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PATHOGENETIC RELATIONSHIP OF ENDOMETRIAL HYPERPLASIA WITH ASEPTIC NONSPECIFIC INFLAMMATORY PROCESS

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Abstract

The aim is to reveal pathogenetic relationship of endometrial hyperplasia with inflammatory process and to show intercellular mechanisms of its realization on the basis of complex morphological analysis. The leading factor in the pathogenesis of simple endometrial hyperplasia is absolute or relative hyperplasia in combination with a defect in the action of progestins. The absence of a direct relationship between the quantitative indicators of serum estradiol, morphology of proliferation and the degree of endometrial atypia, indicates the existence of other mechanisms underlying this pathology. The study of the relationship of local changes with a variety of molecular biological mechanisms involved in the development of endometrial hyperplasia is relevant for improving approaches to treatment, for example, a combination of hormonal and anti-inflammatory therapy, as well as some new forms of anti-oncogenic drugs. Material and methods. A morphological study was carried out on the endometrium in women aged 35-55 years in the following groups: control, simple endometrial hyperplasia without atypia, complex hyperplasia without atypia, adenocarcinoma. Reviewing stains of paraffin sections helped to clarify the diagnosis, and immunohistochemical research revealed the expression of markers of cellular renewal and inflammation. Results. Our studies have shown an increase in the expression of the total leukocyte antigen CD45+, that is, an increase in the activity and severity of inflammation depending on the nature of the hyperplastic process. In all samples of the tissue with endometrial hyperplasia there were cells with CD45 + expression, their number increased during the transition from a simple form of hyperplasia to a complex one. Among other factors, reflecting the degree of severity and nature of inflammatory changes in the endometrium, it included the presence of various subpopulations of lymphocytes, decrease in the intensity of apoptosis of epithelial cells in the glands of the endometrium (unlike stroma) and activation of vascular growth factor. At the same time, it is important to note the mechanism of inflammation as a vascular-stromal, mesenchymal reaction involving connective tissue cells, including various inflammatory cells. Conclusion. The progression of the hyperplastic process led to more severe inflammatory changes in the endometrium. In case of simple endometrial hyperplasia, the more important progressive factor was hormonal imbalance, but in case of complex and atypical hyperplasia, the role of the inflamatory process increased. The formation of inflammation in endometrial hyperplasia can be considered a factor in the development and progression of the pathology, as well as a risk factor for malignancy in hyperplastic processes.

Keywords: *endometrial hyperplasia, inflammation, pathogenesis, oncogenesis, immunomorphology.*

Introduction

The data show an increase of morbidity of endometrial cancer in most countries and a adverse prognosis of its epidemiology for the coming years. At present, this topic is also relevant in connection with reproductive problems and infertility, which were due to the chronic inflammation and hyperplastic processes of the inner lining of the uterus [*Parnitskaya O. I. et al., 2013*]. The possibilities of molecular biological diagnostic methods have expanded the understanding of the pathoge-

