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ANALYSIS OF THE FUNCTIONAL STATE OF DENDRITIC CELLS CD_{56+}, CD_{83+}, CD_{1A+} AND MARKERS OF APOPTOSIS BCL_2, CVC IN HYPERPLASIA OF ENDOMETRIUM

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Summary

The objective: to evaluate the processes of pathomorphosis and relationship between factors of apoptosis and functional state of dendritic cells in the endometrium of the women with endometrial hyperplasia. **Materials and Methods.** The treatment group consisted of 50 women, 25 - with simple hyperplasia (group I) and 25 - with atypical glandular-cystic hyperplasia (group II) of endometrium. Control set included 20 healthy women of the same age. The expression of activation markers CD_{16+}, CD_{56+}, CD_{1a}, CD_{85k} and CD_{123} on the surface of lymphocytes was studied by flow cytometry. Determination of CD receptors in endometrial biopsies performed by immunohistochemical method with the use of monoclonal antibodies LIR, Dardilly (France) to CD_{56+}, CD_{83+}, CD_{1a+}. Identification of tissue antigens was performed by avidin-biotin method. Expression of the antiapoptosis protein BCL-2 protein and proapoptosis protein CVC was observed in the cytoplasm of cells, it differed in intensity of staining. Statistical analysis of the results is made by methods of variance and correlation analysis using software Statistica 10.0. **Results.** In immunohistochemical study of endometrial biopsies of the women with atypical and simple hyperplasia it was established that dendritic cells CD_{56+} and CD_{83+} showed a high level of expression. Differences with control are reliable in group II ($p < 0.05$). At the same time it was found that the degree of expression of factors Bcl-2 and BAX was either weak or moderate (1-2 +; less frequently 3 +) in the women with atypical endometrial hyperplasia and negative - in healthy women and women with simple endometrial hyperplasia. The level of expression of markers of apoptosis in the second group correlated ($r = 0.54$) with the severity of the expression of dendritic cells. This demonstrates the important role of the functional state of dendritic cells

in the regulation of cell proliferation and apoptosis activity in the women with endometrial hyperplasia. The results obtained demonstrate the feasibility of the use in the diagnostic algorithm in patients with endometrial hyperplasia estimation of the functional state of dendritic cells.

Key words: dendritic cell, marker of apoptosis, hyperplasia of endometrium.

РЕФЕРАТ

Запорожан В. Н., Фетеску С. А., Маричереда В. Г. **АНАЛИЗ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ДЕНДРИТНЫХ КЛЕТОК CD_{56+}, CD_{83+}, CD_{1A+} И МАРКЕРОВ АППОТОЗА BCL_2, BAX ПРИ ГИПЕРПЛАСТИЧЕСКИХ ПРОЦЕССАХ ЭНДОМЕТРИЯ (**Одесский национальный медицинский университет, Украина).

Целью исследования была оценка процессов патоморфоза и взаимосвязь между факторами апоптоза и функциональным состоянием дендритных клеток в эндометрии женщин, страдающих гиперпластическими процессами эндометрия**. Материалы и методы**. Основную группу составили 50 женщин, 25 - с простой гиперплазией (I группа) и 25 - с атипической железисто-кистозной гиперплазией (II группа) эндометрия. В качестве контроля обследованы 20 практически здоровых женщин того же возраста. Экспрессию активационных маркеров CD_{16+}, CD_{56+}, CD_{1a}, CD_{85k} и CD_{123} на поверхности лимфоцитов изучали методом проточной цитофлуориметрии. Определение содержания CD рецепторов в биоптатах эндометрия проведено иммуногистохимическим методом с использованием моноклональных антител LIR, Dardilly (Франция) к CD_{56+}, CD_{83+}, CD_{1a+}. Выявление тканевых антигенов проводили авидин-биотиновим методом. Экспрессия антиапоптозного белка BCL_2 и проапоптозного белка BAX исследовали в цитоплазме клеток, отличалась по интенсивности окрашивания. Статистическая обработка полученных результате проведена методами дисперсионного и корреляционного анализа с использованием программного обеспечения Statistica 10.0. **Результаты.** При иммуногистохимическом исследовании биоптатов эндометрия женщин с простой и атипической гиперплазией, установлено, что дендритные клетки CD_{56+} и CD_{83+} демонстрировали высокий уровень экспрессии. Различия с контролем были достоверными во II группе ($p < 0,05$). Вместе с тем установлено, что степень экспрессии факторов Bcl-2 и BAX была слабой или умеренной (1-2 +, реже 3 +) у женщин с атипическойой гиперплазией эндометрия и отрицательной - у здоровых женщин и женщин с простой гиперплазией эндометрия. При этом уровень экспрессии маркеров апоптоза во II группе коррелировал ($r = 0,54$) с выраженностью экспрессии дендритных клеток. Это свидетельствует о существенной роли функционального состояния дендритных клеток в регуляции клеточной пролиферации и активности апоптоза у женщин с гиперпластическими процессами эндометрия. Приведенное свидетельствует о целесообразности применения в диагностическом алгоритме у пациенток с

