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Expression Level of Cyclin-D1 between Endometriomas and Ovarian Carcinomes

Tingkat Ekspresi Cyclin-D1 antara Endometrioma dan Karsinom Ovarium

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Abstract: Endometrioma on the ovaries is a benign gynecological disorder that is often found in women of reproductive age. The approach was made to the malignant transformation through the study of cyclin-D1 expression. This research aim to analyze differences in the level of Cyclin-D1 expression in ovarian endometriosis and ovarian carcinoma associated with the pathogenesis of endometrioma and ovarian carcinoma. Analytical observational study with cross sectional approach to cyclin-D1 expression between endometrioma and ovarian carcinoma with good and bad differentiation. The research subjects were 20 cases of endometrioma, each of the 20 cases of ovarian carcinoma were well and poorly differentiated. Statistical analysis using the ANOVA test on the level of cyclin-D1 expression between groups. The mean cyclin-D1 expression in endometrioma was 67.25. The mean of well-differentiated ovarian carcinoma was 132.41. The mean of poorly differentiated ovarian carcinoma was 128.83. Anova test resulted in a significant difference between the expression of cyclin-D1 endometrioma and ovarian carcinoma with good and bad differences (p 0.00). There is a significant difference between endometrioma and ovarian carcinoma with good and bad differentiation. Endometrioma cyclin-D1 expression was lower than ovarian carcinoma with good and bad differentiation.

Keywords: endometrioma; ovarian carcinoma; cyclin-D1

Abstrak: *Endometrioma pada ovarii merupakan kelainan ginekologi jinak yang sering ditemukan pada wanita usia reproduksi. Pendekatan dilakukan terhadap transformasi ke arah maligna melalui studi ekspresi cyclin-D1. Tujuan penelitian ini untuk menganalisa perbedaan tingkat ekspresi Cyclin-D1 pada endometriosis ovarii dan karsinoma ovarii dikaitkan dengan patogenesis endometrioma dan karsinoma ovarii. Penelitian observasional analitik dengan pendekatan cross sectional terhadap ekspresi cyclin-D1 antara endometrioma dengan karsinoma ovarii berdifferensiasi baik dan jelek. Subyek penelitian ialah 20 kasus endometrioma, masing-masing 20 kasus karsinoma ovarii berdiferensiasi baik dan jelek. Analisa statistik menggunakan uji anova terhadap tingkat ekspresi cyclin-D1 antar kelompok. Rerata ekspresi cyclin-D1 pada endometrioma adalah 67,25. Rerata pada karsinoma ovarii berdiferensiasi baik adalah 132,41. Rerata pada karsinoma ovarii berdifferensiasi jelek adalah 128,83. Uji anova menghasilkan perbedaan yang bermakna antara ekspresi cyclin-D1 endometrioma dan karsinoma ovarii berdifferensiasi baik dan jelek (p 0,00). Terdapat perbedaan bermakna antara endometrioma, karsinoma ovarii berdiferensiasi baik dan jelek. Ekspresi cyclin-D1 endometrioma lebih rendah dibandingkan karsinoma ovarii berdiferensiasi baik dan jelek.*

Kata Kunci: *endometrioma; karsinoma ovarii; cyclin-D1*

INTRODUCTION

Endometriosis is a benign disorder. Based on the theory, both endometrioma and ovarian carcinoma originate from the ovarian surface epithelium (OSE-Ovarian Surface Epithelium). During ovulation, the follicle ruptures and the oocyte is released resulting in physical trauma to the cell surface of the ovary and it needs repair. This incident takes place many times during the reproductive period of women so that the process of damage and repair is repeated many times. OSE cells will show a high degree of resilience resulting in a transition from epithelial to mesensimal phenotype. Physical trauma that occurs in connection with ovulation will recruit inflammatory cells that are involved so that many cytokines play a role and become reactive oxygen species (ROS) and result in DNA damage. DNA damage in OSE cells makes it very vulnerable so that it is easy to transform.[1] Endometriosis is also a complex disease that involves the interaction of multiple genes. Several early studies have shown an increased risk of endometriosis among families with endometriosis. Research on the role of environment and genetic regulation in endometriosis has shown that endometriosis is increasing due to a combination of environmental factors and genetic variation. On the other hand, various molecular techniques including linkage and genome analysis have identified candidate genes that are located in locations associated with the female reproductive tract.[2] In this study, a biomolecular relationship between endometrioma and ovarian carcinoma will be sought, in relation to the transformation towards malignancy. Endometriosis is diagnosed in 30% -40% of women with infertility and pelvic pain. Endometriosis is complex with various etiopathogenesis. Research on the pathogenesis of endometriosis covers 4 research areas, namely: (1) genetics, (2) environment, (3) cancer biology, and (4) immunology.[2]

