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# PROLIFERATIVE ACTIVITY IN OVARIAN ENDOMETRIOMA

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## Abstract

Endometriosis shows features similar to neoplasia, which recently increased interest in the monoclonal antigen Ki-67. The aim of the study was to study the expression of Ki-67 in the operated ovarian endometriomas in women of reproductive age. Materials of the study were 32 patients with ovarian endometriomas and 30 patients with tubal infertility in the control group who underwent surgical treatment. Immunohistochemical study was performed by avidin-biotin-peroxidase method according to the standard method. Evaluated the number of positively colored cells using rabbit monoclonal antibodies to Ki-67. In 68.75% of patients with operated endometriomas, active proliferation in ovarian formations is observed, which is manifested by increased expression of oncoprotein Ki-67 in the glandular epithelium, in the cytogenic stroma of the endometrium and in surrounding vessels. There is a direct correlation between the level of Ki-67 expression and the prevalence of the endometriomas.

**Key words: endometrioma, proliferation, Ki-67, immunohistochemistry.**

Endometriomas are a fairly common gynecological pathology and are registered in 11.3-31.1% of women operated on for adnexal masses [2, 9]. Questions about their morphogenesis and tumor properties remain unexplored [4], although multiple genetic, ecological, immunological, angiogenic and endocrine changes are considered their etiological factors. Despite the fact that endometriosis is evaluated as a benign condition, recent studies have shown that it, as a neoplastic process, has histopathological, molecular and genomic

similarities to cancer. Like the neoplastic process, the reasons why endometriosis is more aggressive and invasive in some patients are unknown [2, 7].

Endometriosis begins with invasion, receives autonomy and progresses with proliferation against the background of decreased contact inhibition of proliferation [9], ultimately damaging the target organ and exhibiting tumor properties. Endometriosis shows features similar to neoplasia, which recently increased interest in the monoclonal antigen Ki-67 [12].

Ki-67 is a non-histone nuclear protein that is present in all phases of the cell cycle, except for the G0 phase, and contains two molecules of 345 and 395 kDa; its gene is located on chromosome 10. Ki-67 is a nuclear protein observed in proliferating cells. Since this is a protein that shows the morphological features of cell proliferation, it is often used in the mitotic index and for the classification of tumors [12]. In immunohistochemical assessments, the percentage of cells that shows positive nuclear staining for Ki-67 indicates a proliferation index. In the study of JS Park et al. (2009) [10], the proliferation of endometrial cells in patients with endometriosis was determined using the Ki-67 proliferation index and was higher than in women without endometriosis.

The proliferative capacity of the endometrioid implants seems to be a prerequisite for long-term persistence, however, the proliferative activity of endometrioid lesions is described with controversial results. Some authors have shown increased proliferation in endometrium foci [8], and in the endometrium of patients with endometriosis [11]. The proliferative cells can be identified by studying the nuclear or perinuclear expression of Ki-67, a protein that is expressed in all phases of the cell cycle, except for the G0 stage, and is a universal marker for cell cycle evaluation. Antibodies to Ki-67 show proliferating cells that are in different phases of the cycle. This is the most reliable and clear marker of proliferation.

The antigen of Ki-67, which is detected by the corresponding monoclonal antibodies, is a short-lived protein, it collapses within 1.5-2 hours. Therefore, antibodies to Ki-67 are detected only by cells that share, since Ki-67 does not manage to accumulate and does not remain in the cells that are resting [1].

A number of studies have shown a probable correlation between Ki-67 expression and the number of mitotic cells. There are changes in mitotic activity in the eutopic and ectopic endometrium during the menstrual cycle [3]. In addition, apoptotic events seem to be reduced in endometrium foci, as well as in the corresponding endometrium [5]. These synergistic effects (enhanced proliferation in combination with reduced apoptosis) can explain the mechanism of growth and existence of tissue of the ectopic endometrium.

The aim of the study was to study the expression of Ki-67 in the operated ovarian endometriomas in women of reproductive age.

**Materials and methods**

Materials of the study were 32 patients of group I with ovarian endometriomas and 30 patients with tubal infertility in the control group who underwent surgical treatment in the University Clinic of the Odessa National Medical University "Center for Reconstructive and Rehabilitation Medicine".

