PROSPECTIVE STUDY OF THE EFFECTIVENESS OF DIFFERENTIATED THERAPY OF ENDOMETRIUM HYPERPLASIA WITHOUT ATYPIA IN WOMEN IN REPRODUCTIVE AGE

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Summary. The paper considers the issues of improving the effectiveness of treatment of endometrial hyperplasia without atypia in women of reproductive age with the use of progestins as a pathogenetic therapy and should be personalized (targeted) taking into account the receptor sensitivity of endometrial tissue to progestins. The positive effects of progestin use are mainly due to the expression of progesterone receptors in the endometrial tissue, which must be taken into account during hormone therapy. A prospective study was performed in 60 patients of reproductive age with abnormal uterine bleeding, who according to the results of histological examination of endometrial tissue was diagnosed with endometrial hyperplasia without atypia. All patients were treated with micronized progesterone at a dose of 400 mg / day continuously for 6 months. To determine the effect of the use of progestins was performed by studying the expression of receptors for estrogen (ER) and progesterone (PR) in histological blocks of the endometrium by immunohistochemistry. In all women there was a significant expression of EP in endometrial cells, which led to its proliferative activity against the background of reduced expression of progesterone receptors by 65%, which caused no effect of therapy in 25% of women. Studies have shown that when deciding on the appointment of micronized progesterone for the treatment of endometrial hyperplasia without atypia, it is recommended to study the expression of progesterone receptors in endometrial tissue to clarify the possibility of a pharmacological effect. Treatment of endometrial...
Hyperplasia without atypia with progesterone drugs is not effective in low expression of progesterone receptors in endometrial tissue. Based on this, we can identify a group of women with progesterone-resistant hyperplasia who require other treatments.

Keywords: endometrial hyperplasia without atypia, immunohistochemistry, progesterone receptors, progesterone therapy, progesterone resistance.

Endometrial hyperplasia (HDE) in women of reproductive age currently occupies an important place in the structure of gynecological morbidity (30 to 55%), second only to inflammatory diseases of the pelvic organs and uterine fibroids. GPE in women of reproductive age and its inherent abnormal uterine bleeding are the most common reason for seeking medical attention and hospitalization of patients [1,2].

Scientific and practical interest in the problem of GPE in women of reproductive age is determined not only by existing cancer risks, but also by frequent detection of persistent recurrences, menstrual disorders, anemia, reproductive dysfunction, which significantly impair quality of life and efficiency of the active population [3,4].

Women with GPE in the reproductive period are a diverse group, more associated with hormonal disorders, which are observed in 85% of women with hormonal ovarian failure, in 24-74% of patients with polycystic ovary syndrome, chronic endometritis, obesity, in 15-95.6% of cases of endometriosis [5-8].

GPE is considered a benign pathology in which conservative treatment with progestogens is practiced, given that progesterone derivatives balance the mitogenic action of estrogen and induce secretory differentiation, as a result of which endometrial cells lose the ability to mitosis. It is proved that the use of progestogens (intrauterine system with levonorgestrel, medroxyprogesterone acetate, micronized progesterone, dydrogesterone) provides a higher rate of disease regression compared to observation alone. But to date, neither the dose nor schedule of progestogens is standardized in published studies, as well as clinical or histological signs suggesting the effectiveness of progestogen treatment and long-term absence of recurrence.

The high frequency of recurrence of GPE and the possibility of their malignant transformation require improvement of diagnostic methods for this pathology [9,10].

**The aim of the study.** Improving the quality of treatment of GPE without atypia in women of reproductive age based on the use of micronized progesterone, taking into account clinical, morphological and immunohistochemical data. To investigate the effect of estrogen and progesterone receptor expression in hyperplasia of the endometrium without atypia in women of reproductive age on the effectiveness of therapy.