Endometrioma (ovarian endometriosis) is a common manifestation of endometriosis. Endometrioma is suspected of having the potential to be a malignancy where the development of ovarian cancer is found in 0.3-0.8% of endometrioma sufferers.[3] Several studies reported a histological transition from endometrioma to ovarian carcinoma including malignant transformation in extraovarial endometriosis. The prevalence of endometrioma in patients with epithelial ovarian malignancies, especially endometrioid and clear cells, is higher than other ovarian malignancies.[4] Young women (10-29 years) with endometrioma have a 3.5-fold risk of developing ovarian cancer. compared with women without endometriosis.[5]

Endometriosis can turn into a malignant ovarian tumor, with a malignancy rate of 0.3% -1.6% and a type of endometrioid or clear cell carcinoma. Several studies have been carried out to see the profile between ovarian carcinoma and endometrioma at Dr. Hospital. Moewardi Surakarta, obtained the following results: there is no difference in the expression of BCL-2 oncogene between ovarian carcinoma and endometrioma,[6] there is no difference in bax expression between carcinoma and endometrioma,[7] there is no significant difference in the expression of hyperphosphorylation Prb Between endometrioma and ovarian carcinoma,[8] there is a significant difference where the c-Myc expression in endometrioma is lower than ovarian carcinoma,[9] there is no difference in intra-nuclear p21 expression between endometrioma and ovarian carcinoma,[10] there is a significant difference in the expression of cytoplastic p21 in endometrioma and ovarian carcinoma, there is a significant difference in the expression level of p 53 mutants between endometrioma and ovarian carcinoma.[11] The p27 expression value did not differ significantly between endometrioma and ovarian carcinoma,[12] there was no difference between HER2 / NEU between endometrioma and ovarian carcinoma,[13] there was no difference in COX-2 expression between endometrioma and ovarian, these results show that the molecular aspects of endometriomas have a role in carcinogenesis. Endometrioma has one of the promotion mechanisms leading to malignant transformation.

Cyclin-D1, which is associated with an increase in the rate of cell proliferation, is one of the cell cycle proteins that is responsible for the transfer (transition) to the S phase of the cell cycle (DNA synthesis). Overexpression of cyclin-D1 is associated with a malignant transformation. Several studies have shown that an estimated 26% of sporadic ovarian cancers have overexpression of cyclin-D1. In addition, cyclin-D1 plays a dominant role in regulating the development of the cell cycle in ovarian cancer cells and that the degradation of cyclin-D1 can induce the cessation of the G1 phase cell cycle. So that by choosing cyclin-D1 as the target of therapy, it is possible to inhibit the development of ovarian cancer molecularly.[14] In this study, we will look for differences in the level of Cyclin-D1 expression between endometrioma and ovarian carcinoma through genetic pathways. The results are expected to provide an overview of the possibility of

endometrioma transformation towards ovarian malignancy. If there are differences, it will affect the treatment of endometriosis women who suffer from endometrioma in the future.

