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CLINICAL-IMMUNOHISTOCHEMICAL CHARACTERISTICS OF ATYPICAL ENDOMETRIAL HYPERPLASIA IN WOMEN OF REPRODUCTIVE AGE

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Цитування: *Медичні перспективи. 2020. Т. 25, № 1. С. 134-141*

Cited: *Medicni perspektivi. 2020;25(1):134-141*

Key words: *non-atypical endometrial hyperplasia, reproductive age, immunohistochemistry, CD-138, chronic endometritis*

Ключові слова: *неатипова гіперплазія ендометрія, репродуктивний вік, імуногістохімія, CD-138, хронічний ендометрит*

Ключевые слова: *неатипическая гиперплазия эндометрия, репродуктивный возраст, иммуногистохимия, CD-138, хронический эндометрит*

Abstract. *Clinical-immunohistochemical characteristics of atypical endometrial hyperplasia in women of reproductive age. Vovk I.B., Zadorozhnaya T.D., Gorban N.E., Kondratiuk V.K. The article presents the analysis of clinical-morphological and immunohistochemical features of endometrial hyperplasia. 60 patients of reproductive age with non-atypical endometrial hyperplasia (EH) were examined. The following morphological distribution of its different types was established: glandular – 56.6%, glandular-cystic – 40.0%, stromal and cystic-atrophic forms – 1.7%, respectively. When carrying out immunohistochemical studies, the expression of CD-138, a modern reliable marker of the presence of an inflammatory process in endometrial tissue was determined. In the group with non-atypical glandular EH, signs of the inflammatory process in the endometrium were verified in 7 (20.59%) patients. In the group with signs of glandular-cystic change of EH – in 11 (45,83%) patients, as well as in the only patient with non-atypical stromal EH (100,0%). In almost one-third of patients with different morphological types of EH in 19 (31.7%) of 60 the presence of structural-morphological signs of chronic endometritis was revealed, which was manifested by signs of lymphoplasmacytic infiltration, indicating the presence of chronic inflammatory process. These data indicate the possible pathogenetic role of inflammation as one of the trigger factors for the hyperplastic transformation of endometrial structures, which allows to confirm the role of infectious factors in the occurrence of these processes in almost one third of patients with non-atypical EH. Thus, the conducted studies indicate that the development of EH is a process based on both the infectious factor and hormonal metabolic disorders, which dictates different personalized approaches to therapeutic tactics.*

Реферат. *Клинико-иммуногистохимическая характеристика неатипичной гиперплазии эндометрия у женщин репродуктивного возраста. Вовк И.Б., Задорожная Т.Д., Горбань Н.Е., Кондратиук В.К. В статье представлен анализ клинико-морфологических и иммуногистохимических особенностей гиперплазированного эндометрия. Обследованы 60 пациенток репродуктивного возраста с неатипической гиперплазией эндометрия (ГЭ). Установлено следующее морфологическое распределение различных её типов: железистая – 56,6 %, железисто-кистозная – 40,0%, и по 1,7 % - стромальная и кистозно-атрофическая формы соответственно. При проведении иммуногистохимического исследования определена экспрессия CD-138 – современного надежного маркера наличия воспалительного процесса в ткани эндометрия. В группе с неатипической железистой ГЭ признаки воспалительного процесса в эндометрии были верифицированы у 7 (20,59%) пациенток. В группе с признаками железисто-кистозного изменения ГЭ - у 11 (45,83%) больных, а также у единственной пациентки с неатипической стромальной ГЭ (100,0%). То есть почти у трети пациенток с разными морфологическими типами ГЭ – у 19 (31,7%) из 60, выявлено наличие структурно-морфологических признаков хронического эндометрита, что проявлялось признаками лимфоплазмоцитарной инфильтрации, свидетельствующими о наличии хронического воспалительного процесса. Эти данные свидетельствуют о возможной патогенетической роли именно воспаления как одного из триггерных факторов гиперпластической трансформации эндометриальных структур, что позволяет утверждать о роли инфекционных факторов в возникновении этих процессов почти у трети пациенток с неатипической ГЭ. Таким образом, проведенные исследования свидетельствуют, что развитие ГЭ – процесс, в основе механизмов развития которого лежит как инфекционный фактор, так и гормональные метаболические нарушения, что диктует разные персонализированные подходы к лечебной тактике.*

The widespread problem of endometrial hyperplastic processes in the female population in terms of reproductive health disorders causes scientific interest in addressing this issue, and the notable tendency of recent years to "rejuvenate" this pathology is not only of medical concern but also is a social problem [1]. It is also worth noting that the endometrium is probably one of the most active organs in a female body, which undergoes regular cyclic changes almost 450 times during a woman's life [10].

