

ASSESSMENT OF THE RECEPTIVITY OF THE ENDOMETRIUM TO STEROID HORMONES IN WOMEN WITH CHRONIC ENDOMETRITIS

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Summary

Chronic endometritis (CE) is one of the main pathologies that lead to female infertility. Although research on the molecular and biological features of the endometrium in this disease has been conducted for a long time, the problem of studying the morphotype of CE remains relevant. Today, there is no universally accepted panel of immunohistochemical markers that would allow to fully evaluate the status of the endometrium before and after treatment, as well as to evaluate the effectiveness of the therapy.

The purpose of our study was to study the features of the expression of markers of estrogen (ER) and progesterone (PR) receptors in order to make a prognosis for the recovery of patients with a possible subsequent pregnancy.

For this, 2 research groups were formed using Peipel biopsies of female patients. Group I included cases before treatment, group II – after treatment. During the study, it was found that the criterion for the success of the treatment is a relative decrease of ER- and PR-positive cells both in the stroma ($p < 0.0001$) and in the epithelium of the endometrium ($p < 0.0001$). It was also found, that the expression of PR by the epithelium of the glands shouldn't exceed 50% ($p < 0.0001$), while for ER the critical level hasn't been established ($p = 0.77$) and it is possible to judge only the relative decrease in the expression of the marker.

Key words: chronic endometritis, estrogen, progesterone, receptors for steroid hormones.

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1. Introduction

Chronic endometritis (CE) is a pathology that is usually associated with female infertility, but there are still no uniform recommendations for a mandatory diagnostic panel and the minimum level of necessary research for a couple who can't become parents for a long time (Kimura, 2019).

The seriousness and actuality of the problem of the study of CE is also reflected in the numbers, because according to various researchers, it is diagnosed in infertile women in 2.8–56.8% of cases and in 9.3–67.6% of women with repeated miscarriages (Kimura, 2019; Espinos, 2021). The gold standard for the diagnosis of chronic endometritis is microscopic examination of endometrial biopsies with the presence of plasma cells (Kimura, 2019) and subsequent immunohistochemical (IHC) confirmation using the marker CD138 (a marker of plasma cells) (Dimitriadis, 2007). But researchers are usually interested in much broader molecular and biological characteristics of the endometrium than simple verification of the diagnosis of CE. In particular, the issue of determining the causative agent of the disease and controlling the quality of treatment is important. There is still no well-defined recommended

standard of the panel of IHC markers in the world, which would allow predicting the outcome of therapy and answering the most important question: whether a woman will be able to get pregnant and give birth to a healthy child.

Among the variety of IHC markers used in the diagnostic process for chronic endometritis, a special place is given to estrogen receptor (ER) and progesterone receptor (PR) markers. The role of estrogen and progesterone for the endometrium has been studied in great detail. The change of phases of the endometrium occurs precisely on the basis of these hormones. Thus, estrogen induces the proliferative phase when interacting with receptors sensitive to it and prepares the endometrium for the synthesis of progesterone receptors, which will then trigger the secretory phase (Yu, 2022; Mylonas, 2004). A study in mice showed, that it is the expression of ER that is critically important for a normal menstrual cycle and subsequent pregnancy (Yu, 2022; Brosens, 2004). Disturbance or abnormal secretion of ER is observed in endometriosis, carcinoma, chronic endometritis (Chantalat, 2020).

The aim of our study was studying changes in the expression of ER and PR receptor markers in women with chronic endometritis before and after treatment with subsequent pregnancy that ended in delivery.

2. Research materials and methods

Clinical characteristics of material

The clinical material in our study was medical histories, paraffin blocks, and scales of patients, who underwent Peipel biopsy of the endometrium on the 7–8th day after ovulation. Cases found in the archive of the immunohistochemical laboratory "Prime-test", which was located on the basis of the Kharkiv Medical Academy of Postgraduate Education, for the period 2014–2021 were subjected to research.

According to the results of the conducted search, 50 cases of CE were selected with appropriate quality scales and a sufficient number and quality of paraffin blocks. In all cases, they had blocks before and after CE therapy with the subsequent onset of pregnancy and childbirth.