гиперпластическими процессами эндометрия оценки функционального состояния антиген дендритных клеток.

Ключевые слова: дендритная клетка, маркер апоптоза, гиперплазия эндометрия

Background of endometrial hyperplastic processes (EHP) is defined by wide prevalence of this disease, its tendency to long - term recurrent course, against which if untreated, may occur malignant lesions of the mucous membrane of the uterus. Unflagging interest to the problem of EHP is determined not only by the need to comply cancer suspicion, but the frequent detection of persistent recurrences, involving women of reproductive age menstrual irregularities, development of anemia, reproductive dysfunctions [1-5].

The basis of cyclic alterations of the endometrium are very peculiar molecular mechanisms of morphological and functional changes of the endometrium in different phases of the normal menstrual cycle [3]. It is known that an important intracellular process in the endometrium is apoptosis, which maintains cellular homeostasis during the menstrual cycle by eliminating ageing cells from the functional layer of the endometrium of the uterus [6-8]. At that disturbance of apoptosis and related pathological changes of cells is the base of the hyperplastic and neoplastic diseases of the endometrium [8]. There is scientific evidence that depending on the functional state of dendritic cells in patients with endometrial hyperplastic processes, apoptosis intensity changes [9].

But finally the relationship between factors of apoptosis and functional state of dendritic cells is still not understood.

In this context, **the objective** of the study was to evaluate the processes and pathomorphosis relationship between factors of apoptosis and functional state of dendritic cells in the endometrium of women suffering from endometrial hyperplasia.

Material and Methods. The research has been conducted at the clinical sites of the Department of Obstetrics and Gynecology №1 of Odessa National Medical University and on the base of Odessa Regional Clinical Hospital in 2010-2011. 50 women with endometrial hyperplasia (25 patients with simple and 25 with atypical glandular-cystic hyperplasia) have been comprehensively examined according to existing clinical protocols regulated by the orders of Ukrainian Ministry of Health Care [9, 10]. The average age of the patients was 33,6 ± 0,8 years. Control set included 20 healthy women of the same age.

The expression of activation markers CD_{16+}, CD_{56+}, CD_{1a}, CD_{85k} and CD_{123} on the surface of lymphocytes was studied by flow cytometry. The device «FACScan» (Becton Dickinson) and direct dial monoclonal antibodies and isotypic controls of the same company were used.

Determination of CD receptors in endometrial biopsies was performed through immunohistochemical method using monoclonal antibodies LIR, Dardilly (France)

to CD_{56+}, CD_{83+}, CD_{1a+}. The material for the study was obtained by biopsy of uterine mucosa of the patients under examination 2-3 days before the predicted time of menstruation. The Paypel curette or fractional medical diagnostic excochleation of the uterus mucous membrane and cervical canal.

