

DOI: <https://doi.org/10.34921/amj.2024.3.013>**ENDOMETRIUMUN HİPERPLASTİK PROSESLƏRİNİN KLİNİK-MORFOLOJİ VƏ
GENETİK XARAKTERİSTİKASI****N.L.Sindrenko, M.S.Lındin, K.O.Sikora, A.V.Şamray,
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Məqalədə endometriumun hiperplastik proseslərinin (EHP) müxtəlif histoloji variantlarının klinik, morfoloji və genetik xarakteristikası haqqında məlumat verilmişdir. Cərrahi müdaxilədən sonra histeroresektoskopiyanın köməyi ilə alınmış toxuma nümunələrində α -estrogen reseptorlarının ($ER\alpha$) və tsiklooksigenaza-2-nin (COX-2) nə dərəcədə yayıldığı immunohistokimyəvi üsulla analiz edilmişdir. Zəncirşəkilli polimeraza reaksiyası (ZPR) vasitəsilə məhdudlaşdırıcı ferment zəncirlərinin uzunluğu və ESR1 (rs2234693) geninin PvuII polimorfizmi öyrənilmişdir.

Epitelial komponentdə estrogenin (ER) mezenximal komponentdəkinə nisbətən əhəmiyyətli dərəcədə artıq ($p < 0,05$) olduğu müşahidə edilmişdir. Lakin endometrial hiperplastik proseslərin (EHP) müxtəlif histoloji variantları arasında ER-in yayılması baxımından ciddi dəyişikliyə rast gəlinməmişdir. EHP əlamətləri olan bütün toxuma nümunələrində COX-2-nin eyni səviyyədə olduğu müşahidə edilmişdir. Tədqiqat göstərmişdir ki, ESR1 geninin PvuII polimorfizmi ilə EHP arasında assosiasiya əlamətləri, həmçinin onun konkret histoloji yarımtipləri ilə ER- α və COX-2 səviyyəsi arasında assosiativ əlaqə yoxdur.

Açar sözlər: tsiklooksigenaza-2, endometriumun hiperplaziyası, endometrium polipləri, estrogen reseptorları, endometriumun hiperplastik prosesləri, PvuII polimorfizmi

Ключевые слова: циклооксигеназа-2, гиперплазия эндометрия, полипы эндометрия, рецептор эстрогена, гиперпластические процессы эндометрия, PvuII полиморфизм

Key words: cyclooxygenase-2, endometrial hyperplasia, endometrial polyps, estrogen receptor, hyperplastic processes of the endometrium, PvuII polymorphism

**CLINICAL-MORPHOLOGICAL AND GENETIC CHARACTERISTICS OF
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Summary. The article presents data on the clinical, morphological, and genetic characteristics of endometrial hyperplastic processes (EHP), which involve different histological variants.

Tissue samples of ninety-five patients obtained post-surgery via hysteroresectoscopy underwent immunohistochemical analysis for estrogen receptor alpha ($ER\alpha$) and cyclooxygenase-2 (COX-2) expression. Polymerase chain reaction (PCR) followed by restriction fragment length analysis (RFLP) was employed to investigate the PvuII polymorphism of the ESR1 gene (rs2234693).

Significantly elevated estrogen (ER) expression was noted in the epithelial component compared to the stromal component ($p < 0.05$). However, no significant differences were observed in ER expression among different histological variants of EHP ($p > 0.05$). COX-2 expression was uniformly detected in the epithelial component of all EHP tissue samples. Notably, no association was found between the PvuII polymorphism of the ESR1 gene and EHP development, nor with specific histological subtypes or expression levels of $ER\alpha$ and COX-2.

Introduction

Endometrial hyperplastic processes (EHP) are characterized by non-invasive abnormal

proliferation of the endometrium, associated with the risk of progression to endometrial cancer [1]. The latter is one of the most

common malignant gynecological diseases in developed countries [2]. EHP are morphologically manifested in the uterus by endometrial hyperplasia (EH) and endometrial polyps (EP) [3].

The most common symptom in EHP is abnormal uterine bleeding. However, they often have an asymptomatic course [4]. EHP develop against the background of long-term exposure to estrogen, which is not counteracted by progesterone [5]. The action of estrogen is mediated by estrogen receptors (ER), which are expressed in certain tissues, including the ovaries, uterus, cervix and mammary glands. By binding to ER, estrogen induces endometrial cell proliferation. Violations of their expression can cause hyperproliferative processes in the uterus, including EHP [6-7].

