



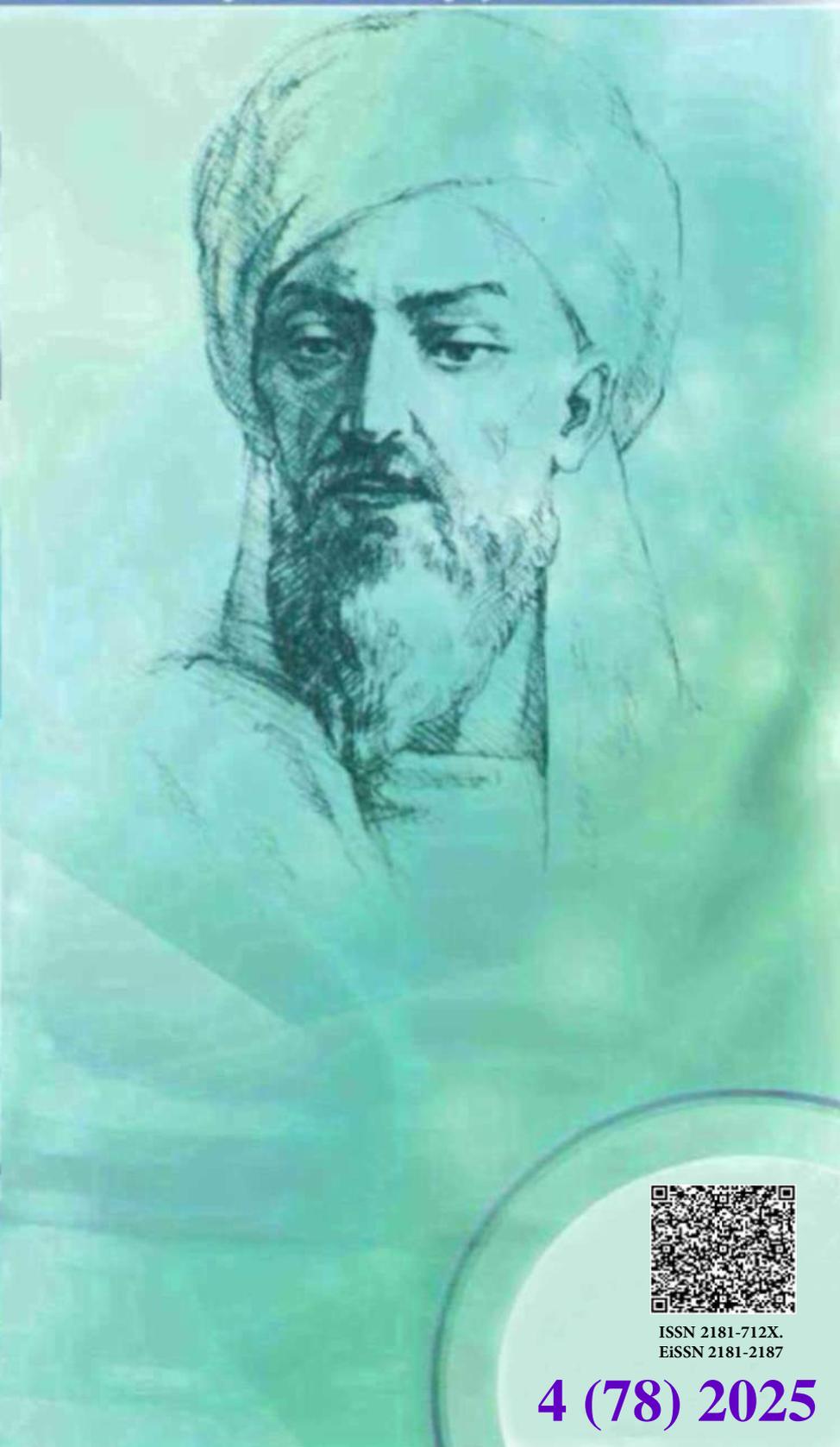
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**ТИББИЁТДА ЯНГИ КУН
НОВЫЙ ДЕНЬ В МЕДИЦИНЕ
NEW DAY IN MEDICINE**

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GENETIC FACTORS OF GENITAL ENDOMETRIOSIS

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✓ Resume

Frequency of occurrence of individual polymorphic genes of estrogen metabolism enzymes CYP1A1 A-4889G (allele G and genotypes GG, AG) and CYP1A2 C-734A (allele A and genotypes CA, AA), cytokine genes IL1B C511T (allele T), IL2 T-330G (allele G), IL4 C-590T (allele T), IL6 G-174C (allele C and genotype CC), IL10 C-592A (allele A and genotypes CA, AA), TNFA G-308A (allele A and genotype AA), TGFB C-509T (allele T and genotype CT) and genes of angiogenesis factors VEGF G-405C (allele C and genotype GC) and G-1154A (allele A and genotypes GA, AA), KDR T-604C (allele C and genotypes TC, CC), Ang2 G-735A (allele A and genotypes GA, AA) in women with external genital endometriosis are higher than in women without endometriosis.