Scrapings from the uterus with intact endometrium in different phases of the menstrual cycle and histological sections of patients with endometrial hyperplasia have been investigated immunohistochemically. The tissue sections (4-5 mc thick) were fixed in neutral formalin and embedded in paraffin. The slices of tissue were placed on the glass coated with poly-E-lysine, or treated with a solution of ovalbumin by 3-4 samples. The sections were stained with hematoxylin and eosin, by MSB (orange, red, blue) to detect disruption of connective tissue, van Gieson's picrofuchsin mixture technique to identify the degree of sclerosis, periodic Acid Schiff reaction to detect neutral and acidic mucopolysaccharides.

Identification of tissue antigens was performed by avidin-biotin technique where biotin-modified secondary antibodies reacted with the appropriate molecules of peroxidase-conjugated streptavidin. The method is highly sensitive and allows to visualize the location of antigens. When setting immunohistochemical reaction the universal DAKO LSAB + kit (Dako corporation, USA) was used. Expression of the antiapoptosis protein Bcl-2 and proapoptosis protein BAX was observed in the cellular cytoplasm and differed in intensity of staining. Evaluation of research results expressed in points: 0 - no reaction, 1 - weak reaction, 2 - moderate reaction, 3 - strong reaction.

Statistical analysis of the results is made by methods of variance and correlation analysis using software Statistica 10.0 [10].

Results.

The course of the disease in the patients with THP was stereotypic. Menometrorrhagiae were observed in 20 (80.0%) women from the 1^{st} and in 21 women (84.0%) from the 2^{nd} group. Hyperpolymenorrhea and algomenorrhea was observed respectively in 32.0% and 36.0% of the first and second group patients. e 6 (24.0%) women of the 1^{st} group and 4 (16.0%)women of the 2^{nd} group mentioned about long-term (over 5 years) wearing of untrauterine device. More than three abortions were recorded in 9 (36.0%) patients with simple glandular hyperplasia of the endometrium and 10 (40.0%) with atypical hyperplasia. Hereditary tained cancer history had 12 (48.0%) patients of the 1^{st} group, and 11 (44.0%) in the second group and in 3 (15.0%) patients in the control group. There were no statistically significant differences between the first and second groups patients ($p > 0,05$).

In the study of lymphocyte subpopulations it has been revealed that the most characteristic phenomena of immunological changes was the increased content of natural killer cells (CD_{16+}, CD_{56+}) to $13.2 \pm 0.6\%$ in the patients of the 2nd group and to $11.3 \pm 0.8\%$ in the patients of the 1st group, as in the control set this index did not exceed $9.4 \pm 0.4\%$ ($p < 0,05$). In addition, for the patients with endometrial hyperplasia changing of the ratio of T suppressor and cytotoxic cells ($0.8 \pm 0.05\%$ in the first group and $0.9 \pm 0.08\%$ in the second group and $1.3 \pm 0.1\%$ in the control set) was characteristic ($p < 0,05$). However, the depletion of population

CD_1 and growth of CD_{85} and CD_{123} was observed, respectively to 0.6 ± 0.03% and up to 9.20 ± 0.9% and 4.4 ± 0.5% in the first group and 0.7 ± 0.05% and up to 9.12 ± 0.8% and 4.3 ± 0.4% in the second group. In the control set the number of CD_{1a+} cells was 1.2 ± 0.05% ($p < 0,05$), CD_{85} – 7.1 ± 0.4%, CD_{123} – 3.9 ± 0.4% ($p > 0.05$).

In healthy women in the early stage of proliferation phase (the 5^{th} -7^{th} day of the menstrual cycle) endometrium is thin with scattered, narrow and straight glands, which are evenly placed in a free stroma consisting of spindle-shaped cells. The glands have the form of straight or slightly convoluted tubules with narrow opening. On cross-section the contours of glands are rounded or oval. The epithelium of the glands is monostichous, low, prismatic-shaped with small, rounded or oval intensely colored with condensed chromatin nuclei, located at the base of the cell. Cytoplasm is basophilic and homogeneous. Apical edge of epithelial cells is even and clearly delineated.

Histological structure of the unchanged endometrium was as follows in the secondary stage endometrial proliferation (8-10 day). The glands are straight and slightly convoluted, due to their elongation, epithelial cells are high and prismatic in shape. The nuclei of epithelial cells are located at different levels (in places), and are slightly enlarged or more increased in comparison with the early stages of proliferation, oval in form, they are less intensely stained and some of them have small nucleoli, mitosis is often observed.