ER has two forms: alpha (ER α) and beta (ER β). ER α is encoded by the estrogen receptor alpha gene (ESR1) gene. The ESR1 polymorphism is of great importance regarding the effect of estrogens on the functioning of a female body and may indicate the development of many pathological conditions [8]. One of the most frequently studied is the PvuII (rs2234693) polymorphism of the ESR1 gene, which occurs as a result of the substitution of thymine (T) with cytosine (C) in the first intron of the gene and can change its activity [9], including causing violations and loss of receptor sensitivity to hormones [9]. It was confirmed that the PvuII polymorphism of the ER1 gene is closely associated with tumorigenesis [12].

Cyclooxygenase-2 (COX-2) is involved in the regulation of cell proliferation and apoptosis, as well as cell differentiation [11]. COX-2 is frequently expressed in many types of cancer [12], including endometrial adenocarcinoma [13]. COX-2 expression is present in the immune cells of the endometrial stroma and tumor microenvironment [14,15].

Materials and Methods

We examined 95 patients with a histologically verified diagnosis of EHP at the Sumy Regional Clinical Oncology Center (Sumy, Ukraine) during 2020-2022. The patients provided written informed consent for the examination of tissues and blood samples and the processing of personal data. The study involving human participants was conducted according to the guidelines of the Declaration of Helsinki, reviewed and approved by the Bioethics Committee of Sumy State University.

All patients were divided into three groups depending on the histological variant of EHP. Group 1 included 29 patients with non-atypical endometrial hyperplasia (EH) (30.5%), group 2 included 11 cases with glandular endometrial polyps (GEP) (11.6%), and group 3 included 55 cases with glandular-fibrous endometrial polyps (GFEP) (57.9%).

Immunohistochemical study of ER α and COX-2 expression was performed on tissue samples obtained after surgical treatment (hysterorectoscopy) according to the manufacturer's protocols for antibody staining and visualization system. Immunohistochemical study of ER and COX-2 expression was performed according to the method described in our previous work [16].

Image analysis and archiving were performed using a Carl Zeiss Primo Star microscope with a Zeiss AxioCam ERc 5 s digital camera and ZEN 2 (blue edition) software (Germany).

The research on the PvuII polymorphism of the ESR1 gene (rs2234693) was performed using the polymerase chain reaction method followed by restriction fragment length analysis (PCR-RFLP) [18].

Mathematical data processing was carried out using Microsoft Excel and the "SPSS Statistics 29.0 for Windows" software package. Student's t-test (t), ANOVA method, Pearson's χ^2 test were determined. Results with $p < 0.05$ were considered statistically significant.

Results

When examining the anamnesis of women with EHP, it was found that they were accompanied by ovarian-menstrual cycle disorders, namely hyperpolymenorrhea in 11 cases (11.6%) and algodysmenorrhea in 7 cases (7.4%); insignificant bleeding during menopause in 17 cases (17.9%). In 66 cases (69.5%),

Immunohistochemical characteristics of EHP

Expression of ER α in tissues with EHP in 18 cases (35.3%) was strongly positive in the epithelial and moderately positive in the stromal components; strongly positive in the epithelial and stromal components in 15 samples (29.4%); moderately positive in the epithelial and stromal components in 8 cases (15.7%); moderately positive in the epithelial and weakly positive in the stromal components in 5 cases (9.8%); strongly positive in the epithelial and weakly positive in the stromal components in 3 samples (5.9%); and in 2 cases (3.9%) it was weakly positive in the epithelial and moderately positive in the stromal components.

According to the results of immunohis-

tochemical examination of samples of group 1 (with non-atypical EH), strongly positive expression of ER α in the epithelial and stromal components was observed in 7 cases (47%); strongly positive in the epithelial and moderately positive in the stromal components – in 3 cases (20%); moderately positive in the epithelial and stromal components – in 2 cases (13%); weakly positive in the epithelial and moderately positive in the stromal components – in 2 cases (13%); moderately positive in the epithelial and weakly positive in the stromal components – in 1 case (7%).

The results of ER α expression in group 2 (with GEP) were distributed as follows: most of samples showed strongly positive expression in the epithelial and moderately positive in the stromal components – 4 (57%); in 2 cases (29%) strongly positive reaction was in the epithelial and stromal components; in 1 case (14%) the expression was moderately positive in the epithelial and weakly positive in the stromal components.