MATERIALS AND METHOD

An analytic observational study with a cross sectional approach showed a difference in the level of expression of Cyclin-D1 in endometrioma (ovarian endometriosis) and carcinoma of the ovary by immunohistochemical staining with monoclonal human antibody anti cyclin-D1. The study was conducted from December 2013 to January 2014 in the Obstetrics and Gynecology department Moewardi Surakarta Hospital and the Laboratory of Pathology Anatomy Faculoty of Medicine Universitas Sebelas Maret, Central Java. After the results of this main research were completed, it will be continued in future years of research with the development of cyclin-D. The number of samples studied in this study were 20 samples of endometrioma, 20 samples of ovarian carcinoma with good differentiation and 20 samples of ovarian carcinoma with poor differentiation. Data obtained from immunohistochemical staining with monoclonal human antibody anti cyclin-D1 and tabulated and displayed in the form of a percentage of cells with negative, weak positive, moderate positive and strong expressions. The mean expression of each sample is then converted into a qualitative value. The data normality test was also carried out using the Kolmogorov-Smirnov test showing normal data distribution so that further statistical analysis was carried out using the Anova test.

RESULT

Qualitative calculations using histological scores were carried out based on the percentage of cyclin-D1 expression in each sample. The mean histological score of endometrioma 67.25 ovarian carcinoma with good differentiation 132.41 and ovarian carcinoma with poor differentiation 128.83 Statistical test for histological score of the endometrioma group using the Kolmogorov-Smirnov test had a mean of 67.25 with a standard deviation of 44.03 The mean of the ovarian carcinoma group was well differentiated 132.41 with a standard deviation of 59.13. The mean ovarian carcinoma group with poor differentiation was 128.83 with a standard deviation of 50.74. The three groups of data were normally distributed so that the analysis was carried out using the Anova test.

Table 1. The Cyclin-D1 Histological Score Table for Endometrioma

Disease Status	N	Average	SD
Endometrioma	20	67.25	44.03
Ovarian Carcinoma			
Good	20	132.41	59.13
Ugly	20	128.83	50.74

Table 2. Table of Mean Percentage of Cyclin-D1 Expression in Endometriomas

			Expression				
			Negative	Weak	Moderate	Strong	Total
Group	Endometriosis	Count	3	2	5	10	20
		% of Total	5.0%	3.3%	8.3%	16.7%	33.3%
	Carcinoma of Ovarri diff Good	Count	1	1	2	16	20
		% of Total	1.7%	1.7%	3.3%	26.7%	33.3%
	Diff ugly Ovarri carcinoma	Count	0	1	3	16	20
		% of Total	.0%	1.7%	5.0%	26.7%	33.3%
Total		Count	4	7	7	42	60
		% of Total	6.7%	11.7%	11.7%	70.0%	100.0%

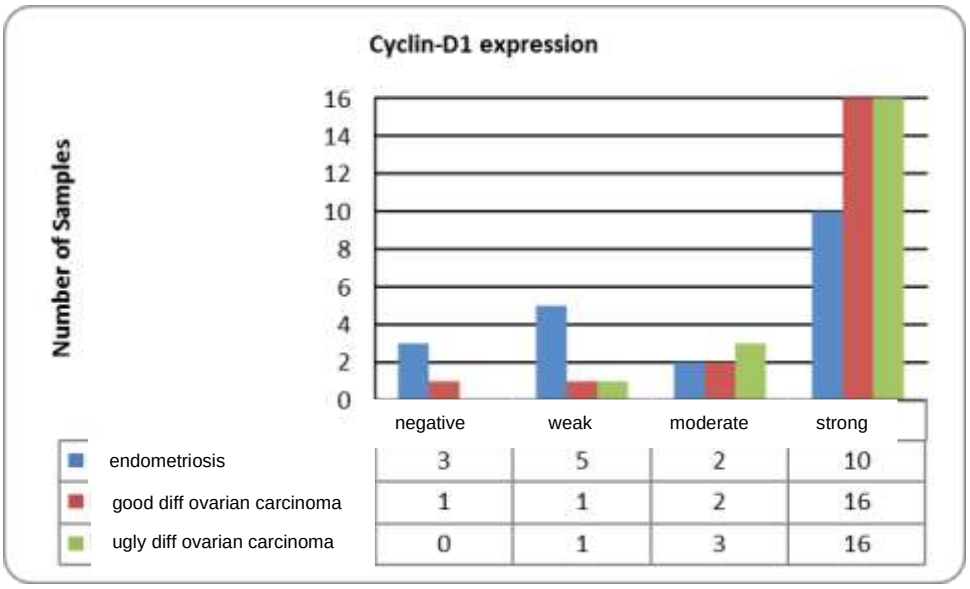
Based on the assessment of cyclin-D1 expression in 20 samples of endometrioma, there was a strong positive expression in the endometrioma group found 10 cases with a mean percentage of 16.7%. Mean 26.7%, whereas in 20 samples of ovarian carcinoma with poor differentiation, strong positive expression was found in the ovarian carcinoma group with poor differentiation found 16 cases with a mean percentage of 26.7%.

