Research Article

Ki-67 Expression is Correlated with Cyst Size and Stage of Endometriosis

Ekspresi Ki-67 Berkorelasi dengan Ukuran Kista dan Derajat Endometriosis

Muhammad Alif, Ruswana Anwar, Adhi Pribadi

Department of Obstetrics and Gynecology
Faculty of Medicine Padjadjaran University/
Dr. Hasan Sadikin Hospital
Bandung

Abstract

Objectives: To analyze the association between Ki-67 expression with the cyst size and stage of endometriosis, and the correlation strength between them.

Methods: A cross-sectional analytic observational study involving 56 paraffin blocks from subjects diagnosed with endometriosis, who had undergone laparotomy or laparoscopic surgery. The study is conducted in Dr. Hasan Sadikin Hospital in September-November 2012.

Results: Shows a significant association between Ki-67 expression and the size of endometriotic cyst ($p < 0.0001$), also with a strong correlation ($r = 0.55$) according to Guilford criteria. There is also a significant association between Ki-67 expression and the endometriosis stage ($p < 0.0001$), with a strong correlation ($r = 0.564$) according to Guilford criteria.

Conclusion: Ki-67 expression is correlated with the cyst size and stage of endometriosis.

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Keywords: endometriosis stage, endometriotic cyst size, Ki-67

Abstrak

***Tujuan**: Untuk menganalisis hubungan antara Ki-67 ekspresi dengan ukuran kista dan tahap endometriosis, dan kekuatan hubungan di antara mereka.*

***Metode**: Penelitian ini merupakan penelitian observasional analitik dengan metode potong lintang menggunakan 56 parafin blok dari subyek didiagnosis dengan endometriosis, yang telah menjalani operasi laparotomi atau laparoskopi. Penelitian ini dilakukan di Rumah Sakit Hasan Sadikin pada bulan September-November 2012.*

***Hasil**: Menunjukkan hubungan yang signifikan antara Ki-67 bereks-presi dan ukuran kista endometriosis ($p < 0,0001$), juga dengan korelasi yang kuat ($r = 0,55$) sesuai dengan kriteria Guilford. Ada juga hubungan yang signifikan antara Ki-67 dan ekspresi tahap endometriosis ($p <0,0001$), dengan korelasi yang kuat ($r = 0,564$) sesuai dengan kriteria Guilford.*

***Kesimpulan**: Ekspresi Ki-67 berkorelasi dengan ukuran kista dan tahap endometriosis.*

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***Kata kunci**: Ki-67, tahap endometriosis, ukuran kista endometriosis*

Correspondence: Mohammad Alif. Department of Obstetrics and Gynecology Faculty of Medicine University of Padjadjaran, Dr. Hasan Sadikin Hospital, Bandung. Telephone: 022-2032530, Email: m_aliefzoel@yahoo.com

INTRODUCTION

Endometriosis is defined as endometrium-like tissue found outside uterus, which causes a chronic inflammatory reaction. This disease is chronic and progressive. Physiologically, there is a significant molecular difference between eutopic endometrial tissue and endometriosis. The difference is observed on the evidence of cellular activity of the lesion, the progressivity, its ability to impair normal physiological process, and also its ability to form a massive invasive mass.[1-3]

Endometriosis is a benign disease with high-proliferative property. Proliferation is associated with the progressivity of the disease, while the progressivity is marked by the ability to form a massive mass, as discussed here it is the endometriotic cyst, and to invade the surrounding tissue, represented by the stage of the disease.

Proliferation is the key marker of tumor progressivity. One of the biochemical factors to measure proliferation is Ki-67, being immunohistochemically examined.[4-9] Ki-67 is a protein marking cell proliferation. In interphases the antigen can be detected in the nucleus, just as protein is on the chromosome in mitotic phases. Ki-67 protein appears in every active phases in cell fission (G1, S, G2, and mitotic) but is undetected in the resting phase (G0), making Ki-67 a good marker to determine cellular growth fraction.[4-9]

METHOD

This is a cross-sectional observational analytical study involving 56 patients diagnosed with endometriosis who had had histopathological examination and laparotomic/laparoscopic cystectomy or salpingo-oophorectomy procedure in Depart-

ment of Obstetrics and Gynecology of Dr. Hasan Sadikin Hospital, aged 20-42 years old, with stage III-IV endometriosis. From the subjects, the expression Ki-67 protein and endometriotic cyst size were then measured.