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netic mechanisms of these changes, showing not only hormone-dependent stimulation of proliferation, but also abnormal dynamics of cell renewal (mitosis and apoptosis) in the development of local chronic inflammation. Proliferative endometrial processes in middle-aged and older women is a high risk of malignancy with the progression of atypia and invasion, accompanied by genetic changes in epithelial cells typical for endometrial cancer. The most significant genes involved in the development of neoplastic processes were PTEN, PGR, NDRG1 and some others genes [*Dumanovskaya M. et al., 2013*]. The researchers noted a significant decrease in the mRNA expression of the tumor growth suppressor gene PTEN and NDRG1 – apoptosis inducer in endometrioid carcinoma tissues [*Chernukha P. et al., 2013; Raffone A., 2018*]. Also important is the state of the CD 63, GNB2L и S100A6 genes that are involved in apoptosis inhibition, cell survival, angiogenesis and endometrial cell proliferation [*Gomes VA et al., 2018*]. Immunology of this tissue is interesting in terms of its contact with the external environment of the genital tract and the growth of infectious lesions, including immunodeficiency conditions. While an acute inflammation may serve the activation of protective forces of the body and stop of tumor growth [*Askeland E., 2012*], chronic inflammation is often a boost in the development of cancerous process as a result of persistent disorders of immunological balance in the tissue [*Korniluk A. et al., 2017*]. Abnormal cytokine environment of the endometrium may be a non-specific consequence of the activation of congenital immunity of the endometrium by exogenous or endogenous stimulation, which leads to a decrease in the receptivity of the endometrium [*Kagramanova J. et al., 2016*]. Thus, the authors showed a significant increase in the level of inflammatory protein NALP-3 and protein ASC, increased activation of caspase-1 and increased levels of IL-1 and IL-18 in the endometrium of women with infertility [*D'ippolito S. et al., 2016*]. Activated leukocytes released active forms of oxygen and intermediate products of active nitrogen, which could cause DNA damage and instability of the genome [*Grivennikov S., 2010*]. Other authors also noted the important role of oxidative stress as a factor stimulating the development of the neoplastic process [*Çınar M. et al., 2016; Popov E. et al., 2016*]. In turn, the increase in the number of mutations led to the probability of genes damage, responsible for the coding of the protein-regulators of the cell cycle [*Korniluk A. et al, 2017*]. It is well known that proinflammatory cytokines stimulate angiogenesis in tissues. The experiments in vitro showed that the joint cultivation of endometrial cells, monocytes and regulatory T-cells could enhance immunosuppression of the latter, especially in the presence of estradiol and (or) progesterone. Actively synthesized IL-1β and TNF-α could synergistically stimulate the expression of IL-8 and VEGF, and thus activate angiogenesis [*Wang X. et al., 2017*]. The high risk of developing neoplastic process on the background of chronic inflammation and increased tissue vascularization makes the prognosis of the disease unfavorable for patients. Mutagenic effects of inflamatory cytokines, their activation of proteolysis systems, violation of local cellular immunity, stromal-vascular dysregulatory reactions, anovulatory cycles as a consequence of adnexites can be considered the most likely pathogenetic causes of the transition from chronic nonspecific inflammation to hyperplastic and neoplastic processes in the tissues of the uterine wall [*Pakharenko L.V. et al., 2015*]. The study of the pathogenic mechanisms of endometrial hyperplasia is important for the justification of the method of therapy, which is personalized. It is very promising and justified in terms of its therapeutic effectiveness and minimization of iatrogenia and complications of the use of pharmacological drugs. Reproduction requires the choice of therapeutic approaches with an understanding of the action on the molecular links of pathogenesis, which demonstrates the relevance of the presented work and the practical significance of its results.

The aim is to reveal pathogenetic relationship of endometrial hyperplasia with inflammatory process and to show intercellular mechanisms of its realization on the basis of complex morphological analysis and investigation of protein expression level in stroma and endometrial gland cells.

Material and methods

Endometrial scraping in women aged 35-55 years were studied, groups were formed on the basis of morphological conclusion: control, simple