The mean percentage of cyclin-D1 expression in endometriomas obtained a strong positive result of 16.7%, moderate positive 8.3%, weak positive 3.3%, and negative 5%. Ovarian carcinoma with good differentiation obtained a strong positive result of 26.7%, moderate positive 3.3%, weak positive 1.7% and negative 1.7%, while ovarian carcinoma had a bad difference obtained a strong positive result 26.7%, moderate positive 5 %, weak positive 1.7%, and negative 0%.

The mean percentage of moderate positive cyclin-D1 expression in carcinoma ovarii good differentiation (10%) is almost 2 times the average percentage of moderate positive expression in endometrioma (5%) and almost 5 times when compared to ovarian carcinoma with poor differentiation. The mean of weak positive endometrioma (45%) is almost 4 times that of well-differentiated ovarian carcinoma (10%) and 3 times that of poorly differentiated ovarian carcinoma (30%).

Calculation of the meaning of cyclin-D1 expression in the endometrioma group, ovarian carcinoma with good differentiation and poorly differentiated ovarian carcinoma shows weak positive qualitative meaning. The distribution of data in the endometrioma group shows the highest value of nine in the eleventh sample and the lowest value in the thirteenth sample with a mean value of four. The average distribution of ovarian carcinoma with good differentiation shows the lowest mean value of four and the highest mean value of five, while in the poorly differentiated ovarian carcinoma group, the distribution of data with the highest value is seven with a mean of seven point thirteen and the lowest value is four with a mean of four point seven.

With the Ft-test, the value of p = 0.000 was obtained so that it means that the cyclin-D1 expression in endometrioma was significantly different from the cyclin-D1 expression in ovarian carcinoma which was well differentiated and cyclin-D1 expression in ovarian carcinoma was poorly differentiated.



Graph 1. The meaning of cyclin-D1 expression between endometrioma, well-differentiated ovarian carcinoma and poorly differentiated ovarian carcinoma

Table 3. Table of Results of the F-test Cyclin-D1 Expression between Endometrioma and Carcinoma Ovaries

Disease Status	N	Mean Diff	p
Endometrioma-Ovarri is good	20	65.1505	0.000**
Endometrioma-Ovarri is bad	20	61.580	0.000**
Ovarri is good-Ovarri is bad	20	3.57050	0.828

Table 4. The Results Table for Multiple Comparisons of Cyclin-D1 Expression between Endometrioma

Disease Status	N	Average	SD	p
Endometrioma	20	67.25	44.03	0.000
Ovarii Good	20	132.41	59.13	
Ugly Ovarii	20	128.83	50.74	

The multiple comparison test for the difference between endometrioma and ovary, the value of p=0,000 was obtained, so it can be interpreted that the cyclin-D1 expression in endometrioma was significantly different from the cyclin-D1 expression in ovarian carcinoma with good differentiation. The difference between endometrioma and bad ovary obtained p = 0.000, so that it can be interpreted that the cyclin-D1 expression in endometrioma is significantly different from the cyclin-D1 expression in ovarian carcinoma with poor differentiation. Whereas the difference between good ovaries and bad ovaries obtained p = 0.828 so that it can be interpreted that the cyclin-D1 expression in well-differentiated ovarian carcinoma did not differ significantly from the cyclin-D1 expression in ovarian carcinoma with poor differentiation.