Histologically, with the help of light microscopy using routine hematoxylin-eosin staining, CE with the presence of a large number of plasma cells was verified in all cases. IHC-confirmation of the diagnosis took place with the involvement of the marker CD138, which was expressed in plasma cells.

Immunohistochemical study

The material for IHC study was fixed with 10% neutral formalin for 24 hours, embedded in paraffin blocks, sections 4 µm thick were prepared, which were applied to highly adhesive Super Frost glass and dried at 37°C for 18 hours. Unmasking was performed by boiling the slices in a citrate buffer (pH 6.0). The UltraVision Quanto Detection Systems HRP Polymer detection system (Thermo scientific) was used to visualize primary antibodies. DAB (diaminobenzidine) was used as a chromogen.

Primary monoclonal antibodies (MCAT) from Diagnostic BioSystems (ER, Estrogen Receptor 1D5) and Dako Cytomation (PR, PgR636) were used.

A semi-quantitative scale of 0–3 + was used to assess the intensity of the immunohistochemical labeling of markers: 0 – no expression, + – weak, ++ – moderate, +++ – pronounced reaction. The percentage of cells expressing markers was also taken into account: 1–10% – weak expression, 11–50% – moderate expression, and more than 50% – strong expression of markers (Chatzipantelis, 2022).

Statistical data processing

Statistical analysis was performed using the Wilcoxon test to assess differences between paired samples by trait level. The Pearson test with Yates correction was used to assess the differences between the studied groups. The significance level was considered to be ≤ 0.05 .

3. Research results and their discussion

The study of the expression of estrogen receptor marker revealed, that in 5 (10%) samples of the 1st study group, the reaction was negative both in the stroma and in the epithelium of glands, and in 1 (2%) case, stromal reaction was absent with its preservation in the epithelium of the glands focally at the level of 50% (Fig. 1A). In the rest of the studied cases of chronic endometritis before treatment, pronounced expression was observed both in the stroma and in the epithelium of glands (Fig. 1B). The average level of ER expression was $70.92 \pm 4.47\%$ in the stromal component of the endometrium with fluctuations from 0% to 96%, in the epithelial component – $84.78\% \pm 3.88\%$ with fluctuations from 0% to 100%.

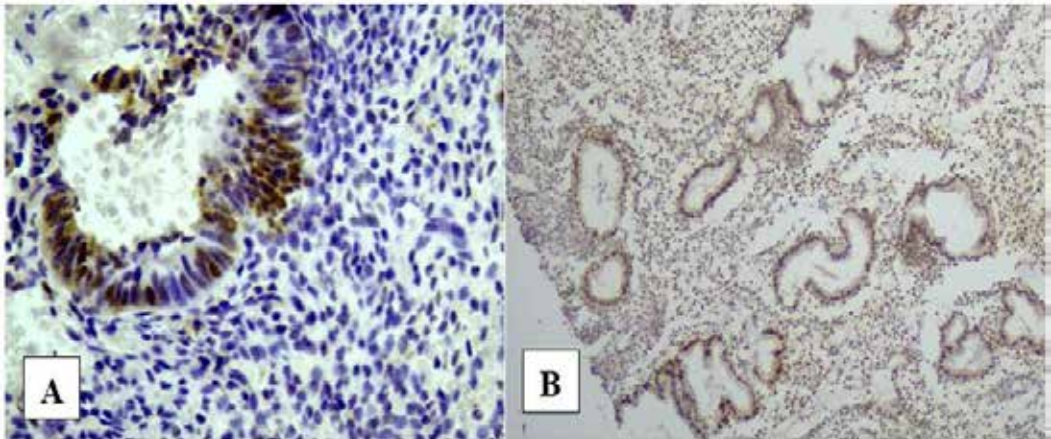


Fig. 1. A – absence of stromal expression of ER and its focal preservation in glandular epithelium (magnification $\times 400$). B – Pronounced expression of the ER marker both in the stroma and in the epithelium of the glands (magnification $\times 200$)