Despite the use of advanced technologies in the diagnosis of endometrial hyperplastic processes, known "gold standards" in the verification of this diagnosis [6], the search for clinical factors and biological markers that would allow the diagnosis and monitoring of endometrial status (to control the effectiveness of treatment and prognosticate recurrence of process), is declared in the last British Protocol of 2016 as the task of modern scientific search [13].

The aim of the work is to study the clinical-morphological and immunohistochemical features of endometrial status in women of reproductive age with non-atypical endometrial hyperplasia.

MATERIALS AND METHODS OF RESEARCH

For an in-depth search for possible etiological and pathogenetic factors that affect the onset of hyperplastic processes in women of reproductive age, we conducted a survey of 60 patients with non-atypical endometrial hyperplasia. Morphological evaluation (van Gieson staining with picrofuxin and hematoxylin-eosin) was made and immunohistochemical studies of samples of hyperplastic endometrium – determination of markers of inflammatory process – detection of plasmacytic cells CD-138: (Ab-2) (Syndecan-1) (Thermo scientific production, mouse antibodies, working dilution 1: 10-1: 20, UltraVision LP detection system) was carried out.

The prevalence and intensity of the reaction in the cytoplasm was evaluated by a semi-quantitative method (from 0 to 3 points), where the prevalence was treated as follows: 0 – no staining; 1 – less than 10% of positively stained cells; 2 – more than 10% and less than 50% of positively stained cells; 3 – uniform staining of more than 50% of cells, and the intensity, respectively: 0 – no visible staining; 1 – weak staining; 2 – moderate staining; 3 – expressed staining.

Histological examination was performed in accordance with the well-known protocol of indicators of normal endometrium, taking into account the phase and day of the menstrual cycle, according to the classical criteria [4] and the Protocol of the study of endometrium, developed in the laboratory of pathomorphology of the SE "Institute of Pediatrics, Obstetrics and Gynecology named after Academician O.M. Lukianova National Academy of

Medical Sciences of Ukraine" (approved by DPC "Pathological Anatomy" of the Ministry of Health of Ukraine and NAMS of Ukraine from 22.01.2010, by the Academic Council of the Institute from 15.02.2018). An Olympus BX 51 light microscope (Japan) was used. All researches were carried out in the setting of pathomorphology laboratory of the SE «Institute of Pediatrics, Obstetrics and Gynecology named after Academician OM Lukianova NAMS of Ukraine », Head – Corresponding Member of NAMS of Ukraine, Professor Zadorozhna TD.

Statistical analysis of the results of the study was carried out in the package EZR v. 1.35 (R statistical software version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria) [11]. This package does not require a license - Open and is recognized. The reliability of the results was taken into account at $p < 0.05$.

RESULTS AND DISCUSSION

The average age in the study group was 37 ± 1.8 years. Distribution of patients according to morphological types of hyperplastically changed endometrial tissue had the following structure: manifestations of non-atypical endometrial glandular hyperplasia were found in 34 (56.6%) of patients, non-atypical cystic glandular endometrial hyperplasia was revealed in 24 (40.0%) of women, one (1.7%) case of stromal and cystic-atrophic forms of non-atypical endometrial hyperplasia each were verified.

The variety of histological pattern of the specimens enabled to distinguish the main stages of formation of hyperplastic rearrangement of endometrial structures. So, we have identified the following changes: glandular non-atypical endometrial hyperplasia; glandular-cystic non-atypical endometrial hyperplasia; non-atypical stromal endometrial hyperplasia; cystic-atrophic type of non-atypical hyperplasia (Fig. 1-3).

According to the results obtained, non-atypical glandular endometrial hyperplasia was marked by the presence of secretory transformation, high prismatic epithelium, glandular-cystic non-atypical endometrial hyperplasia had signs of dyschronosis and focal early secretory glandular transformation, non-atypical stromal endometrial hyperplasia was manifested by a marked dyschronosis of development of endometrial structures with the presence of decidualization and predecidualization of stroma in which areas of multiple vascular bundles and glomerules of spiral arteries with fibrosis of their wall are placed, as well as a small number of glands with a decrease in the proportions of glands in the stroma of hypoplastic glands, the diameter of which is 20-30 microns with the presence of hypo- and atrophic epithelium.