Keywords: polymorphic genes, endometriosis, allele C, allele T, allele G.

ГЕНЕТИЧЕСКИЕ ФАКТОРЫ ГЕНИТАЛЬНОГО ЭНДОМЕТРИОЗА

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✓ Резюме

Частота встречаемости отдельных полиморфных генов ферментов метаболизма эстрогенов CYP1A1 A-4889G (аллеля G и генотипов GG, AG) и CYP1A2 C-734A (аллеля A и генотипов CA, AA), генов цитокинов IL1B C511T (аллеля T), IL2 T-330G (аллеля G), IL4 C-590T (аллеля T), IL6 G-174C (аллеля C и генотипа CC), IL10 C-592A (аллеля A и генотипов CA, AA), TNFA G-308A (аллеля A и генотипа AA), TGFB C-509T (аллеля T и генотипа CT) и генов факторов ангиогенеза VEGF G-405C (аллеля C и генотипа GC) и G-1154A (аллеля A и генотипов GA, AA), KDR T-604C (аллеля C и генотипов TC, CC), Ang2 G-735A (аллеля A и генотипов GA, AA) у женщин с наружным генитальным эндометриозом выше, чем у женщин без эндометриоза.

Ключевые слова: полиморфные гены, эндометриоз, аллель C, аллель T, аллель G.

GENITAL ENDOMETRIOZNING GENETIK OMILLARI

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✓ Rezyume

Estrogen metabolizmining individual polimorf genlari CYP1A1 A-4889G (allel G va genotiplari GG, AG) va CYP1A2 C-734A (allel A va genotiplari CA, AA), sitokin genlari IL1B C511T (barchasi G3-ele), C3-95 (barcha) paydo bo'lish chastotasi T (allel T), IL6 G-174C (alleli C va genotip CC), IL10 C-592A (allel A va genotiplari CA, AA), TNFA G-308A (allel A va genotip AA), TGFB C-509T (alleli T va genotip VEG0C va genotip genotiplari CEG4 va genotiplari) GC) va Tashqi genital endometriozi ayollarda G-1154A (allel A va genotiplari GA, AA), KDR T-604C (allel C va genotiplari TC, CC), Ang2 G-735A (allel A va genotip GA, AA) endometriozi ayollarga qaraganda yuqori.

Kalit so'zlar: polimorf genlar, endometrioz, аллель C, аллель T, аллель G

Relevance

Endometriosis is a genetically determined, chronic, dyshormonal, immune-dependent disease with benign excessive growth of tissue similar in morphological structure and function to the endometrium, outside the uterine mucosa [1,2]. In the structure of diseases of the female reproductive system, it ranks third, including every tenth woman of reproductive age, and is one of the main causes of pelvic pain syndrome and infertility [3,4,5,6].