endometrial hyperplasia (EH) without atypia, complex EH without atypia, adenocarcinoma. There were 5 patients in each group. This separation was consistent with the classifications of EH by WHO in 1994 and 2014 years [*Baak J., Mutter G., 2005; Sobczuk K., Sobczuk A., 2017*]. However, we did not isolate EH with atypia due to the morphology of the received clinical material. It is important to note that the nomenclature of EH forms takes into account pathogenetic components: functional categories (1994) and the presence of genetic changes with the prognosis (2014). I. Classification EH by W HO (1994): 1) EH as a simple non-typic EH associated with hyperestrogenicity; complex hyperplasia without atypia; 2) EIN simple atypical hyperplasia - precancer; complex hyperplasia with atypia; 3) adenocarcinoma - cancer. II. Classification of EH by WHO (2014): 1) EH without atypia (EH), which is benign, simple or complex with a low level of somatic mutations and a low risk of co-presence of invasive endometrial cancer (EC) and progression in EC; 2) EH with atypia (EIN), also simple or complex with many genetic changes typical for EC (microsatellite instability, inactivation of PAX2, mutation PTEN, KRAS and CTNNB1 (β-catenin) with a high level of co-presence and risk of progression of EC. A standard histological examination was carried out in accordance with the generally accepted methodology and instructions for reagent sets the hematoxylin staining of Gill and eosin (BioVitrum, Russia) and studying the tissues by immunohistochemical (IHC) method. Using the antigen-antibody reaction in the tissue, proliferative and inflammatory markers expressed by cells of the endometrial glands and stroma were detected. The IHC study was performed on serial paraffin sections with a thickness of 4 *μm* (automatic rotational microtome Leica RM 2255, Germany) placed on adhesive glasses with a polylysin coating (Menzel–Glazer, Germany), En vision imaging system (Dako, Denmark). In order to control the method, a series of studies using positive and negative samples, which served as standards. To study the parameters of local immunity, monoclonal antibodies (Dako, Denmark) were used to detect the common leukocyte antigen CD 45, the population of natural killer cells (NK) CD 56, and the macrophage marker CD 68. The choice of monoclonal antibodies is justified by publications that indicate the important role of these populations in inflammation process in endometrium, reproductive disorders [*Guo G. et al., 2014; Elfayomy A. et al., 2015; Kubyshkin A. et al., 2016*]. VEGF vascular growth factor and apoptosis were also determined to assess oncogenesis using monoclonal mouse antibodies to Bcl-2 protein (apoptosis regulator NCL-L-BCL-2) and CD 95 proapoptotic antibodies. The reaction was evaluated in a light field at 400x magnification in ten fields of view. The scanner of samples Aperio CS2, Leica (Germany) was used. Markers with cytoplasmic localization (VEGF, Bcl-2, CD95) were assessed by semi-quantitative method separately for glandular epithelium and endometrial stroma. The following scale was used: 0-staining is absent, or there was a weak / medium staining of less than 25% of cells; 1+ - low-intensity staining of more than 25 % of cells, or staining of a strong intensity of less than 25% of cells; 2+ - 25-75% of cells had medium-intensity staining, or 25-50% of cells were highly-intense stained; 3+ - more than 75% of cells were medium-intense stained, or more than 50% of cells were highly-intense stained (Table).

To evaluate CD markers (CD68, CD56), we calculated positively stained cells in the field of view at 400x magnification, estimated 10 random fields of view (the area of one field was 0.1 mm^2) from each slice with the calculation of the arithmetic mean. The calculations were performed using the ImageJ and ImageScope programs. Statistical methods were used to process the results using the program STATISTICA 10. The statistical significance of the differences was determined using the non-parametric Mann – Whitney U-test. Differences were considered significant at $p < 0.05$.

The study was performed in the histological laboratory of the Center for collective use of scientific equipment “Molecular Biology” on the basis

TABLE

Criteria for assessing IHC markers with cytoplasmic localization

Staining	absent	weak	medium	strong
<25 cells	**0**	**0**	**0**	+
25-50 cells	**0**	+	++	++
50-75 cells	**0**	+	++	+++
>75 cells	**0**	+	+++	+++

