

Original Article

## Comparison of Ki-67 proliferative index between eutopic and ectopic endometrium: A case control study

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Accepted 22 July 2011

### Abstract

**Objective:** In this study, the Ki-67 proliferative indices among the stages of the endometriosis were compared to clarify whether the proliferation was increased with increasing disease stage.

**Materials and Methods:** Thirty-eight patients who underwent surgery either by laparotomy or by laparoscopy with the diagnosis of endometriosis and 21 patients, as controls, who underwent hysterectomy with the diagnosis of myoma uteri and without any endometrial pathology at our hospital between 2005 and 2007 were studied. Biopsy specimens of endometriotic foci and endometriomas in study group, and eutopic endometrium of hysterectomy specimens of control group were studied.

**Results:** Fifty-nine patients were divided into Group 1 (21 patients in control), Group 2 (19 patients in stage I and II of endometriosis), and Group 3 (19 patients in stage III and IV). A moderate correlation between the stage of endometriosis and the degree of Ki-67 staining was found. When Ki-67 immunohistochemical staining was considered according to the threshold value for CA-125 (35 U/mL), Ki-67 positivity was increased with the increase in CA-125 value, but this increase was not statistically significant.

**Conclusion:** Endometriosis shows some characteristics of tumors such as high rate of invasion, getting autonomy, and proliferation as the disease progresses with subsequent damage to target organs. When the stage of the disease increases, environment becomes more suitable for increased proliferation and invasion. In this study, the increase in proliferative activity as the severity increases is shown by the increase in Ki-67 index. As more studies are being conducted in this field, pathogenesis will be clarified, which could help in the development of new treatment modalities. Copyright © 2012, Taiwan Association of Obstetrics & Gynecology. Published by Elsevier Taiwan LLC. All rights reserved.

**Keywords:** endometriosis; Ki-67 proliferative index; Ca-125

### Introduction

Endometriosis is a chronic, estrogen-dependent gynecological disorder characterized by the presence of functional endometrial glands and stromal elements outside the uterus, which induces chronic, inflammatory reaction associated with complaints ranging from pelvic pain to infertility [1–6].

Although it is thought to be hormone dependent, there are many theories to explain the pathogenesis: retrograde menstruation, lymphatic and hematogenous spread, immunologic theory, genetic factors, and environmental factors.

Ki-67 is a nuclear antigen present only in proliferating cells except G0 phase of cell cycle. Increase in antigenic expression during cell cycle in both benign and malignant cell lines assessing their proliferative status has also been shown [7–9]. Ki-67 score is now used to predict the prognosis, survival, and even the recurrences. A high level of Ki-67 proliferative index (PI) is associated with aggressive tumoral behavior and metastasis [10–19].

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CA-125 is a high-molecular-weight glycoprotein secreted by Müllerian epithelia. Elevated serum levels have also been recognized in gynecologic malignancies in patients with endometriosis.

CA19-9, a carbohydrate antigen, is elevated in gastrointestinal adenocarcinomas, and pancreatic and ovarian tumors. Like serum CA-125 levels, serum CA19-9 levels seem to be elevated in endometriosis patients [20].

Although it is thought to be a hormone-dependent disease, few recent studies investigating the invasive phenotype of endometriosis resembling the tumors draw attention to Ki-67 [21–23]. In this study, we compared the Ki-67 PIs at different stages of the endometriosis to clarify whether the proliferation was increased with increasing stage and its correlation with tumor markers.

## Materials and methods

Thirty-eight patients who underwent surgery for endometriosis (either by laparotomy or by laparoscopy) and 21 patients who underwent hysterectomy with the diagnosis of myoma uteri and without any endometrial pathology at our hospital between 2005 and 2007 were studied. Among the 38 patients in the study group, categorized according to the revised American Fertility Society (r-AFS) classification, 19 patients were at stage I and II and remaining 19 were at stage III and IV. Diagnosis of endometriosis was established following both macroscopic and postoperative histologic confirmation. Biopsy specimens of endometriotic foci and endometriomas of the study group and eutopic endometrium of hysterectomy specimens of the control group were studied. All patients were operated at early follicular phase of menstrual cycle. Blood samples for tumor markers were also taken at early follicular phase of the menstrual cycle. Patients had not been treated with any hormonal agent. The Ethics Committee of the hospital has also approved this study.