All capsules of the endometriomas and ovarian biopsy were subjected to histological examination. Operating materials were fixed in 10% solution of neutral formalin, poured in paraffin. The research was carried out using the technique of step sections with a thickness of 5 microns. The sections were painted with hematoxylin and eosin.

Immunohistochemical study was performed by avidin-biotin-peroxidase method according to the standard method. Evaluated the number of positively colored cells using rabbit monoclonal antibodies (MAB) to Ki-67 (Clone SP6, code No. SP7 (RM-9106-S), Thermo Scientific, USA). Positive control with known immunoreactivity of the corresponding epitopes when coloring tissues for Ki-67 was the tonsil tissue. The passage of the primary antibody was used as a negative control. Nonspecific staining was not detected.

When evaluating the expression of KI-67, positively colored cells were counted in three fields of view and the percentage of positive cells in relation to all cells of the stroma or glands was calculated. Calculation was made of at least 1000 cellular elements of the stroma or glands.

Microscopy of patterns and all morphometric studies were performed on the Olympus AX70 Provis microscope (Olympus, Japan) using the analysis imaging “Analysis software 3.2 Pro” (Soft Imaging, Germany), according to the manufacturer's software recommendations.

The obtained data was processed using methods of analytical and variation statistics. In the case of a normal distribution of the population sample, the student's criterion (t-distribution) was used to assess the differences in the comparison of averages. In other cases, either the criterion of the values of the abnormal distribution of the sample with pairwise bound variables, or the non-parametric Wilcoxon-Mann-Whitney criterion was used. For the estimation of the differences in the frequency of manifestation of the analyzed index, the method of the Fisher's transformation of the angle and the $\chi^2$-criterion were used. Correlation analysis was used to study the stochastic relationship between indicators.

**Results and discussion**

The average age of patients with endometriomas was 29,50 ± 0,75 years, in women of the control group - 30,16 ± 0,44 years. The average diameter of the endometrioma varied from 3.5 to 8 cm and averaged 5.06 ± 0.62 cm. The endometriomas were removed from the right ovary in 12 (37.50%) and from the left one - in 20 (62.50%) persons

Immunoreactable to KI-67 cells were registered in the glandular epithelium, cytogeneous stroma of endometriomas and stromal vessels around 22 (68.75%) of the 32 patients under study. The number of immunopositive cells to Ki-67 in the glandular epithelium of the endometriomas varied from absence to 10.97%, in cytogenic stroma - from absence to 4.65%, in vessels - from absence to 2.34% (Fig.).