In the late stage phase of proliferation (11-14 day) chalazae have a pronounced tortuosity at their staining with hematoxylin and eosin than in the middle stage, and their lumen is slightly expanded. The epithelium of the glands is tall, prismatic, apical edge of the cell are level, clear. As a result of intensive segmentation and increase of epithelial cells number, their nuclei are at different levels. Thus, the epithelium overlaying the glands is pseudopolynucleated by the location of nuclei at different levels. The nuclei increase in size and become oval, contain small well visualized nucleoli, mitosis are common. Cytoplasm of epithelial cells becomes poor. At the late phase of proliferation in epithelial cells of some glands there are subnuclear vacuoles.

Compared with previous descriptions the endometrial glands were more convoluted at the early stage of secretion (15-18 day) and their lumen was dilated. In the epithelium of the thyroid subnuclear vacuoles. The nuclei were pushed by the vacuoles in the central parts of the cells. The shape of nuclei was rounded. Cytoplasm of the cells was basophilic. Mitosis were rare.

The main characteristics of unchanged endometrium in the middle stage of secretion phase (19-23 day) were as follows. The epithelium of the glands in the middle stage of secretion was monostichous, and the most of the nuclei were located in the basal part of the epithelial cells. All the nuclei were round, light bubble. The apical part of epithelial cells became domed. The lumens of the glands dilated, their walls became folded. The epithelium of the glands was monostichous, with the nuclei located basally. As a result of intensive secretion the cells were low, their apical edge was not clearly expressed, as if they had serrations. There was secret in the lumen of the glands. In the late stage of

secretion (24-28 day) the folding of the glands walls was increased, they had pronounced stellate shape in cross-section and dustlike - in the longitudinal section. The nuclei of some epithelial cells had sights of pycnosis.
In contrast with atypical endometrial hyperplasia the following changes were observed in the phase of proliferation. Glandular component significantly prevailed over stromal. The glands were held tightly to each other, and separated by narrow layers of connective tissue, convoluted, with numerous arborizations, their shape irregular, wavy, scalloped due to papillary outgrowths that went into the lumen of the glands. The cells lining the glands, were large, mainly due to increase in the cytoplasm, which may be basophilic, bright translucent and sometimes got eosinophilic staining. The nuclei of the cells were either large oval or elongated, with distinct clumps of chromatin and large nucleoli, painted pale. Polarity of nuclei location was broken.
At simple hyperplasia the predominance of glandular component, without disturbing of the polarity of nuclei location in the phase of proliferation. It was no noticeable close location of the glands. The glands were rounded and varied in size. Along with small sized glands, large and cysts-like dilated glands were found in different ratios. The glands were lined with highly prismatic epithelium with multi located nuclei and well-rounded apical cell edge. The nuclei were oval or slightly elongated, cigar-shaped, rich in chromatin. The cytoplasm was basophilic, the secret was usually absent in it, although in the ducts of the glands a small amount of mucicarmine stained mucus might be found. In glandular and stromal cells figures of mitosis were often met. It indicates active proliferation of both glandular and stromal elements.
At immunohistochemical study of endometrial biopsies of women with simple and atypical hyperplasia (Table), it had been revealed that dendritic cells $CD_{56\ (+)}$ and $CD_{83\ (+)}$ demonstrated high expression. Differences with control were reliable in group II ($p < 0.05$).
Table

Expression of markers antigenpresenting cells in patients with endometrial hyperplasia

indicators	**simple hyperplasia**	**atypical hyperplasia**	**control**
CD_{1a+}	1.3 ± 0.2	2.2 ± 0.2	0.9 ± 0.1
CD_{56+}	1.5 ± 0.2	1.9 ± 0.2	1.3 ± 0.1
CD_{83+}	1.6 ± 0.1	2.1 ± 0.2	1.2 ± 0.1

*Note: * - differences from control are significant (p <0.05)*

At the same time it has been revealed that the degree of expression of Bcl-2 and BAX factors was either weak or moderate (1-2 +, at least 3 +) in women with

atypical endometrial hyperplasia and negative in healthy women and women with simple endometrial hyperplasia (Fig. 1) .