A

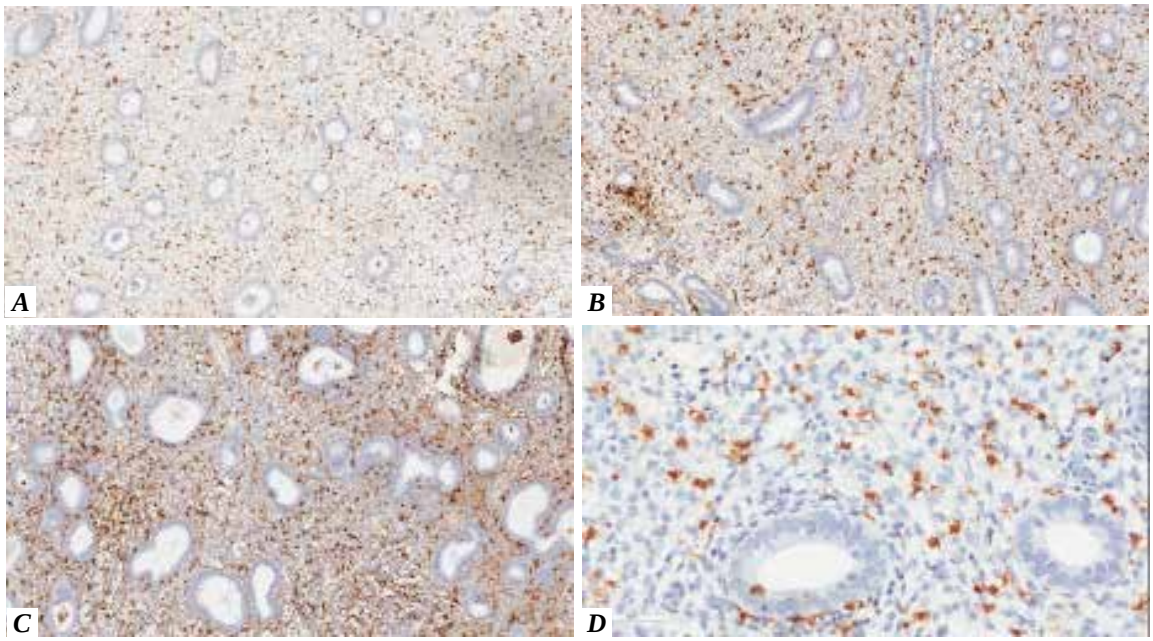

Figure 1. *Fragments of human's endometrium (scraping). Paraffin sections. IHC reaction with antibody to CD 45 receptors - a common leukocyte antigen (A, B, C, magnification 100) and CD-56 receptors (D, magnification 400), visualization in the En vision system. A. Normal. B. Simple hyperplasia. C. Complex hyperplasia of the endometrium without atypia. D. Complex endometrial hyperplasia without atypia. Endometrium is abundantly infiltrated with CD 56+ NK cells (30.1%).*

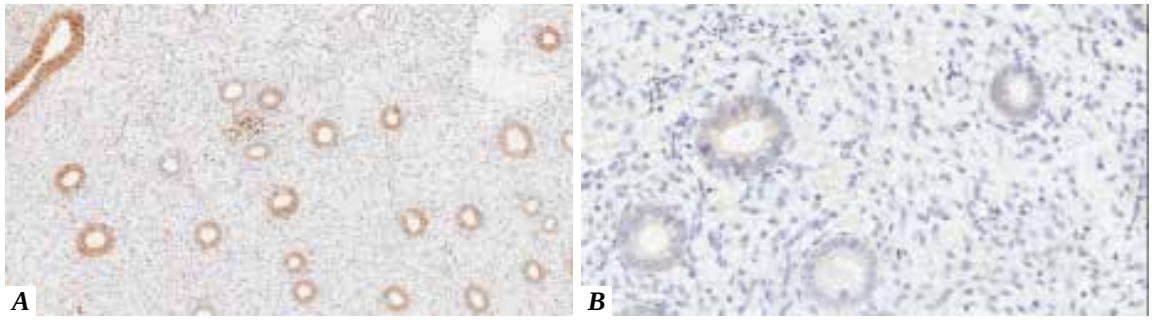

Figure 2. *Fragments of human endometrium (scraping). Paraffin sections. IHC reaction with antibodies to Bcl-2, apoptosis regulator, visualization in the En vision system. A Control, magnification ×100. Intense reaction in glands (3+) and weak – in stroma (1+). B. Complex endometrial hyperplasia without atypia, magnification 400x. Weak cytoplasmic staining of low intensity in glandular epithelial cells, single intensive nuclear staining of less than 25% of stroma cells (1+/0).*

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Results and discussion

The growth of total leukocyte antigen indicated the inflammatory origin of hyperplasia of any form. A number of drugs demonstrate a high level of endometrial infiltration of CD 56+ by NK cells and other lymphocyte and plasma cell subsets. (Fig. 1).

IHC reaction with antibodies to Bcl-2 were usually quite intensive, but in some cases with complex hyperplasia weak staining, indicating a low reaction of apoptosis in the tissue (Fig.2).