DISCUSSION

The results above show that the cyclin-D1 expression in endometriomas is lower when compared to ovarian carcinoma with good and bad differentiation. This is because ovarian carcinoma occurs, so there is more ovulation trauma than endometrioma, and in accordance with the epidemiology of ovarian carcinoma which often occurs at menopause compared to endometrioma which often occurs at reproductive age.[15] In addition, there is also differentiation and metaplasia, which makes the epithelium complex similar to the mulberry duct organ. If the DNA damage in these cells is not repaired, it is possible for neoplastic transformation to occur and eventually to ovarian carcinoma.[16]

Cyclin-D1 binds with Cdk4 and Cdk6 to form a pRB kinase. Against phosphorylation, pRB loses repressive activity for transcription factor E2F, which then activates the transcription of several genes required for the transition from the G1 to S phase and for DNA replication. The cyclin-D1 gene is rearranged and overexpressed in centro cytic lymphoma and parathyroid tumors and then amplified and / or overexpressed in most human tumors of various cancers. The ectopic overexpression of cyclin-D1 in fibroblast culture shortens in the G1 phase of the cell cycle. Furthermore, it has been shown that the introduction of an anti-sensecyclin-D1 into carcinoma cell lines, in which the cyclin-D1 gene is amplified and overexpressed, causes reversion of the malignant phenotype. Thus, increased cyclin-D1 expression can play an important role in tumor development and in the maintenance of the malignant phenotype. However, this amount is insufficient to change the nature of the main or definite fibroblasts.

Cyclin-D1, which is associated with an increase in the rate of cell proliferation, is one of the cell cycle proteins that is responsible for the transfer (transition) to the S phase of the cell cycle (DNA synthesis). Overexpression of cyclin-D1 is associated with a malignant transformation. Several studies have shown that an estimated 26% of sporadic ovarian cancers have overexpression of cyclin-D1. In addition, cyclin-D1 plays a dominant role in regulating the development of the cell cycle in ovarian cancer cells and that the degradation of cyclin-D1 can induce the cessation of the G1 phase cell cycle. So that by choosing cyclin-D1 as

the target of therapy, it is possible to inhibit the development of ovarian cancer molecularly.[14] In addition, cyclin-D1 also has a role in triggering the development of malignancy which is related to the activity of tumor-suppressing genes, namely p21 (cyclin dependent kinase inhibitor 1 A) and p16 (cyclin dependent kinase inhibitor 2A). In normal people, p21 products play an active role in the regulation of cell complexes. Through transcription activation of the p21 gene followed by inhibition of cyclin dependent kinase, it will stop the cell cycle in the G1 phase so that it will prevent entering the S phase.The p21 gene also inhibits the activity of the cyclin group with cyclin dependent kinase (cdk2) and cyclin dependent kinase (cdk4) which acts as a regulator during the DNA replication process taking place in the S phase. In cases of endometriosis when compared to normal people, it appears that there is a decrease in the expression of the p21 gene and conversely there is an increase in cyclin-D1 so that the normal gene suppression process as described above will be inhibited.[17] The Hallmark of cancer also shows a decreased sensitivity to apoptotic signals, which is more common in ovarian carcinomas than in endometriomas. Multistep tumor progression also indicates endometrioma is in promotion stage and ovarian carcinoma is in progression stage. Based on the above points, it can be concluded that in this study there is a significant difference in the level of cyclin-D1 expression between endometrioma and ovarian carcinoma with good and bad differentiation. This difference illustrates that the endometrioma still indicates the possibility of being malignant.

The limitation of the research because we only assessed in cross sectional approached, with limited number of patients. Future investigation requires the development of cyclin-D1 expression with a larger sample, cases of severe and longitudinal assessment of endometriosis.

CONCLUSION

The expression of cyclin-D1 between endometrioma and ovarian carcinoma with good and bad differentiation can be an indication of an influential genetic pathway. Cyclin-D1 expression was lower in endometrioma than ovarian carcinoma with good and bad differentiation

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CONFLICTS OF INTEREST

The authors declare that have no conflict interest

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