**Research design.** A prospective study was performed in 60 patients of reproductive age (35-46 years) with abnormal uterine bleeding (AMC). Histological examination of endometrial scrapings in 45 patients was diagnosed with endometrial hyperplasia without atypia, which formed the main group of the study. This group was divided into 2 subgroups according to the level of expression of progesterone receptors: 1 group with low levels and 2 groups with high levels. The
control group consisted of 15 women of reproductive age, in whom the study revealed the transformation of the endometrium according to the phase of the menstrual cycle. All patients received treatment with micronized progesterone at a dose of 400 mg/day continuously for 6 months, followed by histological examination of the endometrium by scraping the uterine cavity in the late secretory phase and repeated immunohistochemical examination of PR expression. The effectiveness of therapy was evaluated by morphological criteria. Restoration of physiological trophism of the endometrium was considered a satisfactory result of treatment.

The study of progesterone receptors was performed by immunohistochemical method in the laboratory of immunohistochemistry DDMA. To do this, the biomaterial of scrapings of the uterine cavity was fixed in a 10% solution of buffered formalin for 12-24 hours, and after standard treatment was poured into paraffin. Immunohistochemical study of progesterone receptors was performed by streptavidin-biotin-peroxidase method on serial paraffin sections with a thickness of 5 μm using monoclonal antibodies (firm "DAKO") with preliminary high-temperature unmasking of antigens in tissues by boiling in boiling water. Next, the sections were incubated with normal non-immune serum, primary (specific) antibodies, secondary biotinylated antibodies and streptavidin-biotin-peroxidase complex. Detection of peroxidase was performed using diaminobenzidine (firm "DAKO"). The manifestation of the reaction was monitored under a microscope for 3 min, followed by washing in distilled water, additional staining with Mayer's hematoxylin, dehydration of sections and immersion in Canadian balm [11].

A positive color of the nuclei in brown was considered a positive result. Quantitative assessment of the degree of expression of progesterone receptors was performed according to the system Hystochemical score (H-score) [12].

The counting system was performed on the intensity of immunohistochemical staining, which is assessed on a 3-point scale and the percentage of stained cells. The count was performed in three cohorts of 100 cells in different fields of view with a lens × 40. The color intensity was estimated as follows:

0 - no staining, 1 - weak staining, 2 - moderate and 3 - strong staining.

Calculation formula:

\[ H \text{ score} = \sum P_i \times i, \]

where:

\( P_i \) is the percentage of cells stained with different intensities,
\( i \) is the intensity of staining, expressed in points from 0 to 3.

The degree of expression of progesterone receptors was judged by the H-score: 0-100 - low, 101 or more - high.

Significance of differences was assessed by Student's test, assuming statistically significant values of \( p < 0.05 \). Correlation coefficients were calculated by the Pearson method, the differences were considered significant at \( p < 0.001 \).

Research results. To clarify these issues, we examined, treated and monitored over the next three years 101 women of late reproductive age (36-45 years) with a confirmed histological diagnosis of GPE (group II), who were registered at the dispensary in the medical institution where the research. The choice of the object of study in women of this age category was justified by us in section 2 based on the results of a retrospective study.
All women of group II after histological confirmation of the diagnosis of GPE received progestogens, depending on the type of which they were divided into 2 subgroups. Subgroup IIa included 47 women who received oral micronized progesterone (200 mg per day) for 6 months, and subgroup IIb included 54 women who received dydrogesterone (20 mg per day) in the same way. The age of women in the randomized subgroups did not differ significantly (respectively 38.7 ± 2.3 and 39.2 ± 2.1; p> 0.05). Thirty morphological samples of eutopic endometrium (EUE) obtained from women of the appropriate age were used as controls.

The criterion for the effectiveness of GPE treatment was considered to be obtaining at least two negative results of endometrial biopsy in a row with an interval of 6 months, as well as the absence of recurrence of the disease during three years of follow-up. Endometrial samples were examined by histological and IHC methods using monoclonal antibodies to key molecular antigens of cell cycle inhibition according to the criteria described in section 2.2. In addition, ultrasound was performed to determine the thickness of the M-echo and the structure of the endometrium with an interval of 6 months.

Control histological examination of endometrial biopsies obtained after 6 months of progestogen use showed a positive treatment result in 35 (74.5%) patients of subgroup IIa and in 44 (81.5%) patients of subgroup IIb, ie, the frequency of positive results in women who took oral forms of micronized progesterone or dydrogesterone, did not differ significantly.