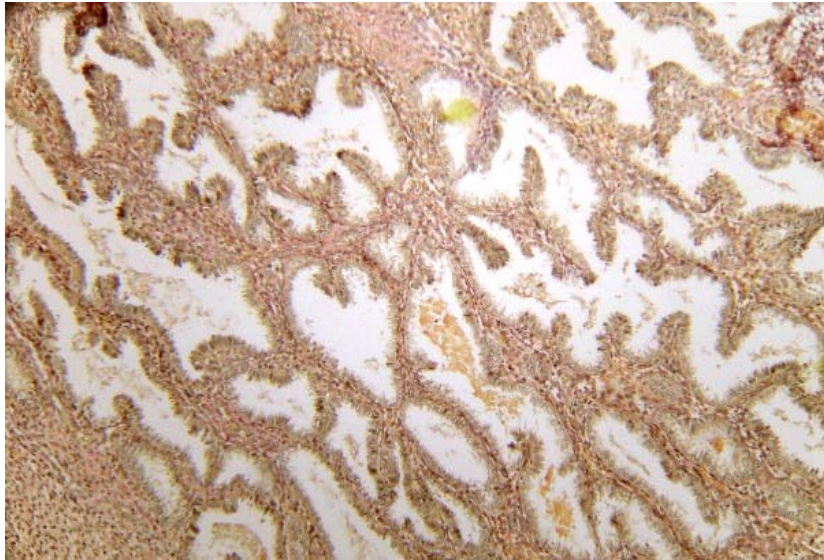


Fig. 1. Non-atypical glandular endometrial hyperplasia. Micrograph. Staining by Van-Gieson. 3p. 200

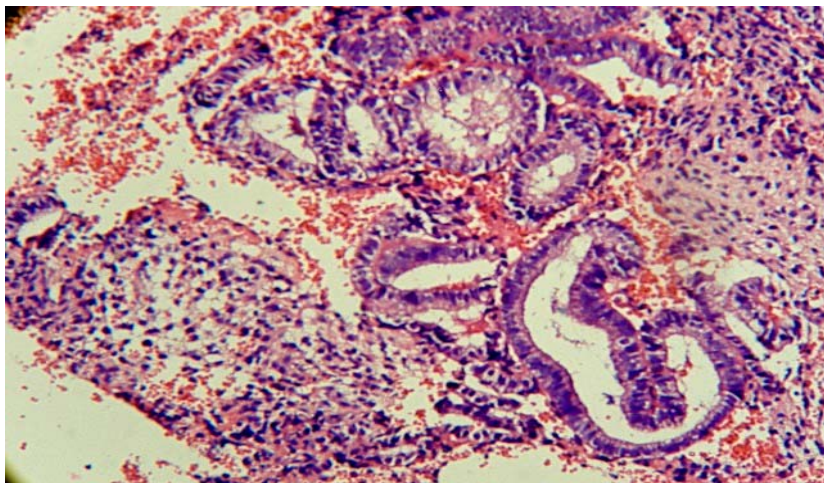


Fig. 2. Non-atypical glandular-cystic endometrial hyperplasia. Micrograph. Staining with hematoxylin-eosin. 3p. 200

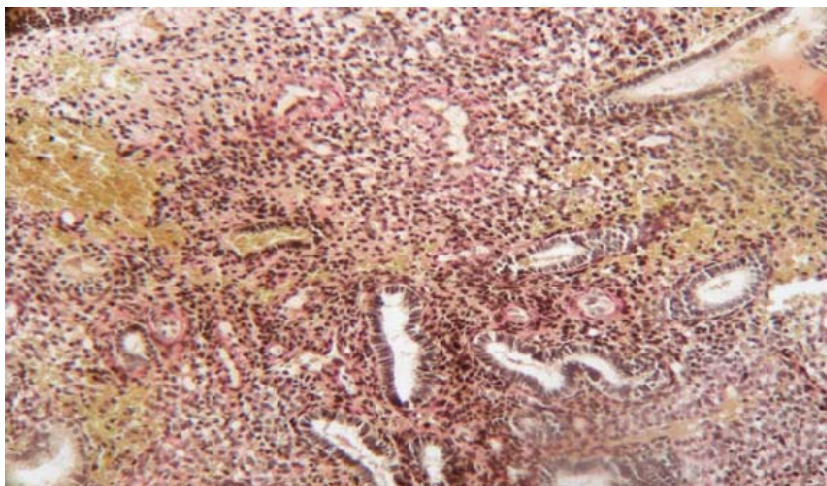


Fig. 3. Non-atypical stromal endometrial hyperplasia. Micrograph. Staining with hematoxylin-eosin. 3p. 200

All specimens examined were verified according to the latest revision of classification by World Health Organization (2014), as endometrial hyperplasia without atypia (no signs of cytologic atypia).