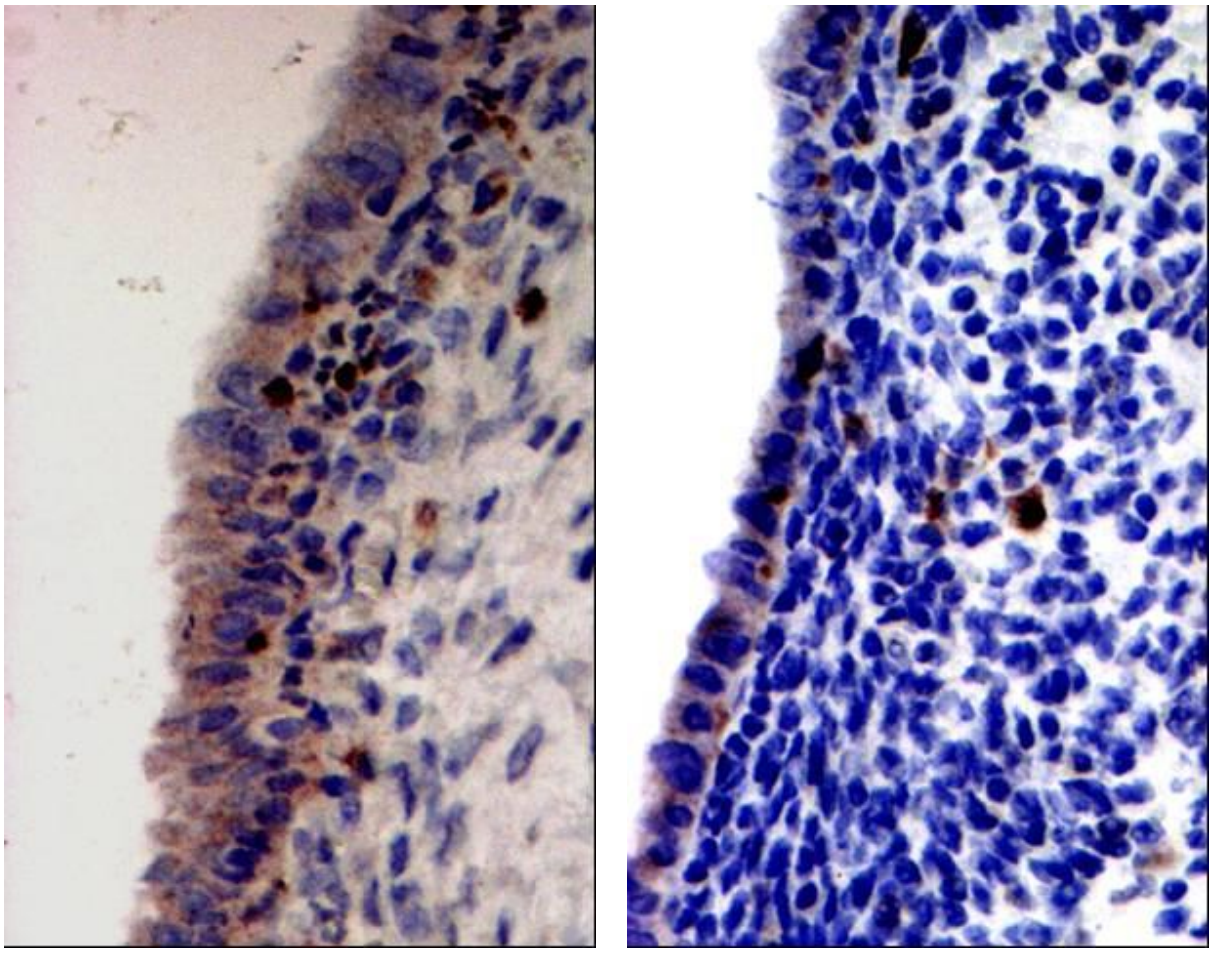

Fig. Immunohistochemical study. Different levels of Ki-67 in the glandular epithelium and cytogenic stroma of the ovarian endometriomas. Immunohistochemistry with MAB to KI-67. × 150

The average number of immunopositive cells to KI-67 in the glandular epithelium of the endometriomas was 8.95 ± 0.15% in the right ovary, 9.12 ± 0.19% – in the left ovary, and in the cytogenic stroma respectively – 3.87 ± 0,09 and 3.91 ± 0.11%, in vessels – 1.82 ± 0.04 and 1.96 ± 0.07%. The expression level of Ki-67 in the control group in the stroma of the ovary was 0.035 ± 0.003%, in the vessels – 0.028 ± 0.001%.

The direct correlation dependence between the level of Ki-67 expression and the prevalence of the endometriomas (mono-, bilateral) is established - $r = 0.35$, $p<0.04$.

Ki-67 is an oncoprotein, the level of expression of which reflects the processes of proliferation in the tissue. When Ki-67 production is elevated, tumors tend to be more aggressive with vascular invasion, proliferation and metastasis, which are commonly observed in these patients [12]. Recent studies emphasize that endometriosis exhibits tumor-like behavior [2, 7], and therefore among researchers increased interest in the relationship between Ki-67 expression and endometriosis. Our data show that more than two thirds of operated patients with ovarian endometriomas show the activity of proliferation processes in the glandular epithelium and cytogeneic stroma of endometriomas, as well as in surrounding vessels.

I. Kahyaoglu et al. (2012) [6] compared the Ki-67 proliferation index in the eutopic and ectopic endometrium and found an increase in the proliferation index with an increase in the incidence of endometriosis. In addition, there was a correlation between the extent of the spread of endometriosis and the levels of CA125. In our study, we also established a direct correlation between the expression level of KI-67 and the prevalence of the endometrium (one- and two-sided).

Thus, cells of the ectopic endometrium with an increased proliferative potential receive autonomy in the same way as tumor cells, and therefore, endometriosis damages the surrounding tissues, violates anatomy and causes the formation of connective tissue.

**Conclusions**

In 68.75% of patients with operated endometriomas, active proliferation in ovarian formations is observed, which is manifested by increased expression of oncoprotein Ki-67 in the glandular epithelium, in the cytogeneic stroma of the endometrium and in surrounding vessels. There is a direct correlation between the level of Ki-67 expression and the prevalence of the endometriomas.

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