ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

DOI: https://doi.org/10.26565/2617-409X-2023-12-01
UDC 618.14-002.28

D. A Khaskhachykh [A, B, C, D], **V. O.Potapov** [E, F]
O. V. Poslavska [B,C] **I. S. Garagulia** [C]
docdhas@gmail.com

STUDY OF IMMUNOHISTOCHEMICAL MARKERS IN RECURRENCE OF ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA IN WOMEN OF REPRODUCTIVE AGE AFTER TREATMENT WITH PROGESTINS

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

ABSTRACT. The high frequency of hyperplastic processes of the endometrium (EH), the lack of proper effectiveness of hormonal therapy, and the possibility of their malignancy place EH among the most relevant issues in modern medicine. The clinical significance of EH lies in the fact that they are one of the most common causes of uterine bleeding and hospitalization of women. It is known that along with hormonal disorders, other activators of proliferative activity, such as growth factors, proliferation and apoptosis markers, and extracellular matrix components, play a significant role in the developmentofEH.

The study investigated the immunohistochemical markers in the endometrial tissue of reproductive-aged women with endometrial hyperplasia without atypia who were diagnosed with recurrent hyperplasia without atypia after 6 months of continuous therapy with a daily dose of 200 mg of progesterone. The following markers were selected for the study: PR, ER, p21, dcl-2, KI-67, eNOS, cycl-D1, BAX, b-catenin, E-cadgerin i Caspasa3, whose expression was examined by immunohistochemical methods before and after therapy. The control group consisted of women with secretory changes in the endometrium. The expression of receptors for PR, ER, p21, dcl-2, KI-67, eNOS, cycl-D1, BAX, b-catenin, E-cadgerin i Caspasa3 was studied primarily in women with non-neoplastic endometrial lesions (hyperplasia without atypia) and may be of greater significance in predicting the risk of progression and recurrence.

Objective. The aim of the study was to determine changes in the expression of immunohistochemical markers in the endometrium in cases of hyperplasia without atypia before and after the use of progesterone therapy, and to identify the most predictive markers for therapy response.

Results. The histological examination revealed significant changes in the endometrial biomarkers after therapy in women with no response to the treatment. The expression of receptors in the endometrium after therapy showed the following indicators: **ER:** a 20 % increase in glandular cells compared to baseline and a 63.3 % increase compared to the control group. In stromal cells, there was a 63.3% increase compared to baseline. **PgR**: An 85 % decrease compared to baseline. An 85 % decrease compared to the control group. **p21**: A 114 % increase in glandular cells compared to baseline and a 5% increase in stromal cells. Overall, there was a 29.3 % increase in expression compared to the control group. **bcl-2**: An 80 % decrease compared to baseline in glandular cells and a 90 % decrease compared to baseline in stromal cells. **Ki-67**: A 114 % increase compared to baseline in glandular cells and an expression more than 67% higher than the control group. **eNOS**: A 69 % increase compared to baseline in glandular cells and an 85% increase compared to baseline in stromal cells. Cyclin D1: A 15% increase compared to baseline in both glandular and stromal cells. **BAX**: A 10 % increase compared to baseline in both glandular and stromal cells. **Beta-catenin:** Remained stable compared to baseline in both glandular and stromal cells. **E-cad**: A 50% increase compared to baseline in glandular cells and a 60% increase compared to baseline in stromal cells. **Caspasa3**: Showed a 76 % increase compared to baseline and an 80 % increase after therapy in stromal cells, which may be associated with increased apoptosis processes.

Conclusions. 1. Markers ER, PgR, b-catenin, p21, cyclin D1, Ki-67, Caspase-3 demonstrated differences between the non-glandular endometrium (EH) group and the control group in the glandular component, and ER, PgR, b-catenin in the stromal component (all $p<0.05$). This provides a basis for their use as primary diagnostic markers. 2. Markers ER, b-catenin, p21, cyclin D1, Ki-67, eNOS showed differences between the NGE group after treatment and the control group in the glandular component, and ER, b-catenin, and eNOS in the stromal component (all $p<0.05$). This supports their use as primary diagnostic markers. 3. Markers PgR, Ki-67, Caspase- 3, eNOS demonstrated differences between the NGE group before therapy and the control group in the

glandular component, and eNOS in the stromal component (all p<0.05). This indicates their potential as primary diagnostic and prognostic markers. 4. Bcl-2 and BAX markers did not show statistically significant differences in the study groups, suggesting their inability to be used individually as diagnostic or prognostic markers for endometrial hyperplastic processes. Interpretation of the expression results of these markers should consider them in conjunction with other indicators.