In order to determine the signs of inflammatory process in the examined groups of patients, we performed immunohistochemical determination of the CD-138 marker as the most current and absolute marker of chronic endometritis, even with a single verification in the material, sensitivity and specificity of which are at the level of 78% and 65%,

respectively, recently regulated as the "gold" standard in the diagnosis of chronic endometritis [2, 7, 8, 12] (Table). Thus, in the group of patients with non-atypical endometrial glandular hyperplasia only in 7 (20.59%) of patients signs of inflammatory process in the endometrium were verified, whereas this indicator more than doubled due to morphological signs of glandular cystic restructuring of hyperplastic tissue and made up 11 (45.83%) of cases respectively, and was observed in only one patient with non-atypical stromal endometrial hyperplasia.

Degree of expression of inflammatory process marker CD-138 in tissues of hyperplastic endometrium of reproductive age patients in presence of its different morphologic types (n, %)

Morphologic type of endometrial hyperplasia	CD-138 positive		CD-138 negative		Significance level
	n	%	n	%	
Non-atypical glandular EH, (n=34)	7	20.59	27	79.41	*
Non-atypical glandular-cystic EH, (n=24)	11	45.83	13	54.17	
Non-atypical stromal RH, (n=1)	1	100	-	-	
Non-atypical cystic-atrophic EH, (n=1)	-	-	1	100	

Note. * – difference from CD-138 positive – significant.

Thus, out of 60 patients with different morphological types of non-atypical endometrial hyperplasia, in 19 (31.7%) of them, that is, one third, the presence of structural-morphological signs of chronic endometritis was established, which was

manifested by signs of lymphoplasmacytic infiltration (Fig. 4), this by both classical and modern canons [3, 5] may indicate the presence of a chronic inflammatory process.

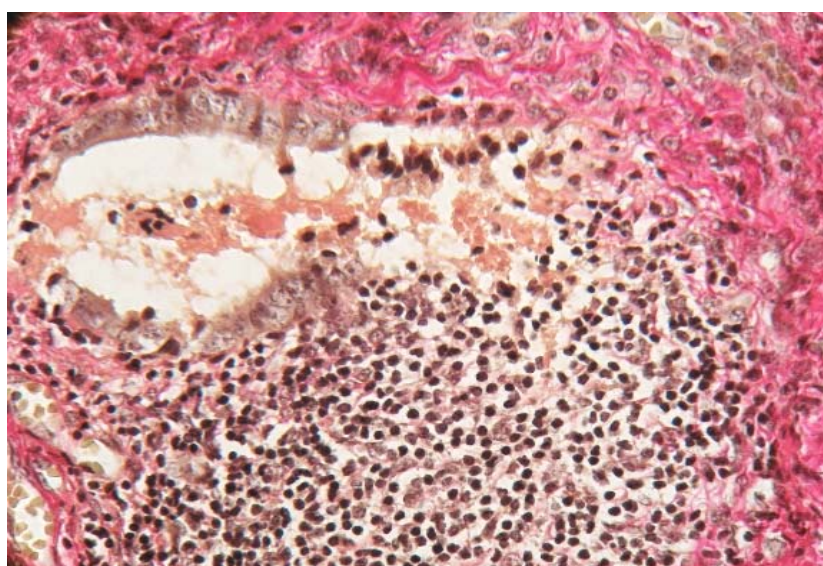


Fig. 4. Non-atypical glandular endometrial hyperplasia against inflammatory process – lymphoplasmacytic infiltration. Micrograph. 3p. 200

In the course of the study we established the presence of endometrial epithelium desquamation, focal impairment of its pseudostratification (Fig. 5) and lysis of individual epithelial structures of the glands (Fig. 6), which is also an evidence of chronic inflammatory process in the studied tissue [8, 14].

The above results indicate the possible pathogenetic role of the inflammatory process itself as one of the triggers of the more complex hyperplastic rearrangement of endometrial structures, especially in the case of a persistent process [9].