The paraffin blocks of endometriotic cyst obtained from the subjects after laparotomic/laparoscopic cystectomy or salpingo-oophorectomy procedure are examined as histopathologic specimen and stained with the immunohistochemical Ki-67 dye. Cells expressing Ki-67 would have been dyed brown, while normal cells would appeared as pale blue in color. The specimens showing Ki-67 were then examined under Olympus CX 21 light microscope with 400x magnification, to be counted for each 100 cells in five high power field, measured for the Ki-67 expression.

The data were documented and then analyzed using SPSS version 18.0 for Windows. The categorical data were analyzed with Gamma correlation test for homogenous data and Somers'd correlation test for inhomogeneous data. After the hypothesis testing and significant result achieved, the correlation strength would be determined with Guilford criteria (1956). P value <0.05 reflects significant result.

RESULT

The study was conducted from September to November 2012 with 56 subjects who met the inclusion criteria. Data that were collected included age, parity, cyst size upon surgery, and endometriosis stage (stage III-IV). Paraffin blocks of endometriotic cyst were then examined for Ki-67 expression in the anatomical pathology laboratory of Dr. Hasan Sadikin Hospital. Subjects of this study were not compared with controls, due to the difficulty in finding samples of normal ovarian tissue.

Table 1. Characteristic of Patients with Endometriotic Cyst by Age and Parity.

	Age (years old)	Parity
Mean ± SD	35.50 ± 7.54	1.18 ± 1.22
Minimum	19	0
Maximum	49	5
Range	30	5

Table 1 shows the descriptive data of the subjects' age and parity. Age of the subjects ranged between 19-49 years old, with mean of 35.50 (± 7.54) years old. Parity of the subjects ranged between 0-5, with mean of 1.18 (± 1.22).

Table 2. Cyst Size and Ki-67 expression in Endometriotic Cyst.

	Age (years old)	Parity
Mean ± SD	8.96 ± 5.68	35.71 ± 30.63
Minimum	27	10
Maximum	2	90
Range	29	80

Table 2 shows the descriptive data of cyst size and Ki-67 expression. The size of the cysts are 2-29 cm, with mean of 8.96 (± 5.68) cm. while the Ki-67 expression ranges 10-80%, with mean of 35.71 (± 30.63) %.

Table 3 shows a significant correlation between Ki-67 expression and endometriotic cyst size, with p value of <0.0001 ($p < 0.05$), and correlation coefficient of 0.55 which reflects strong correlation according to Guilford criteria.

Table 3. Correlation between the Increase of Ki-67 Expression and Cyst Size.

Ki-67	n (%)		Correlation Coefficient	Significance
	Cyst Size < 6 cm	Cyst Size > 6 cm		
Low	14 (50%)	14 (50%)	r = 0.550	p < 0.0001*
Moderate	2 (10%)	18 (90%)		
High	0 (0%)	8 (100%)		

*Note: * Somers'd correlation test for inhomogeneous categorical data; p< 0.05 (significant).*

Table 4. Correlation between the Increase of Ki-67 Expression and Endometriosis Stage.

Ki-67	n (%)		Correlation Coefficient	Significance
	Stage III Endometriosis	Stage IV Endometriosis		
Low	20 (71.4%)	8 (28.6%)	r = 0.564	p < 0.0001*
Moderate	6 (30%)	14 (70%)		
High	0 (0%)	8 (100%)		

*Note: * Somers'd correlation test for inhomogeneous categorical data; p< 0.05 (significant).*

Table 4 shows a significant association between Ki-67 expression and endometriosis stage. Statistical analysis with Somers'd test results in p value of < 0.0001 (p < 0.05) and correlation coefficient of 0.564, reflecting a significantly strong correlation based on Guilford criteria.

DISCUSSION

The result of this study shows a significant correlation between Ki-67 expression and endometriotic cyst size, with p value of <0.0001 (p<0.05), and correlation coefficient of 0.55 which reflects strong correlation according to Guilford criteria.

No other study had reported the association between Ki-67 expression and endometriotic cyst size. However some studies had analyzed the association between Ki-67 and size of other tumor such as breast tumor.