The most indicative in our work was the marker of apoptosis CD-95, the intensity of its expression decreased in a number of cases of complex hyperplasia and, especially, at the appearance of atypia (Fig. 3). In the control group, the intensity of the reaction is high, as in simple hyperplasia.

The dynamics of cytoplasmic expression of vascular growth factor was also considerable (Fig.4).

It is well known that the leading factor of the pathogenesis of a simple EH is absolute or relative hyperestrogenia, in the combination with a defect in the action of progestins [*Tabakman Yu et al., 2016*]. However, the absence of the direct relationship between the quantitative parameters of serum

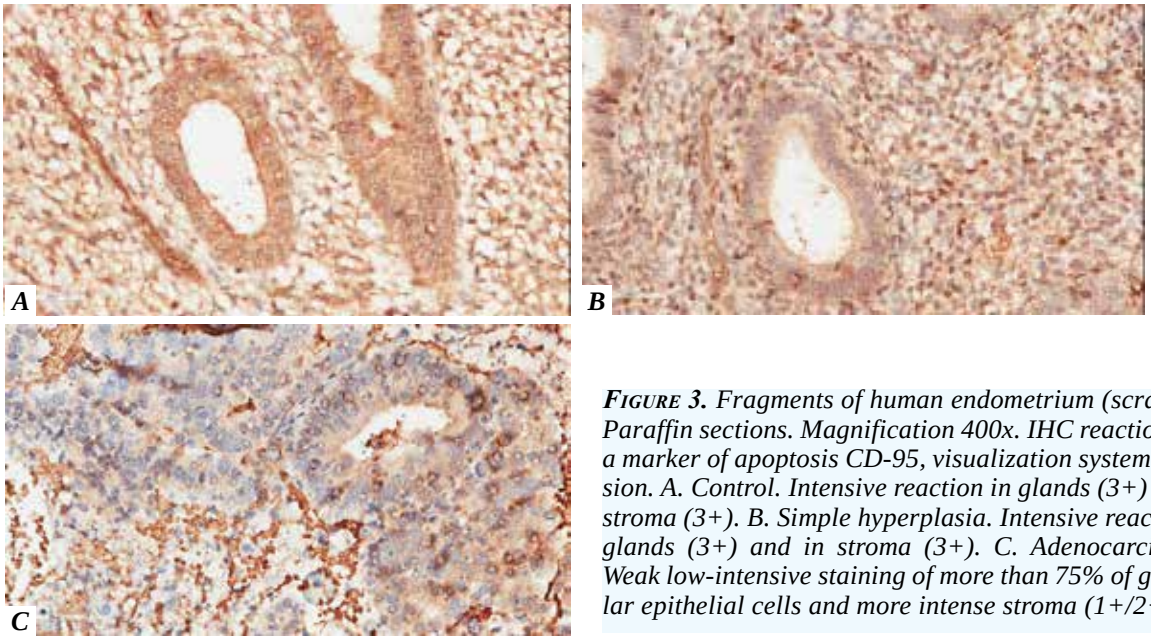

FIGURE 3. *Fragments of human endometrium (scraping). Paraffin sections. Magnification 400x. IHC reaction with a marker of apoptosis CD-95, visualization system En vision. A. Control. Intensive reaction in glands (3+) and in stroma (3+). B. Simple hyperplasia. Intensive reaction in glands (3+) and in stroma (3+). C. Adenocarcinoma. Weak low-intensive staining of more than 75% of glandular epithelial cells and more intense stroma (1+/2+).*