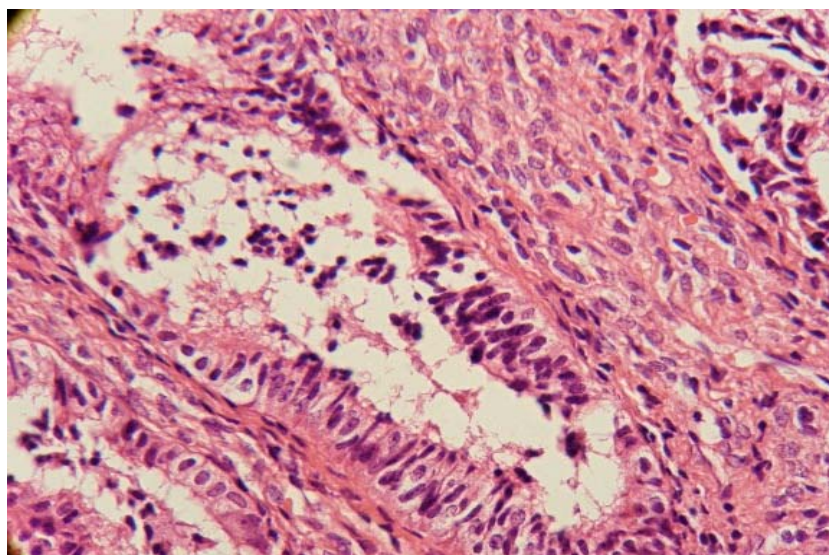


Fig. 5. Non-atypical glandular endometrial hyperplasia against inflammatory process - impairment of pseudostratification and desquamation of epithelium. Micrograph. 3p. 250

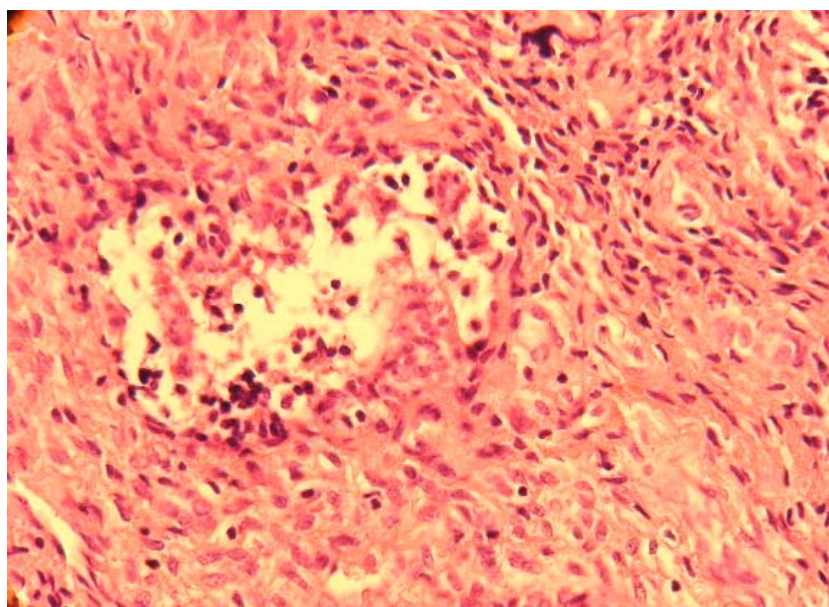


Fig. 6. Non-atypical glandular endometrial hyperplasia against inflammatory process - lysis of structural elements of glandular epithelium. Micrograph. 3p. 100

In immunohistochemical study of specimens with signs of chronic endometritis we have established the following features: reaction to CD-138 is positive in cases of non-atypical glandular-cystic endometrial hyperplasia - expression in plasmocytes,

total distribution, clump of positive intensity 3 points; focus is more diffuse; nucleus is hollow, cytoplasm is of brown color (Fig. 7). In case of non-atypical endometrial hyperplasia of stromal type (Fig. 8) lymphoplasmacytic infiltration of the stroma

of uneven character and marked positive expression of CD-138 in plasmocytes was revealed.

The above data on the presence of objective immunohistochemical markers of the inflammatory process - plasmocytes - in hyperplastic endometrial

tissue in one third of women of reproductive age indicate the involvement of the inflammatory factor in the etiopathogenesis of hyperplastic changes as one of the triggers of its hyperplastic transformation [9].