A prospective study conducted by Johnston, et al. on breast cancer patients who had chemotherapy and hormonal therapy reported a strong correlation between Ki-67 and breast tumor size. In the study, Ki-67 was used as the proliferative index to reflect the therapy efficacy. Low level of Ki-67 reflects a low proliferation rate and tumor regression after therapy.[10-12]

The association of Ki-67 and respond to chemotherapy is obviously concluded. Five of six studies have reported that Ki-67 predicts respond to chemotherapy clinically and histopathologically in patients with breast cancer. High level of Ki-67 is associated with good response to therapy, being clinically marked by the diminished size of tumor and histopathologically by the tumor grading. On the other hand, high level of Ki-67 which is associated with good response to therapy, also gives a poor prognosis. While good response clinically and moreover pathologically reflecting a complete response, is associated with a better long term prognosis. This reflects the need of stratification based on the level of Ki-67 which would improve the prognosis of clinical response to neoadjuvant chemotherapy.[11-14]

The increase of Ki-67 expression in endometriotic cyst shows that endometriosis is highly proliferative. The high proliferation is caused by the endometrial cells outside uterine cavity which trigger inflammatory reaction, these cells invaginate and form cystic mass. Chronic and progressive inflammation is able to form invasive mass, such as a massive endometriotic cyst in this case.[8,15]

This study also shows a significant association between Ki-67 expression and endometriosis stage. Statistical analysis with Somers'd test results in p value of <0.0001 (p<0.05) and correlation coefficient of 0.564, reflecting a significantly strong correlation based on Guilford criteria.

The more advanced stage of the endometriosis, the higher is the Ki-67 expression. This has been proven by Li, et al.[16] The study reported different expression of Ki-67 in each stages, given only few of (+3) and (+4) staining in early stage, even really close to those of the control group. This explains that in the early stage of proliferation, the infiltration or invasion are low, consistent with the finding from surgery that the endometriotic lesions were superficial with not much of adhesion. The Ki-67 expression was different in the later stage, in which there were more of (+3) and (+4) staining results. There is a positive correlation between endometriosis stage and Ki-67 expression, this explains the importance of proliferation in progressivity of the disease.[15,16] In more advanced stage of disease, according to AFS score, there would be deeper endometriotic lesions, more adhesion, and obliteration in cavum douglasi. Consistent with the etiopathogenesis of endometriosis, as endometrial cells reach the implantation sites outside uterine cavity by menstrual blood retrograde, lymphogenic, or

hematogenic, these cells would adhere and invade ovary or peritoneal structures. The invasion would be deeper with adequate vascularization and release of cytokines.[8,15]

In advanced stage of endometriosis, proliferation is enhanced, as seen from the increased expression of Ki-67. Whenever the cellular proliferative property is increased, the cell will grow uncontrolled like tumor does. These cells will attack the surrounding structures, causing adhesion which can lead to organ distortion.[1,8,15]

Endometriosis has been recognized to be hormonal-dependent. It is has some properties of malignancy, such as the high rate of proliferation, growth, and invasion, which causes injury of organs, according to the stage of the disease. [1,8,15,17]

Nissole, et al. in 1997 reported that Ki-67 depends on the menstrual cycle.[18] In normal endometrial cell, the proliferative activity increases in proliferative phase of the cycle, and declines in the secretory phase. Endometriotic cell shows similar activity, in which its proliferation enhances in proliferative phase of the cycle. However, it only shows a little insignificant decline of the proliferative activity in the secretory phase.

Park, et al. in 2008 also reported the similar result.[19] The expression of Ki-67 is associated with the menstrual cycle, that the endometriotic cells proliferation is more extensive in the proliferative phase of menstruation, but with a significantly lower activity in the secretory phase.

Working on the same topic, Li et al. in 1993 compared the proliferative activity of endometriotic cells through menstrual cycle. The activity increases in the end of proliferative phase of menstrual cycle, in the middle and the end of secretory phase.

Those former studies indicated that the proliferative activity of endometriotic cells depend on the menstrual cycle. This is consistent with the result of this study. However, one subject shows a low Ki-67 expression, while having a large cyst with advanced stadium. This might be caused by the subject was in her secretory phase of menstrual cycle, in which the proliferative activity is lower than its activity in the proliferative phase.

CONCLUSSION

There is a strong positive correlation between Ki-67 expression and the size of endometriotic cyst. There is also a strong positive correlation between Ki-67 expression and the stage of endometriosis.

SUGGESTIONS

Another study is needed to prove the role of Ki-67 in endometriosis, so that the pathogenesis of endometriosis will be better explained. Ki-67 is used as an immunohistochemical marker in patients with endometriosis to predict the severity of the disease. Further study having Ki-67 to predict recurrence in patients with high expression of Ki-67 who had undergone cystectomy, is encouraged.

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