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Стаття надійшла 28.12.2020 р.

DOI 10.26724/2079-8334-2021-4-78-141-145
UDC 618.11-006:577.275.6-055.2(045)

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MORPHOLOGICAL FEATURES OF ESTROGEN AND PROGESTERONE EXPRESSION LEVELS IN ECTOPIC ENDOMETRIUM IN WOMEN WITH ENDOMETRIAL CYSTS

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Endometrial cysts occupy one of the leading positions among endometrial neoplasms (up to 30 % of women of reproductive age have a history of this disease). The purpose of the study was to evaluate the immunomorphological features of ovarian endometrial tissue in women with infertility to assess their prognostic and diagnostic value. Our study has shown lower levels of estrogen and progesterone receptors in eutopic endometrium in women with ovarian endometrial cysts than in women without genital pathology. We associate this with the expression variants of the ER and PR receptor systems. In the main group, this ratio was 1 (11 out of 50 women had a characteristic histochemical picture for this pathology); in the comparison group, this figure was 14 cases out of 50, and in the control group – in 18 patients out of 50 studied. This allows us to consider the problem of sex hormones dysregulation as a predictor and, at the same time, a potential point of influence on the pathogenesis of cysts of endometrial origin.

Key words: endometrial cyst, infertility, estrogen receptor, progesterone receptor.

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МОРФОЛОГІЧНІ ОСОБЛИВОСТІ РІВНЮ ЕКСПРЕСІЇ ЕСТРОГЕНУ ТА ПРОГЕСТЕРОНУ В ЕКТОПІЧНОМУ ЕНДОМЕТРІЇ У ЖІНОК З ЕНДОМЕТРІОЇДНИМИ КІСТАМИ

Ендометріюїдні кісти займають одну з головуючих позицій серед новоутворень ендометрію (до 30 % жінок репродуктивного віку мають дане захворювання в анамнезі), тому на меті дослідження стояло вивчення імуноморфологічних особливостей тканини ендометрію яєчників у жінок із безпліддям для оцінки їх прогностичного і діагностичного значення. У ході наукової роботи було виявлено нижчий рівень експресії естрогенових та прогестеронових рецепторів еутопічного ендометрію серед жінок з ендометріюїдними кістами яєчників, ніж у жінок без генітальної патології. Ми пов'язуємо це з варіантами експресії рецепторних систем ER і PR, які розподілились наступним чином: у основній групі це співвідношення прямувало до 1 (11 з 50 жінок мали характерну для даної патології гістохімічну картину), у групі порівняння цей показник склав 14 випадків з 50, а у групі контролю – 18 пацієнтів з 50 досліджуваних. Це дозволяє говорити про проблему порушення регуляції статевих гормонів як про предиктор і водночас потенційну точку впливу на патогенез кіст ендометріюїдного походження.

Ключові слова: ендометріюїдна кіста, безпліддя, естрогеновий рецептор, прогестероновий рецептор.

The study is a fragment of the research project "Improving methods of prevention, diagnosis and treatment of diseases of the female reproductive system using the latest medical and molecular genetic technologies", state registration No. 0117U007494.

Endometriosis is a benign chronic disease in which the endometrium is formed outside the uterine mucosa. This can lead to the formation of superficial and deep endometriotic foci, in particular ovarian endometrial cysts [13]. This problem's genetic, cytological, immunological, endocrine, and inflammatory aspects have been intensively studied in recent years. Particular attention is drawn to the hormonal

mechanism of homeostasis of the uterine mucosa, which can lead to the onset of pathogenesis. Immune and inflammatory links and secondary mechanical alteration are pronounced a little later. The presence of several histopathological and laboratory changes that can be included in monitoring programs for this disease provides an opportunity to perform practical algorithms for diagnosis and treatment. These changes include delayed transformation of the endometrium from proliferative to secretory one, lack of united secretory response of glands, impaired distribution of glycogen and Periodic acid-Schiff positive substances, improper distribution of nucleic acids and alkaline phosphatase, etc. [4].