In group 3 (with GFEP), the largest number of samples (11 cases (38%)) had a strongly positive expression of ER α in the epithelial and moderately positive in the stromal components; in 6 cases (21%) – strongly positive in the epithelial and stromal components; in 6 cases (21%) – moderately positive in the epithelial and stromal components; in 3 cases (10%) – moderately positive in the epithelial and weakly positive in the stromal components; in 3 samples (10%) there was a strongly positive expression of ER α in the epithelial and weakly positive in the stromal components of the endometrium.

COX-2 expression was detected in the epithelium of all tissue samples with EHP. The average value was 4.22 ± 1.11 points. In the general group with EHP, the majority of cases (50%) exhibited a moderately positive expression. At the same time, in 39% of cases, a strongly positive expression of COX-2 was observed. Weakly positive expression was detected in only 11% of cases. In group 1, the results of COX-2 expression were distributed as follows: the majority of samples were moderately positive – 66%; 17% of cases – with strongly positive and 17% – with mildly positive expression. In the group with GEP, COX-2 expression was strongly positive in 50%

of cases, moderately positive – in 33% of samples, and mildly positive – in 17% of cases. In the group with GFEP, the results of COX-2 expression were equally distributed: in 50% of cases, there was a strongly positive and a moderately positive reaction.

Frequency of the PvuII polymorphism of the ESR1 gene in patients with EHP

As a result of the genotyping of patients with EHP according to the PvuII polymorphism of the ESR1 gene, we determined the frequency of T and C alleles to be 0.56 and 0.44, respectively. When analyzing the distribution of genotype variants according to the PvuII polymorphism of the ESR1 gene in patients with EHP, the following results were obtained: patients homozygotes for the main T/T allele – 30 cases (31.6%), heterozygotes for the T/C allele – 47 cases (49.5%), homozygotes carriers of the minor C/C allele – 18 cases (18.9%). Thus, the T/C heterozygote variant was the most frequent, which was 1.6 times more than the T/T homozygote variant, and 2.6 times more than the C/C homozygote variant.

Connection of morphological changes of the endometrium depending on the genotype according to the PvuII polymorphism of the ESR1 gene in EHP

The T/C heterozygotes predominated among patients with non-atypical EH and GFEP. The homozygotes for the main T/T allele predominated among patients with GEP (5.3%). The lowest number among all histological variants of EHP were patients homozygotes carriers of the minor C/C allele. Calculation of data according to Pearson's χ^2 test showed that the histological variant of EHP does not depend on the presence of the studied PvuII polymorphism of the ESR1 gene ($\chi^2 = 4.14$; $P=0.387$).

Connection of the level of ER α and COX-2 expression with the PvuII polymorphism of the ESR1 gene

Analyzing the degree of ER α expression in the epithelial and stromal components depending on the distribution of allelic variants of the PvuII polymorphism of the ESR1 gene, we found that there is no statistically significant difference in these indicators between the groups ($\chi^2 = 3.84$; $P = 0.428$ and $\chi^2 = 1.47$; $P=0.832$, respectively) (Tables 1, 2).

Table 1. Frequency of allelic variants of the ESR1 gene according to the PvuII polymorphism with different degrees of ER α expression in the epithelial component in EHP

Genotype	The level of ER α expression in the epithelial component, n (%)			Total
	Strong-positive reaction	Moderate-positive reaction	Weak-positive reaction	
T/T	25 (26,2)	5 (5,3)	0 (0)	30 (31,6)
T/C	32 (33,7)	13 (13,7)	2 (2,1)	47 (49,5)
C/C	11 (11,6)	6 (6,3)	1 (1,1)	18 (18,9)
Total	68 (71,5)	24 (25,3)	3 (3,2)	95 (100)
$\chi^2 = 3,84$; $p = 0,428$				

Note: n – number of individuals; p – statistical significance of differences by χ^2 -criterion

Table 2. Frequency of allelic variants of the ESR1 gene according to the PvuII polymorphism with different degrees of ER α expression in the stromal component in EHP

Genotype	The level of ER α expression in the stromal component, n (%)			Total
	Strong-positive reaction	Moderate-positive reaction	Weak-positive reaction	
T/T	11 (11,6)	16 (16,8)	3 (3,2)	30 (31,6)
T/C	15 (15,8)	24 (25,2)	8 (8,4)	47 (49,5)
C/C	5 (5,3)	9 (9,5)	4 (4,2)	18 (18,9)
Total	31 (32,6)	49 (51,5)	15 (15,8)	95 (100)
$\chi^2 = 1,47$; $p = 0,832$				

Note: n – number of individuals; p – statistical significance of differences by χ^2 -criterion

We investigated the relationship of allelic variants of the ESR1 gene by the PvuII polymorphism depending on the different degrees of COX-2 expression (**Table 3**). We found no statistically significant difference in this indicator between the groups ($\chi^2 = 3.8$; $P = 0.434$).