A wide variety of localizations of endometriosis foci has led to the emergence of many hypotheses about its origin [1,7,8,9]. It is known that more than 90% of women experience retrograde menstruation. At the same time, the prevalence of endometriosis in the general population is 6-10% [2,10,11,12,13]. Such a discrepancy shows that certain genetic factors and factors associated with a violation of the body's homeostasis predispose to the development of the disease are important in the development of endometriosis [1,14,15,16]. From a genetic point of view, endometriosis is a multifactorial disease with a chronic, recurrent course, which occurs due to the complex interaction of a large number of genes with various environmental factors. In recent years, the leading role in the development of this pathology has been given to genetic factors in combination with dysfunction of the main regulatory systems of the body - nervous, endocrine and immune [2,17,18,19]. Heterotopias can arise from embryonic cells or endometrial cells that have retrogradely moved into the abdominal cavity during menstruation. However, for the development of endometrioid implants, activation of the immune response and the development of inflammation are necessary, as well as an increased ability of the endometrioid cells themselves to survive and invade in an atypical environment. This, in turn, is ensured by general hormonal and local growth factors [1,20]. It should be recognized that today the main and most effective method of diagnosing and treating endometriosis is surgical, the purpose of which is the maximum removal of all visible and palpable foci [2,21]. At the same time, the surgical method of treatment does not always ensure the complete elimination of endometrioid foci and does not prevent the recurrence of the disease [1]. In this regard, modern tactics in the treatment of patients with endometriosis implies a combination of the surgical method and hormone-modulating therapy [2,22]. Hormonal therapy is aimed at creating a hypoestrogenic state in the woman's body during the entire treatment period. Progestogens, antigonadotropins, combined oral contraceptives and gonadotropin-releasing hormone agonists are most often used in hormonal therapy of GE [1]. However, it should be noted that no drug eliminates the morphological substrate of endometriosis, but only has an indirect effect on its activity [2]. The absence of a long-term clinical effect after the end of hormone therapy served as an incentive to search for genetic markers of immunological and hormonal imbalance in endometriosis. Determination of the role of cytokines, growth factors and enzymes of hormone and xenobiotic metabolism in the mechanisms of endometrioid foci formation has seemed to be the most promising in recent years. It is known that their production is genetically determined and is determined by single nucleotide polymorphism, the study of which allows assessing the individual risk of disease development, as well as determining individual sensitivity to drugs [21]. At the same time, the results of studying the polymorphism of genes involved in the pathogenesis of endometriosis are not systematized and are contradictory. A relationship has been established between the concentration of IL-1 and the stage of endometriosis [22]. At the same time, increased levels of tumor necrosis factor (TNF) α in peritoneal fluid have been found in endometriosis [2]. At the same time, data on the role of genetically determined variability of individual characteristics of the response of the immune cell system in the development of endometriosis are presented fragmentarily, and the results obtained to date are contradictory. Peritoneal macrophages produce proliferotropic and angiogenic growth factors (EGF – epidermal growth factor, VEGF – vascular endothelial growth factor, TGF- β – transforming growth factor β), the concentration of which in the peritoneal fluid positively correlates with the degree of spread and severity of clinical manifestations of endometriosis [9]. Of the many proangiogenic factors involved in physiological and pathological angiogenesis, VEGF is the most important mediator of vascular endothelial growth. The development of endometriosis is closely associated with the creation of perfusion conditions necessary to ensure hyperproliferation of endometrioid heterotopias and the formation of connective tissue [7]. The assumption about the direct participation of ectopic endometrioid cells in proliferative processes and disease progression should be considered fair [1].

A significant contribution to the development of endometriosis is made by estrogen metabolism, carried out by a group of enzymes of the cytochrome P450 (CYP) family and thermostable sulfotransferases SULT1A1 and SULT1E1, with the formation of biologically inactive metabolites -

estrogen sulfates [2]. Changes in the production or activity of these enzymes can lead to an increase in the concentration of estrogens and the accumulation of their metabolites (hydroxyestrogens), which have a proliferative effect [8]. Hyperestrogenism in combination with dysfunction of immune surveillance factors and vascularization of the resulting endometrioid foci is an important condition for the development of endometriosis.

Thus, the current principles of diagnosis and treatment of endometriosis need to be optimized. In addition, the establishment of molecular genetic markers of endometriosis can become the basis for creating a diagnostic panel of predictors of the effectiveness of pathogenetic based treatment.

The above in combination determines the relevance of the planned study based on the methodology of an integrated approach to solving the problem of fundamental justification of new tactics in the treatment of GE.

Purpose of the study: Improving the diagnosis of genital endometriosis based on markers of apoptosis and regulators of angiogenesis in women of reproductive age.

Material and methods

To achieve the set goal and solve the problems, 116 women aged 18 to 49 years were selected, in whom genital endometriosis was detected during laparoscopy, or there was no organic pathology, who signed informed consent to participate in the study.

The study was conducted using a specially developed comprehensive questionnaire, including information on age, social status, life history and disease, and the results of the diagnostic search. All examined women were divided into 2 groups: the main group and the control group.

The main group included 116 patients suffering from genital endometriosis.

The criteria for including women in the study were:

- reproductive age (18-49 years);
- diagnosis of "genital endometriosis", confirmed laparoscopically and histologically;
- informed consent of the woman to participate in the study. The division was carried out retrospectively, depending on the stage of genital endometriosis, determined using the R-AFS classification (Revised Classification of American Fertility Society, 1985).