The urgency of the problem has increased over the years. Endometriosis is the most common benign hormone-dependent disease in women of fertile age and a nosological unit with one of the most difficult diagnostic algorithms. In turn, the endometrial cyst is one of the most common pathological consequences of this persistent disease. This increases the average age of diagnosis verification, which is 28 years [13]. However, some data suggest the possibility of diagnosis several months after the first menstruation [15], in some cases even before the first menstruation. The social significance of the problem is exacerbated by the fact that a significant proportion of women with ovarian endometrial cysts suffer from infertility. In patients with external endometriosis, primary and secondary infertility is one of the most common complications [8]. In internal endometriosis, secondary infertility is 4 times more common than primary infertility, while more than 50 % of infertile patients have a certain number of endometriotic foci in the form of endometrioma [10]. Based on modern literature, establishing a plausible link between endometrial cysts and infertility may provide new perspectives in the treatment of this condition [12].

An analytical approach to the pathogenesis of the disease can identify a component such as hormonal imbalance. Therefore, we were interested in the levels of estrogen and progesterone expression in both the glands and the stroma of the ectopic endometrium. It is likely that ovarian endometrial cysts, causing this imbalance, directly impact infertility in women.

The purpose of the work was to study the immunomorphological features of ovarian endometrial tissue in women with infertility to assess their prognostic and diagnostic significance.

Materials and methods. The scientific study was performed at the clinical basis of the Department of Obstetrics and Gynecology No. 1 of Odessa National Medical University. The sample was standardized by age and clinical parameters. The study program included 130 women. The main group included 50 women with ovarian endometrioma who received the proposed methods of diagnosis and treatment. The comparison group consisted of 50 women who received conventional methods of diagnosis and treatment. 30 women in the control group were examined at the Reproductive Centre and applied for male infertility in the couple. The age category of all groups was 22–39 years; the median age was 30.5 ± 8.5 years. All patients were comprehensively examined in accordance with the requirements of the current clinical protocols regulated by Orders of the Ministry of Health of Ukraine No. 582 of 15.12.2003 and No. 676 of 31.12.2004. In our work, we followed the ethical principles of the Declaration of Helsinki of the World Medical Association (World Medical Association Declaration of Helsinki, 1964).

Endometrioid cysts were detected by ultrasound, which was performed on all patients using transabdominal and transvaginal sensors. The size of cysts at least 4 cm was an inclusion criterion for the study group.

A standard pathohistological examination (macroscopic and microscopic) was performed. We processed the surgical material. It included tissues of 100 ovarian endometriomas of women who were examined and received comprehensive treatment on the basis of the University Clinic of Odessa National Medical University in the period from 2015 to 2018, and pipeline biopsies of the endometrium taken on the 18th–20th day of the menstrual cycle according to Noyes criteria [14]. Next, the biopsy material was fixed in neutral 10 % buffered formalin (pH 7.0). Standardised dehydrating in alcohols was performed according to the protocol for ethanol and isopropanol in the Histos 5 Rapid Microwave Histoprocessor (Milestone, Italy), then we performed paraffinisation and pouring into paraffin. Standard sections with a thickness of 4 μ m were made on the Leica RM 2500 Rotary Microtome (Germany), which were placed on ordinary slides (for viewing pathohistological staining) or on adhesive slides “SUPER FROST PLUS” (Menzel, Germany) for immunohistochemical (IHC) studies.

Monoclonal Antibody, Clone SP1 for estrogen receptor detection and Monoclonal Antibody, Clone YR85 for progesterone receptor detection were used for IHC staining. Reaction imaging was performed using the Reveal Polyvalent HRP-DAB Detection System and subsequent Mayer hematoxylin staining. The results of the IHC reaction were evaluated using a Leica DM 750 light microscope (Germany), and micrographs of the preparations were performed using a Camedia C5060WZ Olympus digital camera (Japan). External quality control was breast tissue for estrogen and progesterone.