Specimens were fixed with 10% formalin, sent to pathology laboratory, and embedded in paraffin blocks. Five-micron-thick serial sections were made and studied after staining the specimens with hematoxylin and eosin. Immunohistochemical analysis was performed by means of indirect biotin–streptavidin-amplified system, using DAKO immunostaining kit and Ki-67 MiB1 antibodies. Briefly, deparaffinized sections were washed with phosphate buffer saline (PBS) solution, heated for 5 minutes in Antigen AR 10 solution at 700 W three times in a microwave, and washed with PBS again. Ki-67 MIB-1 antibodies, link antibodies, and labeling reagents were applied after they were washed with PBS each time. Degree of positive staining was calculated by counting 100 endometrial epithelial cells: 0–5 cells with nuclear staining were evaluated as negative, 6–25 cells as (+), 26–50 cells as (++) , 51–75 as (+++), and 76 and more as (++++) . All specimens were examined by the same pathologist.

## Results

Fifty-nine patients were divided into group 1 (21 patients in control), group 2 (19 patients in stage I and II), and group 3 (19 patients in stage III and IV). Characteristic features of the groups are shown in Table 1. Mean serum concentration of

CA-125 was  $17.7 \pm 6.3$  U/mL in control group,  $41.6 \pm 35.1$  U/mL in group 2, and increased to  $86.6 \pm 111.5$  U/mL in group 3. The difference between the mean values was statistically significant ( $p < 0.001$ ). When the groups were compared in pairs, the only statistically significant difference was detected between the mean values of CA-125 of control group and that of group 3 ( $p < 0.001$ ), but not between group 1 and 2 or between group 2 and 3. Mean concentration of serum CA19-9 values in control group, group 2, and group 3 were  $10.5 \pm 9.4$ ,  $26.3 \pm 23$ , and  $40.3 \pm 44.5$  U/mL, respectively. The difference between the mean values was statistically significant ( $p < 0.001$ , Kruskal–Wallis test). When *post hoc* tests were performed, differences between the mean CA19-9 values of control group and group 2 ( $p < 0.01$ ) and between those of control group and group 3 ( $p < 0.001$ ) were found to be statistically significant, but no significant difference was found between early stages and late stages of endometriosis (group 2 and 3).

Table 2 shows the Ki-67 immunoreactivity distribution in the groups. It was interesting that neither 3(+) nor 4(+) staining was found in group 1 and group 2, and negative staining percentages were similar in those groups (71.4% and 73.7%, respectively). Also distribution of Ki-67 staining between group 1 and 2 is not statistically significant ( $p = 0.669$ ).

The distribution in group 3 is very different. Three cases (15.8%) were stained as 4(+) four cases (21.0%) were stained as 3(+), and three cases (15.8%) had negative staining. As group 1 and 3 were compared, a statistically significant difference was found ( $p = 0.004$ ). The difference was due to the high rate of the negatively stained cases in group 1.

When group 2 and 3 were compared, the most noticeable finding was that 14 patients (73.7%) in group 2 and three patients (15.8%) in group 3 were found to be not stained. The difference between these groups was statistically significant ( $p = 0.001$ ). A moderate correlation was found between the stage of endometriosis and the degree of Ki-67 staining ( $r = 0.5$ ,  $p < 0.001$ , Spearman correlation analysis).

As shown in Table 3, when Ki-67 immunohistochemical staining was distributed according to the threshold value for CA-125 (35 U/mL), no statistical difference was found in the staining distribution between below and above threshold levels of serum CA-125 ( $p = 0.561$ , dual-sampling Kolmogorov–Smirnov test). Also, no statistically significant difference was observed between positive and negative staining (according to the distribution below and above threshold level) ( $p = 0.113$ , chi-square test). However, when a correlation

Table 1  
Characteristic features of the groups.