In the II group of the study, a complete lack of expression was observed in 3 (6%) cases, in 7 (14%) cases only a stromal reaction was absent with preservation of ER nuclear expression in the glands. In the remaining observations, moderate or pronounced expression of ER was noted both in the epithelial component of the endometrium and in the stroma. In the stroma ER marker was expressed at the level of $48.8 \pm 3.54\%$ with a range of values from 0% to 78%, in the epithelium the average expression level was $61.22 \pm 3.05\%$ with a maximum expression of 90%. At the same time, for both groups, the more characteristic level of expression in the stromal component is 51–100% with an increase in the number of cases of moderate expression (11–50%) in the II group ($\chi^2 = 4.68$, $p = 0.03$). The epithelial component is characterized by expression of more than 50% in both study groups without a tendency to increase number of cases of moderate expression ($\chi^2 = 1.13$, $p = 0.29$). A tendency to decrease in both stromal ER expression ($p < 0.0001$) (Fig. 2A) and epithelial expression ($p < 0.0001$) (Fig. 2B) was observed, which is indicated on the dot-line diagrams.

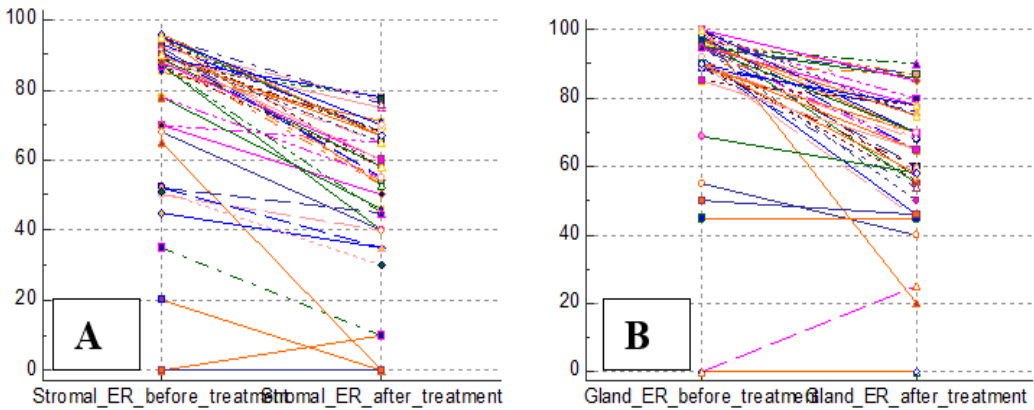


Fig. 2. A – a trend towards a decrease in ER expression in the stromal component of the endometrium after treatment. B – a tendency to decrease ER expression in the epithelial component of the endometrium after treatment

Regarding the expression of PR receptor marker, in group I, 12 (24%) cases of absolutely negative samples were found, and in 17 (34%) cases only stromal expression of PR was noted. In the remaining cases, moderate and focally weak expression of the marker was observed in the stroma and glandular epithelium of the endometrium (Figs. 3A and 3B).