The results of IHC reactions were evaluated by the point system of the continuous staining method to determine the ER and PR status (Allred D. C. et al., 1998) and the recommendations of the College of American Pathologists [9]. According to the point system, the assessment of intensity degrees was carried out as follows: 0 – no; 1 – low; 2 – moderate; 3 – severe. While the prevalence of cell staining as a result of the immunohistochemical reaction was assessed as follows: 0 – no stained cells; 1 –stained cells less than 1/100; 2 –the number of stained cells from 1/100 to 1/10; 3 – the number of stained cells from 1/10 to 1/3; 4 – the number of stained cells from 1/3 to 2/3; 5 – the number of stained cells more than 2/3. The total score was obtained by adding the staining prevalence score to the intensity score. If the sum of scores was 0–2, the ER-, PR-status was considered negative; for the sum of scores above 2– ER-, PR-status was positive.

Statistical processing was performed using the program “Statistica 10.0” (StarSoftInc., USA). The hypothesis of the normality of the distribution of the studied values was tested using the Shapiro-Wilk test. Quantitative data are distributed according to the normal distribution law, so the reliability of the differences in the mean values was determined using Student's t-test.

Results of the study and their discussion. Unilateral endometrioid cysts were diagnosed in 72 % of cases, and bilateral cysts were diagnosed in 28 % (8 in the main group and 6 in the comparison group). In all cases, the diagnosis was confirmed histologically.

Macroscopically, endometrial cysts were round in shape, with an outer surface with numerous foci of the fusion process in 36 cases (17 in the main group and 19 in the comparison group), bilateral cysts in all patients had a fusion process on the serous integument. Often the capsule of endometrial cysts was fused by an adhesive process with the fallopian tubes (41.2 % with the adhesive process in the main group and 42.1 %, respectively, in the comparison group), serous rectum (17.6 % with the adhesive process of the main group and 26.3 %, respectively, in the comparison group). There were also adhesions with the posterior surface of the uterus (11.8 % with the adhesive process of the main group and 15.8 % in the comparison group, respectively), the rectouterine pouch (11.8 % with the adhesive process the main group and 5.3 % of the comparison group, respectively). All endometrial cysts had a dense grey-brown capsule from 0.2 to 0.9 cm thick.

The content of cysts varied from brownish-grey to brownish-red was homogeneous, dense in consistency with foci of compaction and the formation of brittle layered yellowish-brown masses near the walls. The inner surface of the ovarian endometrium was uneven and rough in all cases, but the cyst cavity had no membranes or outgrowths.

The study of endometrial biopsies, conducted in all groups of patients, had the following results. All women did not have endometrial hyperplasia and polyps (inclusion criterion), and the endometrium itself had no morphological signs of chronic endometritis. However, regarding the compliance of the endometrium with the phase of the cycle, we obtained the following data: in 44 (88 %) women of the main group and in 45 (90 %) women of the comparison group, the endometrium fully corresponded to the phase of the cycle (secretion phase). In 11 (22 %) cases (12 % of the main group and 10 % of the comparison group), there was a slight focal “lag” of the morphological picture of the endometrium from the phase of the cycle (fig. 1).

Regarding assessing estrogen receptor status of cells of the glands of eutopic endometrium, a positive reaction was observed in 100 % of cases; as for the intensity of staining, moderate (46 %) and high (32 %) prevailed. Endometrial stroma cells also had a positive estrogen receptor response in a significant proportion of cases (76 %).

In ectopic foci of ovarian endometrioid cysts, low estrogen (ER) expression was verified in 9 cases out of 18 (50 %) and moderate in 4 (22.22 %). There was no high expression, but it was absent in 5 (27.78 %) cases. In the control group, high (48 %) and moderate (36 %) levels of ER expression were found, with a positive response recorded in 95 % of glandular cells and 88 % of endometrial stroma cells.

We found a decrease in the ER expression of the eutopic endometrium of women with ovarian endometrial cysts compared with the endometrium of women without genital pathology. The ER ratio in eutopic endometrial glands to ER in the stroma was 5:1 in the main group and 5.2:1 in the comparison group and 4.6:1 in the control group. The ratio of ER in ectopic endometrial glands to ER in the ectopic endometrial stroma in the main group was 3:1 and 2.8:1 in the comparison group.

Thus, the ectopic endometrium is characterized by a predominant increase in ER expression in the stroma compared with the immunomorphological features of ectopic foci. Eutopic endometrium is characterized by a principal location of ER in the glands. The number of steroid receptors in the eutopic endometrium of the main group and the comparison group had no significant differences; the same applies to the ectopic endometrium in relation to these groups.