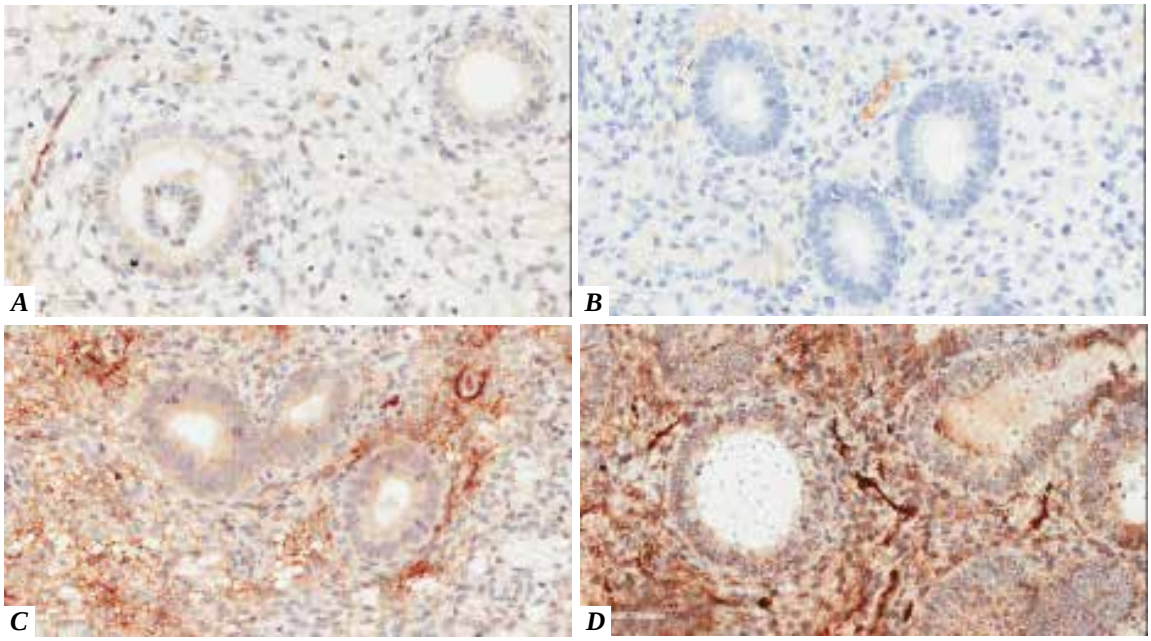

FIGURE 4. *Fragments of human endometrium (scraping). Paraffin sections. Magnification 400x. IHC reaction with VEGF vascular growth marker, visualization in the En vision system. A. Control. Weak staining of 25-50% glandular cells, very weak staining of less than 25% of stromal cells (1 + / 0). B. Simple hyperplasia. Lack of staining of glandular cells, weak focal staining of stromal cells in less than 25% of the area. (0/0). C. Complex hyperplasia without atypia. Uneven staining. In a part of the preparation, the glands have a color of medium intensity of more than 75% of the cells, the stroma cells are stained slightly with foci of extremaly positive staining (2-3 + / 2-3 +). D. Adenocarcinoma. Medium intensity staining of more than 75% of glandular cells, highly intense cytoplasmic staining of more than 2/3 of stromal cells. (3 + / 3 +).*

estradiol, morphology of proliferation and the degree of endometrial atypia, indicated the existence of other mechanisms underlying that pathology [*Singh L et al., 2018*]. In this regard, the study of the relationship of local changes with a variety of molecular biological mechanisms involved in the development of endometrial hyperplasia, is very important for improving approaches for treatment, for example, a combination of hormonal and anti-inflammatory therapy, as well as some new forms of anti-oncogenic drugs. Our studies have shown an increase in the expression of the total leukocyte antigen CD45 +, that is, an increase in the activity and intensity of inflammation depending on the

origin of the hyperplastic process. In all samples of the studied tissue with HE, there were cells with CD45 + expression, their number grows during the transition from a simple form of HE to complex. Other factors reflecting the severity and nature of inflammatory changes in the endometrium include the presence of various lymphocyte subpopulations, a decrease in the intensity of apoptosis of epithelial cells in the endometrial glands (unlike stroma), and activation of vascular growth factor. At the same time, it is important to note the mechanism of inflammation like the vascular-stromal, mesenchymal reaction involving connective tissue cells and various inflammatory cells.

Conclusion

The obtained data prove the idea that in complex forms of hyperplasia the key mechanism of the development of the hyperplastic process is the inflammation. The progression of the hyperplastic process led to more severe inflammatory changes in the endometrium. With simple EH, the more important progressive factor could be hormonal imbalance, but with complex and atypical EH, the role of the inflammatory process increased. It can be considered the factor in the development and progression of EH, as well as a risk factor for malignancy in EH.

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