	Control ( $n = 21$ )	Stage I and II ( $n = 19$ )	Stage III and IV ( $n = 19$ )	P value
Age (y)	$44.5 \pm 4.1$	$41.4 \pm 7.3$	$42.1 \pm 6.3$	0.238 <sup>a</sup>
Gravidity	$3.7 \pm 2.0$	$3.2 \pm 2.5$	$2.4 \pm 2.3$	0.077 <sup>b</sup>
Parity	$2.4 \pm 1.3$	$2.3 \pm 1.6$	$1.8 \pm 1.2$	0.282 <sup>b</sup>
CA-125	$17.7 \pm 6.3$	$41.6 \pm 35.1$	$86.6 \pm 111.5$	<0.001 <sup>b</sup>
CA19-9	$10.5 \pm 9.4$	$26.3 \pm 23.0$	$40.3 \pm 44.5$	<0.001 <sup>b</sup>

<sup>a</sup> One-way variance analysis.

<sup>b</sup> Kruskal–Wallis test (Bonferroni *post hoc* test).

Table 2  
Ki-67 proliferative index distribution according to the groups.\*

Ki-67	Control	Stage I and II	Stage III and IV
Negative	15 (71.4%)	14 (73.7%)	3 (15.8%)
1+ staining	4 (19.1%)	2 (10.5%)	6 (31.6%)
2+ staining	2 (9.5%)	3 (15.8%)	3 (15.8%)
3+ staining	—	—	4 (21.0%)
4+ staining	—	—	3 (15.8%)

\*Pearson chi-square test,  $p = 0.002$ .

analysis is made, as the CA-125 value was increased, Ki-67 positivity was increased, but this increase, although very close, was not statistically significant ( $r = 0.256$ ,  $p = 0.051$ ).

## Discussion

The etiopathogenesis of endometriosis, which is common in infertile population, is largely unknown [24–27]. Chronic and recurrent progress, proliferative, infiltrative and invasive phenotype of the lesions shares the similar aspects of tumoral process [28–30]. This new perspective creates an interest on Ki-67 monoclonal antibody. Ki-67 is a nuclear antigen present only in proliferating cells. Increase in antigenic expression, meaning high Ki-67 PI, is associated with aggressive tumoral behavior and metastasis [7–19].

Li et al compared the PI of eutopic and ectopic endometrial cells. PI of endometriotic cells was found to be constantly higher than normal endometrium irrespective of the hormonal status during both menstrual cycle and postmenopause. PI of postmenopausal ectopic endometrium was high compared to the low PI at atrophic endometrium [31]. This supports the hypothesis that, in addition to hormonal stimulus, proliferation at ectopic implantation sites is controlled by other factors. The results were supported by Toki et al [32], Nisolle et al [33], and Matsumoto et al [34]. Park et al [29] observed higher proliferative activity in the endometrial cells of the patients with endometriosis than in patients without endometriosis. On the contrary, Jones et al observed low PI along the cycle of ectopic endometrial cells, similar to the observations of Suzuki et al [35].

A literature search confirms that our research is the first to study the relation between the stages of endometriosis and Ki-67 PI, although the number of cases is limited. Only Toki et al [20] studied serum CA-125 and CA19-9 values, degree of immunohistochemical staining of tissue CA-125 and CA19-9, and Ki-67 at endometriosis stage III and IV. They observed a positive correlation between CA-125 and Ki-67 immunohistochemical staining, indicating that proliferative (Ki-67 PI high) cells were actively secreting CA-125. However in our study, as the CA-125 value was increased, Ki-67 positivity was increased, but this

increase, although very close, was found to be not statistically significant ( $r = 0.256$ ,  $p = 0.051$ ).