When studying the expression of progesterone receptors (PR) in the eutopic endometrium of women in the main group, a moderate response was found in 10 cases (55.56 %) out of 18 (fig. 2) and a

low one in 6 patients (33.33 %). Distinct expression was documented in 2 cases (11.11 %), and no cases of negative expression were recorded.

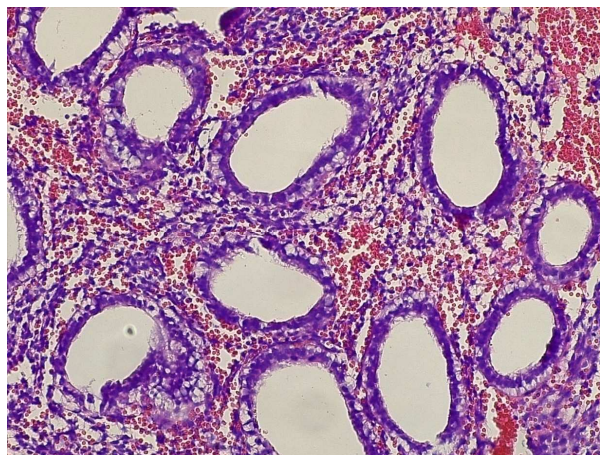


Fig. 1. Endometrium morphologically corresponds to the late stage of the proliferation phase – focal transition to the early stage of the secretion phase (18th day of the cycle). Hematoxylin and eosin staining. Magn.: x 100.

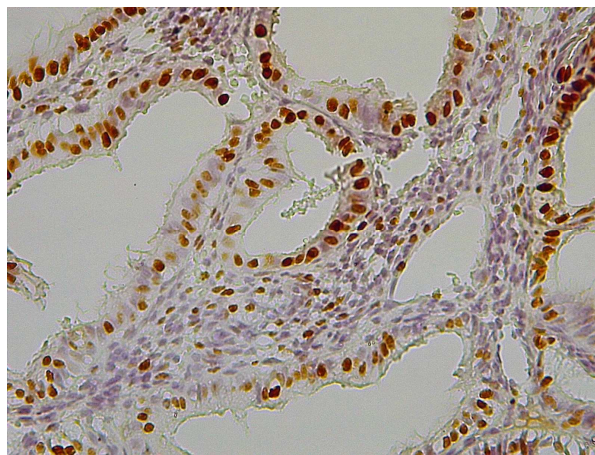


Fig. 2. Moderate level of PR expression in glands and low level of PR expression in ectopic endometrial stroma of a 31-year-old patient in the comparison group. Mo a-HuEr, Clone PG-M1. Magn.: x 100.

In ectopic endometrial cells, the vast majority of patients (12 cases, 66.67 %, respectively) had a low PR expression, moderate was in 4 cases (22.22 %), and negative was in 2 patients (11.11 %). An explicit expression of PR in the tissues of endometrial cysts was not detected. The comparison group had the following results: moderate PR expression in 9 cases (50 %), pronounced expression in 4 cases (22.22 %), and low expression in 5 patients (27.78 %). There was no negative reaction in these cases. At the same time, in the control group, we documented high (71.4 %), moderate (22.9 %) and low (5.7 %) levels of PR expression in the endometrial glands – 65.7 % with a high PR response, 25.7 % with moderate and, respectively, 8.6 % with low). In stromal cells, 80 % of cells had a level of PR expression (of which 77.1 % with a high PR response, with moderate – 17.1 % and, respectively, 5.8 % with low).

The ratio of PR in the ectopic endometrial glands to PR in the stroma was 4.5:1 in the main group, 4.7:1 in the comparison group, and 3.4:1 in the control group. The ratio of PR in the ectopic endometrial glands to PR in the ectopic endometrial stroma in the main group was 2.2:1 and 2.6:1 in the comparison group.

Thus, ectopic endometrium is characterized by a predominant location of PR expression in the stroma, compared to eutopic endometrium. The number of PRs in the eutopic endometrium of the main group and the comparison group had no significant differences; the same applies to the ectopic endometrium of these groups.