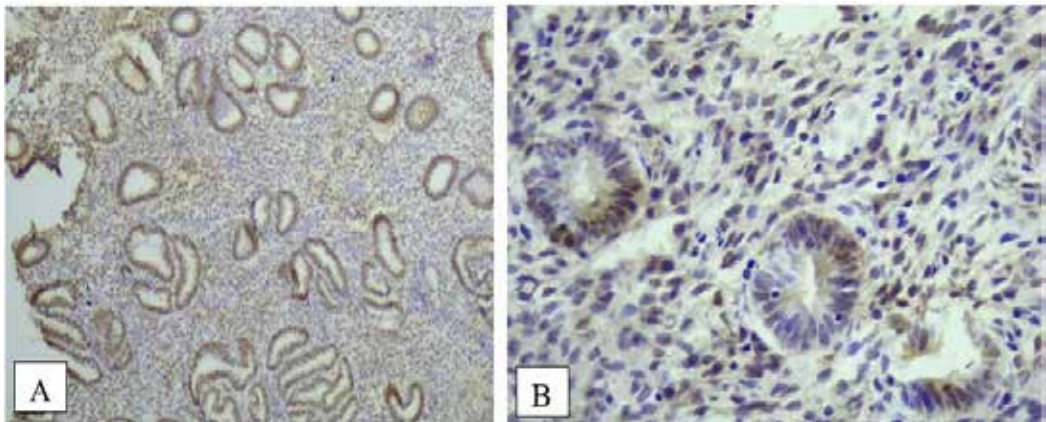


Fig. 3. A – hyperexpression of PR in the epithelium of endometrial glands and in the stroma (magnification $\times 200$). B – focal moderate expression of PR in endometrial glands (magnification $\times 400$)

The mean level of stromal expression in the pretreatment group was $39.42 \pm 3.41\%$ with a range from 0% to 73% and $35.26 \pm 3.78\%$ in the epithelial component of the endometrium with maximum expression value of 67%. After treatment, the average level of marker expression in stroma was $16.98 \pm 2.44\%$, in epithelium – $15.22 \pm 2.51\%$. There is a significant decrease in PR expression in both the stromal ($p < 0.0001$) and epithelial ($p < 0.0001$) components, as shown in Figures 4A and 4B, respectively.

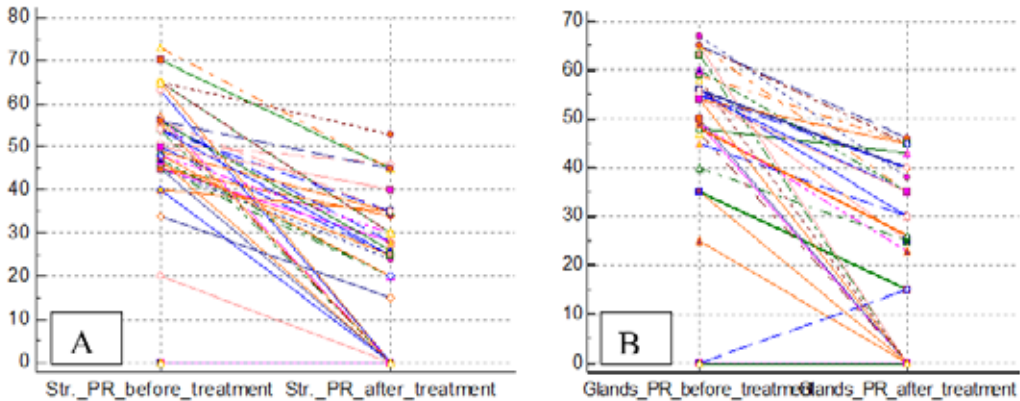


Fig. 4. A – a tendency to decrease the expression of PR in stromal component of endometrium after treatment. B – a tendency to decrease PR expression in epithelial component of endometrium after treatment

In the course of the study, it was also found that after treatment, the characteristic level of PR expression in the epithelial component is within 0–50% ($\chi^2 = 25.69$, $p < 0.0001$), while no threshold value was found for the stromal component ($\chi^2 = 0.08$, $p = 0.77$).

When comparing results of our work with other researchers, it was found that a similar conclusion regarding a decrease in the level of ER and PR expression in women with treated chronic endometritis was reached in their work by Kiran Mishra and co-authors (*Mishra, 2008*) and other researchers (*Kazachkov, 2019; Mylonas, 2005*). That is, in the inflammatory process in the endometrium, hyperexpression of both markers was noted, while in other pathologies, in particular, in the thinning of the endometrium, there was a decrease in the expression level of the markers (*Gao, 2019*).

4. Conclusions

In the course of the work, it was found that the study of the receptivity of the endometrium using expression of ER and PR markers is undoubtedly important for monitoring the quality of treatment and assessing the possibility of pregnancy. In the case of successful treatment of chronic endometritis, a decrease in the expression of both markers should be observed compared to primary endometrial tissue samples, while it is possible to judge the characteristic level of expression of PR in the epithelium in the range of 0-50%, while the level of expression of ER remains high (over 50%) and it can be argued only about a relative decrease in the expression of the marker both in the epithelial and in the stromal component of the endometrium.

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