The control group consisted of 32 women in whom organic pathology was not detected during laparoscopy. Indications for laparoscopy: surgical sterilization of women with realized reproductive function.

The exclusion criteria for both groups were:

- age under 18 and over 49 years;
- other pathology of the pelvic organs (inflammatory diseases in the acute phase, uterine myoma, endometrial hyperplastic processes, functional cysts and ovarian cystomas, adenomyosis);
- fibrocystic disease of the mammary glands;
- anomalies in the development of the genital organs;
- male factor infertility;
- severe extragenital diseases in the decompensation stage;
- oncological diseases;
- chromosomal diseases;
- woman's refusal to continue the study.

In patients of the main group, the surgical material after laparoscopy was subjected to histological examination. All women had their medical history studied, general and gynecological (bimanual) examinations, and instrumental examinations were performed.

During the gynecological examination, attention was paid to the condition of the external genitalia and vagina, cervix and cervical canal, uterus and its appendages (size, consistency, mobility, soreness), parametrium and adjacent organs.

When studying the medical history, the duration of the disease, the hereditary nature of endometriosis, and previous surgical interventions on the pelvic organs were taken into account. The menstrual cycle was analyzed taking into account the age of menarche, the duration and volume of blood loss, and soreness.

Reproductive function was assessed by the number of pregnancies, their course, outcome, presence of complications, characteristics of childbirth and the postpartum period. The clinical manifestations of

genital endometriosis were determined and pain intensity was assessed according to the scale of C.M. MacLavery, R.W. Shaw (1995).

Results and discussion

The obtained frequencies of the studied polymorphic variants of the genes were tested for compliance with those expected under Hardy-Weinberg equilibrium. In women with endometriosis, the distribution of genotypes ($\chi^2=35.79$; $p<0.001$; $\phi =3.2^*$; $p<0.001$) and alleles ($\chi^2=37.52$; $p<0.001$) of the A-4889G polymorphism of the CYP1A1 gene differed significantly from the control group. Among patients with GE, there was an increased frequency of the G allele (24.7%), homozygous genotype GG (8%), heterozygous genotype AG 143 (33.5%) and a decreased frequency of the homozygous genotype AA (58.6%) compared to the control group (5.1, 0.9, 8.4 and 90.7%, respectively). In female patients carrying the GG and AG genotypes, the risk of developing GE was 9.18 and 5.48 times higher, respectively, than in women without GE. Carrying the G allele of the A-4889G polymorphic region of the CYP1A1 gene also predisposed to GE (OR = 6.05), while the AA genotype (OR = 0.15) had a protective effect on the development of the disease. When conducting a molecular genetic examination during the analysis of the prevalence of the C-734A polymorphism of the CYP1A2 gene in women with GE, a statistically significant ($p < 0.05$) decrease in the frequency of the CC genotype (71.3%) was revealed, as well as an increase in the incidence of the CA (21.92%) and AA (6.8%) genotypes compared to the group of women without GE (89.7, 9.3 and 0.9%, respectively). Comparison of the frequency of occurrence of the allele A also showed a statistically significant ($p<0.05$) increase in the main group (17.7%) compared to the control group (5.6%). According to the results of the OR calculation, it was revealed that carriage of the allele A (OR=3.63), genotypes CA (OR=2.72) and AA (OR=7.70) predisposes to GE, and the homozygous genotype CC of the C-734A polymorphism of the CYP1A2 gene, on the contrary, causes a protective effect on the development of the disease (OR=0.28). According to the results of the study, the homozygous genotype for the G allele of the G-638A polymorphism of the SULT1A1 gene in patients with endometriosis (82.1%) and women without this pathology (89.7%) occurred with almost the same frequency. The distribution frequencies of alleles and genotypes of the polymorphic variant G-638A of the SULT1A1 gene did not have statistically significant differences ($p> 0.05$).

Analysis of the distribution of alleles and genotypes of the C-174T polymorphism of the promoter region of the SULT1E1 gene also did not reveal statistically significant differences between the control and main groups of examined women ($p> 0.05$).

The homozygous genotype CC was found in 80% of cases, while the genotypes CT and TT were detected less frequently.

Thus, the carriage of the G allele and the AG and GG genotypes of the A-4889G polymorphism of the CYP1A1 gene, as well as the A allele and the CA and AA genotypes of the C-734A polymorphism of the CYP1A2 gene predisposes women to the development of GE. The protective effect against the development of the disease is possessed by: genotype AA of the A-4889G polymorphism of the CYP1A1 gene; genotype CC of the C-734A polymorphism of the CYP1A2 gene.