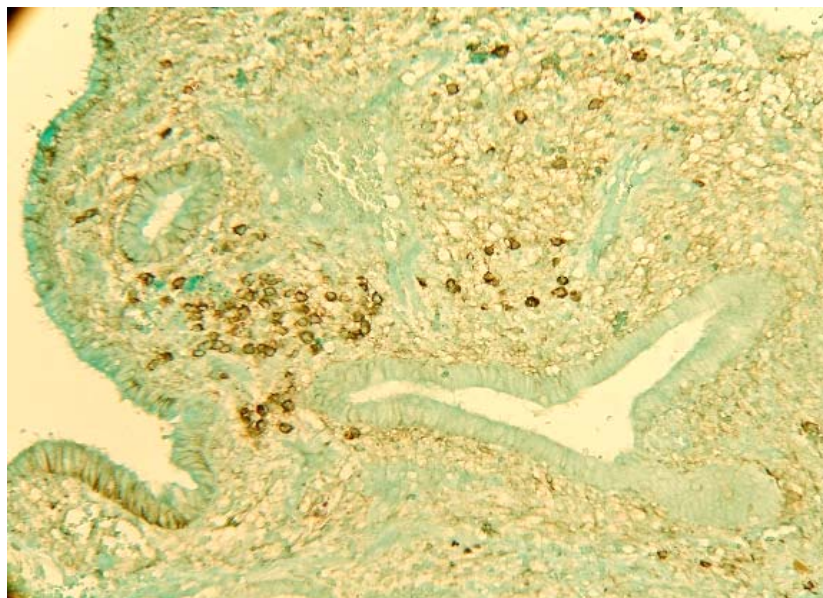


Fig. 7. Non-atypical glandular endometrial hyperplasia against inflammatory process. Positive expression of CD-138 in clump of plasma cells. Micrograph. 3p. 100

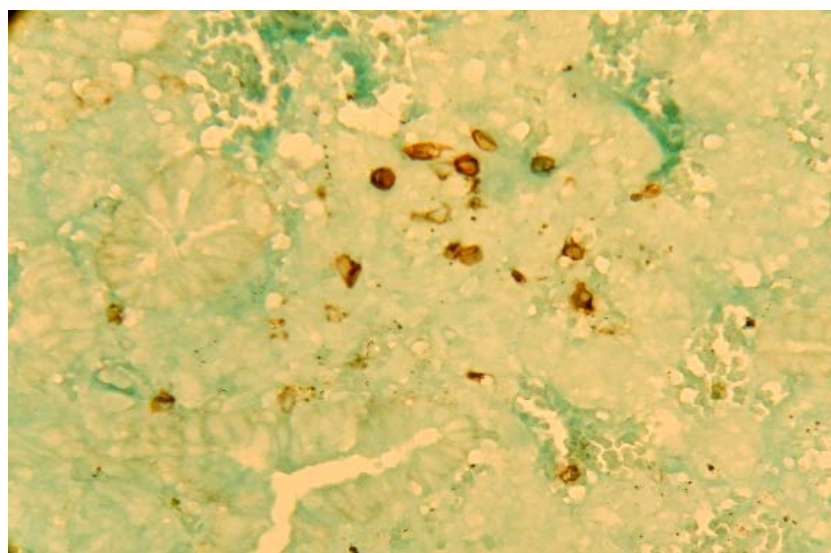


Рис. 8. Non-atypical glandular endometrial hyperplasia with area of stromal hyperplasia against inflammatory process. Positive expression of CD-138 in plasma cells. Micrograph. 3p. 200

In view of the pathogenetic mechanisms of development of hyperplastic processes, the data obtained prove the role of infectious factors in the occurrence of these processes in one third of patients with non-atypical endometrial hyperplasia.

The use of modern immunohistochemical studies enables to reliably confirm the concept which is based on new approaches to the treatment of such pathology, the main focus of which is the elimination

of infectious agent and the restoration of normal cyclic transformation of endometrial structures.

Thus, immunohistochemical studies indicate that the development of non-atypical hyperplastic processes is a complex process, mechanisms of its development is based both on an infectious factor and hormonal-metabolic disorders, which dictates various personalized approaches to therapeutic tactics.

CONCLUSIONS

1. In women of reproductive age with endometrial hyperplastic processes without atypia in almost a third (31.7%) of cases histologically structural-morphological signs of chronic endometritis

were revealed, which was confirmed by the immunohistochemical marker CD-138 – its positive expression is an evidence of inflammatory process participation in the etiopathogenesis of hyperplastic changes.

2. The use of modern immunohistochemical methods of study (CD-138) in the presence of hyperproliferative pathology of endometrium in women of reproductive age should be widely implemented in the practice of gynecologists to identify the signs of the influence of infectious factor and to settle the question of phasing personalized therapeutic approach.

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The article was received
2019.09.04