Analyzing the statistical relationship of the average indicators of the expression levels of ER α in the epithelial and stromal components, as well as COX-2, depending on the variants of the PvuII allelic polymorphism of the ESR1 gene, we found no statistically significant difference in these indicators between the groups ($F = 1.48$; $P = 0.233$; $F = 0.17$; $P = 0.845$ and $F = 1.13$; $P = 0.327$, respectively).

Discussion

It is known that EHP can be precursors of

endometrioid adenocarcinoma of the endometrium [1]. ER α is a key mediator of estrogen influence in endometrial tissue [14-15]. At the same time, other proteins are also involved in the pathogenesis of endometrial diseases. Thus, positive expression of COX-2 can be associated with endometritis [11]. These proteins (together with some other biomarkers) can participate in the regulation of development and EHP and be an indicator of the early stages of carcinogenesis [16,17]. This contradicts the previous study, where the asymptomatic course was only in 16.8% of cases in EP. A.K.Elfayomy and B.S.Soliman in their study, which included 150 women with EP, found that 62% had an asymptomatic course [18].

We found a positive ER expression in all examined tissue samples with EHP both in the

Table 3. Degrees of COX-2 expression depending on allelic variants of the ESR1 gene according to the PvuII polymorphism in EHP

Genotype	The level of COX-2 expression, n (%)			Total
	Strong-positive reaction	Moderate-positive reaction	Weak-positive reaction	
T/T	15 (15,8)	14 (14,7)	1 (1,1)	30 (31,6)
T/C	16 (16,8)	28 (29,5)	3 (3,2)	47 (49,5)
C/C	9 (9,5)	7 (7,4)	2 (2,1)	18 (18,9)
Total	40 (42,1)	49 (51,6)	6 (6,3)	95 (100)
$\chi^2 = 3,8$; $p = 0,434$				

Note: n – number of individuals; p – statistical significance of differences by χ^2 -criterion

epithelial and stromal components, which is consistent with the previous study. A higher degree of ER α expression was also observed in the epithelial component compared to the stromal component in each group with EHP [14].

In our study, we found positive COX-2 expression in all tissue groups. It was significantly higher than the indicators of intact tissues [10-12], indicating the possibility of their participation in the dyshormonal transformation of the endometrium. Thus, our study has an important scientific and practical aspect and can contribute to solving the issue of prevention and early diagnosis of these diseases in the local population [19,20].

Conflict of interest

The authors report no conflict of interest.

Conclusions

Currently, there is a tendency towards an increase in the asymptomatic course of endometrial hyperplastic processes, which are characterized by variability in ER and COX-2 expression. At the same time, we did not find a correlation between the PvuII polymorphism of the ESR1 gene and the histological variant of EHP, as well as the degree of ER and COX-2 expression in the epithelial and stromal components.

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КЛИНИКО-МОРФОЛОГИЧЕСКАЯ И ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ГИПЕРПЛАСТИЧЕСКИХ ПРОЦЕССОВ ЭНДОМЕТРИЯ

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Резюме. В статье представлены данные о клинических, морфологических и генетических характеристиках эндометриальных гиперпластических процессов (ЭГП), которые включают в себя разные гистологические варианты.

Тканевые образцы, полученные после хирургического вмешательства с помощью гистерорезектоскопии, прошли иммуногистохимический анализ на выражение рецептора α -эстрогена (ER α) и циклооксигеназы-2 (COX-2). Была использована полимеразная цепная реакция (ПЦР), за которой последовал анализ длины фрагментов ограничения (RFLP), для изучения полиморфизма PvuII гена ESR1 (rs2234693).

Значительно повышенное выражение эстрогена (ER) было отмечено в эпителиальной компоненте по сравнению с мезенхимальной компонентой ($p < 0,05$). Однако существенных различий в выражении ER среди различных гистологических вариантов ЭГП не наблюдалось ($p > 0,05$). Выражение COX-2 было равномерно обнаружено в эпителиальной компоненте всех образцов ткани ЭГП. Замечено, что не было обнаружено ассоциации между полиморфизмом PvuII гена ESR1 и развитием ЭГП, а также с конкретными гистологическими подтипами или уровнями выражения ER α и COX-2.

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