At the same time, the level of ER and PR of the ectopic endometrium is significantly lower than the level of expression of the same eutopic endometrial receptors in the same patients. It was found that there is a decrease in the expression of estrogen and progesterone receptors in the eutopic endometrium of women with ovarian endometrial cysts compared to the endometrium of women without genital pathology. Decreased expression of ER and PR in patients with endometrial glandular hyperplasia compared with the endometrium of women without genital pathology has been documented. Ectopic endometrium showed a significant decrease in ER and PR expression levels compared to eutopic endometrium. The following distribution variants of the ER and PR receptor systems were established: $ER/PR < 1$, $ER/PR = 1$, $ER/PR > 1$. In the main group, only in 11 of 50 women in the epithelial cells of the eutopic endometrium, we documented the ratio of ER and PR, which was up to 1; in the comparison group, this figure was 14 cases, and in control – 18. As for stromal cells, such a division was found in only 7 of 50 cases of the main group, 8 cases of the comparison group, and 16 in the control group. In stromal cells, a decrease in ER was more often observed against the background of an increase in PR ($ER/PR < 1$) – in 29 of 50 patients in the main group, in the comparison group, this figure was 34 cases in the control – 8. The glandular component of the ectopic endometrium in 31 of 50 women of the main group, 32 cases from the comparison group and only 9 cases from the control group was characterized by an increase in PR and a decrease in ER ($ER/PR < 1$). Most women had a pronounced degree of expression of progesterone receptors. A similar pattern with a predominance of PR was observed in stroma cells in the main and comparison groups. $ER/PR > 1$ ratio was documented in epithelial cells in only 8 patients in the main group, in 7 in the comparison group and 7 of the control group and 14 of 50 in the stromal cells of the main group, in 8 cases of the comparison group and 10 cases of the control group. The predominance of ER may indirectly indicate possible hyperestrogenemia and relative hypoprogesteronemia of these foci.

Scientific discussion on the role of estrogen and progesterone in the development of hyperplastic endometrial processes continues. In particular, the predominance of ER in several studies indirectly indicates possible hyperestrogenemia and relative hypoprogesteronemia among the subjects, which was confirmed in our research and several works [2, 3].

The role of the stromal component in endometrial cyst remains debatable. Our immunohistochemical studies proved the presence of ER in the glands of the eutopic endometrium and in the stroma, which is consistent with the data of other researchers, in particular, Kurik OG, Kalenskaya OV and Afinogenov EA, Cherstvyi ED. [1, 5]. Regarding the cell composition, among estrogen-positive stroma cells, we have documented single lymphocytes and macrophages, which presence correlates with pathology according to Kuryk OG, Kalenskaya OV, and may indicate the effect of steroid hormones on immunocompetent cells [5].

Variable indicators of progesterone receptor activity among endometrial pathological foci in patients with endometrioid cyst create another field for research. High expression of PR receptors in these areas was found in our study and correlates with the data of Kuryk OG and Meliksetyan et al. [5, 6].

High estrogen levels and low progesterone levels are common features of all tumours in this class, but different researchers have shown differences in receptor expression levels [7, 11].

The question of the modern classification of ovarian cysts remains open, so we see prospects in building a classification based on the profile of steroid hormone receptors.

Conclusions

1. It has been found that in the vast majority of cases, foci of ectopic endometrium (tissues of ovarian endometrial cysts) show the expression of estrogen and progesterone receptors, which may be important for both the occurrence and clinical manifestations and course of ovarian endometrial cysts. Estrogen and progesterone dependence of ectopic endometrial foci is confirmed by verifying IHC response with ER and PR in most women.

2. It has been shown that in women with ovarian endometrial cysts, the eutopic endometrium has a lower level of estrogen and progesterone receptor expression than in women without genital pathology. There was a decrease in the expression of estrogen and progesterone receptors of the ectopic endometrium (tissue of ovarian endometrial cysts) in accordance with the eutopic endometrium.

3. The dysregulation of sex hormones, which is one of the manifestations of endometriosis or its development factor, requires further study.

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Стаття надійшла 21.11.2020 р.