker the expression of progesterone receptors, the higher the degree of cell histology (poor differentiation/ G3) and the higher the stage of endometrial carcinoma, meaning that examining the expression of progesterone responders can be the first step in determining the prognosis of endometrial carcinoma patients to determine whether these patients need adjuvant therapy, namely radiation.

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