CA-125 is a tumor marker used in diagnosis and follow-up process, but its sensitivity is low in early stages and use is limited in late stages [36]. Toki et al could not show any correlation between CA-125 and the disease stage. In our study, especially in later stages, CA-125 values were significantly higher than in controls. However, no significant difference was noted between controls and early stages of the disease, which supports Toki's results that CA-125 values do not correlate with the disease stage. Unlike other studies, we also found a positive correlation between the endometrial stage and Ki-67 PI. Fifteen out of 21 patients (71.4%) in control group had no staining, and only two (9.5%) had 2(+) staining, for whom noticeable adhesions were reported during surgery. These two patients likely had microscopic endometriotic foci that could not be detected during surgery. In early-stage group (group 2), high rate of negative staining (73.7%) and absence of 3(+) and 4(+) staining were similar to those in control group, which could be explained by the hypothesis that proliferation, infiltration, and also invasion are low in early stages, as characterized by the superficial lesions and filmy adhesions. But the distribution of Ki-67 PI was completely different in stage III and IV; as the stage increased, the number of 3(+) and 4(+) staining patients was increased. A positive correlation was observed between the stage of endometriosis and the positivity of staining, which indicated that proliferation was important in the progression of the disease. As the severity of the disease increases, according to the AFS scores, deep endometriotic lesions, dense adhesions, and cul-de-sac obliteration are added to the whole picture of the disease. When the etiopathogenesis of the disease is remembered, it is reasonable that endometrial cells reaching the implantation sites, either via retrograde menstruation or via hematogen or lymphatic ways, begin to attach and invade peritoneal surfaces or ovaries. When the vascularization increases and cytokines are liberated to environment, invasion deepens.

Gaetje et al [21] showed that cells from peritoneal endometriotic lesions and a metastatic bladder carcinoma cell line had similar invasion indices, whereas normal endometrial cells and nonmetastatic cells were noninvasive. They also studied E-cadherin expression of the cells from biopsies of endometriotic lesions and endometrium [22]. E-cadherin is an invasion suppressor molecule found in epithelial cells holding them together. *In vitro* invasive endometriotic cells were shown to be E-cadherin-negative nonmalignant cells; E-cadherin-negative cell fraction was increased in sections of endometriotic lesions but not in the eutopic endometrium. It has been proposed that these E-cadherin negative cells migrate to ectopic locations *in vivo*, which is important in pathogenesis.

Table 3  
Distribution of Ki-67 staining according to the CA-125 threshold value.\*

CA-125 value	Ki-67 negative	Ki-67 (+)	Ki-67 (++)	Ki-67 (+++)	Ki-67 (++++)	Total
Below 35 U/mL	23 (62.1%)	5 (13.5%)	7 (19%)	1 (2.7%)	1 (2.7%)	37 (100%)
Above 35 U/mL	9 (40.9%)	7 (31.8%)	1 (4.6%)	3 (13.6%)	2 (9.1%)	22 (100%)
Total	32 (54.2%)	12 (20.3%)	8 (13.6%)	4 (6.8%)	3 (5.1%)	59 (100%)

\*Dual-sample Kolmogorov–Smirnov test,  $p = 0.561$ .

Starzinski-Powitz et al [23] also studied the factors increasing the invasive potential. They have shown that cytokeratin-positive and E-cadherin-negative endometriotic cells have an invasive phenotype *in vitro*, similar to metastatic carcinoma cells; the invasiveness of endometriotic cells can be stimulated by a heat-stable protein present in peritoneal fluid but not the eutopic endometrial cells.

In summary, when the stage of the disease increases, proliferation is increased. The increase in activity as the severity of disease increases is shown by an increase in the Ki-67 index. When the cells increase their proliferative potential, they get autonomy as the tumoral cells. They attack the adjacent tissue, and at the end dense adhesions occur and anatomy is distorted. Postmenopausal endometriosis cases and increases in *bcl-2*, a gene that suppresses the cell death, and Ki-67 prove this autonomy [23, 32]. Although it is regarded as hormone dependent, endometriosis shows some characteristics of tumors such as high rate of invasion, getting autonomy and proliferation, and subsequent damage to target organs with the progression of the disease. With more studies being conducted in this field, pathogenesis will be clarified and perhaps new treatment modalities could be developed.

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