At the same time, persistence of GPE on the background of progestogen therapy for 6 months was detected in 12 (25.5%) patients receiving micronized progesterone (subgroup IIa) and in 10 (18.5%) patients receiving dydrogesterone (IIb subgroup).

At the end of a three-year follow-up of women with late-onset reproductive GPE treated with micronized progesterone, a positive result of no recurrence persisted in 32 (68.1%) of the 47 women (group Ila) included in the study. Among women who received dydrogesterone (group IIb), the absence of recurrence of GPE for the same time was confirmed by histological studies of control endometrial samples in 41 (75.9%) of 54 patients (Fig. 1).

Fig. 1. Dynamics of GPE regression during 36 months of observation of women after treatment with various forms of progestogens,% of cases (MP-micronized progesterone, DG-dydrogesterone)
Thus, almost every third woman of reproductive age who underwent GPE treatment with the use of hestegen drugs, in the following years there was either a recurrence of the disease - 28 (27.7%) cases, or progression to hypertension - 6 (5.9%) cases. All women with recurrent GPE were subsequently given 6 injections of GnRH agonists as second-line therapy at 4-week intervals. In 6 (5.9%) women with disease progression to hypertension, surgical treatment was performed in the amount of pangisteroectomy.

The therapeutic effects of progestogens are known to be related to their biological effects on progesterone-dependent genes that control cell division and differentiation. The culmination of this signaling pathway is the binding of progestogens to the corresponding nuclear receptor (PGR). Therefore, to determine the reasons for the lack of regression of GPE in women from the use of progestogens, we analyzed the results of the study of nuclear PGR expression in endometrial samples obtained at the screening stage before treatment in 28 women who received a positive result of progestogen treatment and 22 - with negative morphological result after treatment with micronized progesterone and dydrogesterone.

We found that in the endometrium of women with GE resistant to progestogen therapy, the expression of PGR in glandular cells (50.8 ± 0.7) and stroma (47.3 ± 0.8) was significantly lower than in the corresponding structures in samples of GPE with a positive result of progestogen therapy (respectively 183.7 ± 3.1 and 166.4 ± 2.3; p <0.05) (Fig. 2).

![Fig. 2. Indicators of H-index PGR expression in progestogen-sensitive (GPE +) and hormone-resistant (GPE -) cells compared to normal endometrium](image)

The different intensity of staining of GPE samples due to IHC reaction in sensitive and progestogen-resistant endometrium is also shown in Figure 8.3, which clearly shows a significant decrease in the number of cells with positive staining of PGR receptors (Fig. 3, a) compared with a significant number of PGR + cells. 3, b). Differences in the expression of PGR in hormone-resistant (PGR-) endometrium in comparison with the unchanged proliferative morphotype were detected by us in both glands (respectively 50.8 ± 0.7 and 193.2 ± 8.5) and stroma (respectively 47.3 ± 0.8 and 140.2 ± 4.4; p <0.05). Significant differences were also determined when comparing the expression of PGR in hormone-resistant (PGR-) endometrium with...
unchanged secretory endometrium - respectively $50.8 \pm 0.7 \ 178.7 \pm 6.3$ (p <0.05) in the glands and respectively $47.3 \pm 0.8$ and $116.6 \pm 3.1$ in the stroma.

Fig. 3. Expression of PGR in cells of GE samples: a) in hormone-resistant (GPE -) and b) progestogen-sensitive (GPE +) endometrium. IGH, DAKO EnVision visualization system. Coll. x 400

Thus, the resistance of GPE to progestogen exposure found by us in 12 patients from subgroup IIa and 10 from subgroup IIb can be explained by low expression of PGR in endometrial cells before treatment.

Conclusions. Studies have shown that when deciding on the appointment of micronized progesterone for the treatment of endometrial hyperplasia without atypia, it is recommended to study the expression of progesterone receptors in endometrial tissue to clarify the possibility of a pharmacological effect. Treatment of endometrial hyperplasia without atypia with progesterone drugs is not effective in low expression of progesterone receptors in endometrial tissue. Based on this, we can identify a group of women with progesterone-resistant hyperplasia who require other treatments.

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