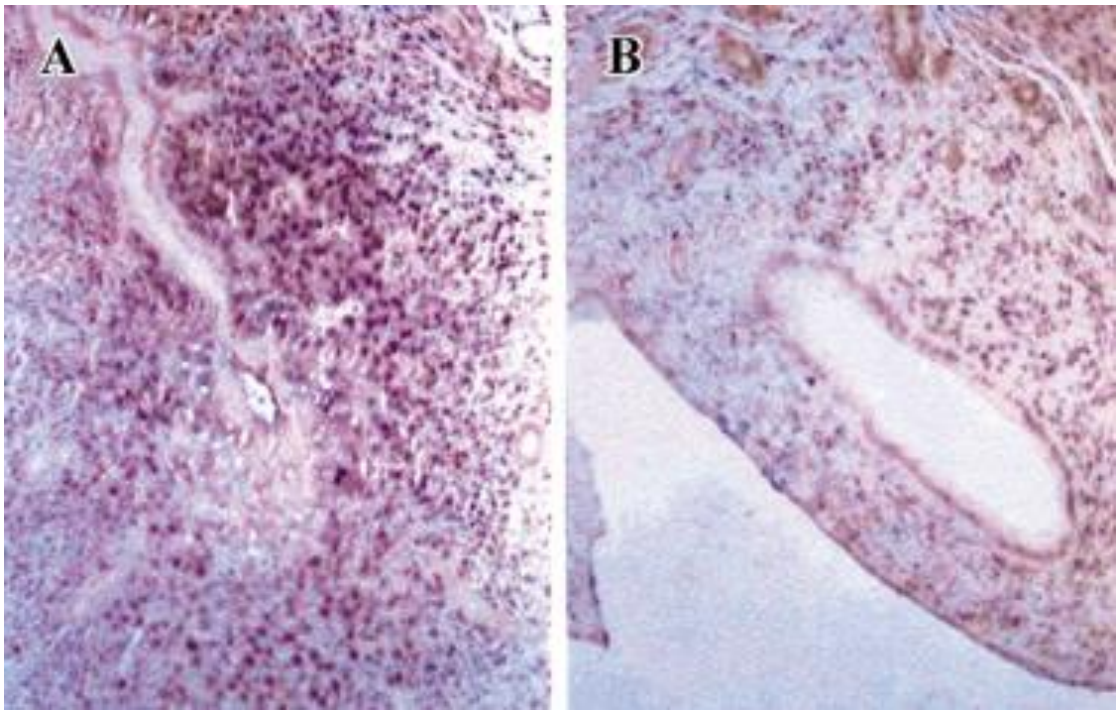

Figure 1. Expression of apoptosis markers in the second group of women (A - Bcl-2, B - BAX). Rev. 10, approx. 20

The level of expression of apoptosis markers in the second group correlated (r = 0.54) with the severity of the expression of dendritic cells. This demonstrates the important role of the functional state of dendritic cells in the regulation of cell proliferation and apoptosis activity in women with endometrial hyperplasia. In other words, the activation of apoptosis provides a "recycling" of atypical endometrial cells.

Conclusions:

1. Pathomorphosis at atypical endometrial hyperplasia is characterized by increase correlation between stromal and glandular component with predominance of glandular one, increased expression of markers of dendritic cells and apoptosis.
2. Dendritic cells in the patients with atypical endometrial hyperplasia demonstrated significantly higher expression level compared with healthy women and patients with simple endometrial hyperplasia.
3. The level of expression of markers of apoptosis in patients with atypical endometrial hyperplasia correlated (r = 0.54) with the severity of the expression of dendritic cells.
4. The results obtained demonstrate the feasibility of use in the diagnostic algorithm in patients with endometrial hyperplasia assessment of the functional state of antigenpresenting dendritic cells.

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