For a comprehensive assessment of the influence of polymorphic variants A-4889G of the CYP1A1 gene, C-734A of the CYP1A2 gene, G-638A of the SULT1A1 gene and C-174T of the SULT1E1 gene on the development of GE, we conducted an analysis of the prevalence of their combinations. The study resulted in the identification of genotype combinations predisposing to the development of the disease:

CYP1A1AG/CYP1A2CC/SULT1A1GG/SULT1E1CC (in 40 (15.9%) women with GE and in 8 (7.4%) women without endometriosis) ($\chi^2=4.03$; $p=0.045$) (OR=2.37).

When conducting a comparative analysis of the distribution of alleles and genotypes of cytokine gene polymorphisms in women with GE, we found an increased frequency of the T allele (in 21.7%) of the C511T polymorphism of the IL1B gene ($\chi^2=5.68$; $p=0.020$) and the G allele (in 32.5%) of the T-330G polymorphism of the IL2 gene ($\chi^2=5.53$; $p=0.019$) compared to the control group, the carriage of which predisposed to the development of GE (OR=1.70 and OR=1.58, respectively).

When assessing the distribution of genotypes of the T-330G polymorphism of the IL2 gene in women of the main and control groups, no statistically significant differences were found, while the TT genotype of the C511T polymorphism of the IL1B gene was determined only in women with GE (in 10.8% of cases).

When studying the C-590T polymorphism of the IL4 gene in women without endometriosis, the prevalence of the CC genotype (in 70.1%) over the CT genotype (in 29.9%) was revealed, while the homozygous genotype for the T allele was not detected at all.

In women with GE, the CC genotype was also predominant (in 51%), and the TT genotype was detected in 11.6% of cases. Comparative evaluation of the frequencies of genotypes and alleles of the C-590T polymorphic region of the IL4 gene in women with endometriosis revealed a decrease in the frequency of the CC genotype ($\chi^2=18.40$; $p<0.001$) and an increase in the frequency of the T allele (30.3%) ($\chi^2=11.85$; $p=0.001$). A positive association of GE with the T allele (OR=2.47) of the C-590T polymorphism of the IL4 gene was shown. The CC genotype (OR=0.44), on the contrary, had a protective effect on the development of GE. During the analysis of the prevalence of the G-174C polymorphism of the IL6 gene in women with GE, a statistically significant ($\chi^2=13.67$; $p<0.001$) decrease in the frequency of the GG genotype (in 59.4%), as well as an increase in the frequency of the CC genotype (in 13.2%) ($\phi=4.7$; $p<0.001$) was recorded compared to the group of women without GE (respectively, in 72 and 0.9%). Comparison of the frequency of the C allele also showed its increase in the main group (in 26.9%, $p<0.001$) compared to the control group (in 14.5%). Based on the OR calculation results, it was found that carriage of the C allele (OR=2.17) and the CC genotype (OR=16.05) predisposes to the development of GE, while the homozygous GG genotype of the G-174C polymorphism of the IL6 gene causes a protective effect against the disease (OR=0.57).

When studying the frequency of occurrence of the C-592A polymorphic region of the IL10 gene, it was found that in women with GE, the frequency of the A allele of this polymorphism (in 32.9% of patients; $\chi^2=74.60$; $p<0.001$) and its CA and AA genotypes (in 35.5% and 15.1% of patients, respectively; $\phi=7.7$ and $\phi=5.2$ ($p<0.001$)) was significantly higher compared to those in the control group. Their positive association with GE was revealed (OR=16.97 for allele A, OR=14.15 for genotype CA and OR=18.91 for genotype AA). The CC genotype of the C-592A polymorphism of the IL10 gene was significantly more common ($\chi^2=68.02$; $p<0.001$) in women of the control group (in 95.3%) and had a protective effect on the development of the disease (OR=0.05).

Conclusion

Before performing laparoscopy in patients with genital endometriosis, it is recommended to determine the combinations of genotypes of polymorphic variants of the genes of estrogen metabolism enzymes CYP1A1AA/CYP1A2CC/SULT1A1GA/SULT1E1CC, cytokines IL1BCC/IL4CC/IL6GG/IL10CA/TGFBC and angiogenesis factors VEGF405GG/KDRCC/Ang2GG and VEGF405CC/KDRTC/Ang2AA to predict the stage of disease spread and plan the scope of surgical intervention.

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