Key words: *endometrium, receptors, endometrial hyperplasia, immunohistochemistry, receptors, resistance to progesterone, relapse*

For citation: Khaskhachykh DA, Potapov VO, Poslavska OV, Garagulia IS. STUDY OF IMMUNOHISTOCHEMICAL MARKERS IN RECURRENCE OF ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA IN WOMEN OF REPRODUCTIVE AGE AFTER TREATMENT WITH PROGESTINS. Actual problems of modern medicine. 2023;12:3-13. DOI: https://doi.org/10.26565/2617-409X-2023-12-01

Information about author
Dmytro Anatolievich Khaskhachikh, PhD, Associate Professor of the Department of Obstetrics and Gynecology at Dnipro State Medical University, 9 Volodimira Vernadskoho str., Dnipro, Ukraine, 49044; e-mail: docdhas@gmail.com; ORCID ID: http://orcid.org/ 0000-0001-5097-6667
*Valentin Oleksandrovich Potapov***,** MD, Professor of the Department of Obstetrics and Gynecology at Dnipro State Medical University, 9 Volodimira Vernadskoho str., Dnipro, Ukraine, 49044; e-mail: potapov250352@gmail.com; ORCID ID: https://orcid.org/0000-0001-7498-7416
Olesandra Volodimirovna Poslavska, MD, Associate Professor of the Department of Morfology at Dnipro State Medical University, 9 Volodimira Vernadskoho str., Dnipro, Ukraine, 49044; e-mail: alexandraposlavskaya@gmail.com, ORCID ID: https://orcid.org/0000-0002-3133-8413
Irina Stepanivna Garagulia, Phd , Associate Professor of the Department of Obstetrics and Gynecology at Dnipro State Medical University, 9 Volodimira Vernadskoho str., Dnipro, Ukraine, 49044; e-mail: nataliyadubenchuk@gmail.com, ORCID ID: https://orcid.org/0009-0000-0256-7658

Introduction

Endometrial hyperplasia (EH) is a condition of the endometrium characterized by excessive proliferation of glandular and stromal components, often clinically manifesting as abnormal uterine bleeding. EH has clinical significance, especially in women of reproductive age, and the reversal of hyperplasia to normal endometrium is a key goal of conservative treatment, crucial for preventing EH recurrence and progression to adenocarcinoma. Currently, cyclic progesterone therapy in a prolonged regimen is an effective method of treating EH without atypia, significantly improving the effectiveness of reversing the endometrium compared to expectant management [1]. However, definitive treatment standards for EH have yet to be established. This is because there are increasing reports of a certain percentage of treatment failures in EH using pathogenetically justified progesterone therapy. Such failure rates can exceed 20%, leading to recurrences or even disease progression [3-6]. An unresolved issue at present is why pathogenetically justified therapy is sometimes ineffective for non-atypical endometrial hyperplasia.

Primarily, the treatment of endometrial hyperplasia should be aimed at controlling symptoms such as heavy bleeding and the associated anemia, preventing recurrences, and halting the progression to endometrial cancer (EC) [7]. However, the risk factors predicting recurrence and progression EC of in women with endometrial hyperplasia are inadequately studied. Therefore, this study was directed towards investigating molecular markers and factors that influence the recurrence of endometrial hyperplasia, leading to decreased sensitivity to progestin therapy.

In reality, most EH without atypia are benign proliferations due to continuous exposure to estrogen, whereas atypical endometrial hyperplasia and EC are neoplastic lesions characterized by specific underlying mutations [11].

For this reason, there has been a growing interest in recent years in studying clinical, visual, histological, and molecular factors that may influence treatment outcomes [12]. Immunohistochemistry, which is the most commonly used tool for assessing tissue markers for diagnosis, prognosis, and treatment of various diseases [13], has played a crucial role in this field. Although a large number of immunohistochemical markers have been evaluated, their utility remains unclear. The expression of receptors for some markers typically changes in different forms of EH with atypia/EH compared to EH without atypia [14,15]. In some studies, it is reported that the expression of sex hormone receptors and molecular markers of intercellular interaction can be used in predicting the recurrence of endometrial hyperplasia and the development of endometrial cancer [8-10]. The study of the expression of receptors for PR, ER, p21, dcl-2,

KI-67, eNOS, cycl-D1, BAX, b-catenin, E-cadgerin i Caspasa3 was mainly conducted in women with neoplastic lesions of the endometrium (EH with atypia and EC) and may be more relevant in women with EH without atypia for predicting the risk of progression and recurrence.

Objective

The aim of this research is to determine changes in the detection of immunohistochemical markers in the endometrium in patients with endometrial hyperplasia without atypia before and after the use of progestins. Specifically, this study focuses on those patients who experienced a recurrence of the condition within 6 months of progestin therapy.

The primary objectives are to identify predictive markers for the response to progestin therapy and to compare the immunohistochemical profile of the endometrium before and after progestin treatment, assessing potential changes in the expression of various markers and evaluating the effectiveness of progestin treatment for endometrial hyperplasia. Additionally, the study aims to establish potential correlations between identified immunohistochemical markers and disease recurrence.

Materials and methods

The study was conducted on morphological material obtained from endometrial diagnostic biopsies of women with abnormal uterine bleeding (AUB) in the gynecological department of City Clinical Hospital No. 9 in Dnipro, between 2020 and 2022. The study design and all methodologies used in the research were approved by the ethics committee of the mentioned institution and complied with the Helsinki Declaration.

Inclusion criteria for the study cohort were as follows: women aged 32 to 45 years (mean age 38.4±2.55), the presence of endometrial hyperplasia without atypia confirmed by histological examination performed in a certified ISO 9001-2000 standard laboratory of pathomorphology and immunohistochemistry at Dnipro State Medical University, using standard techniques. Exclusion criteria included the presence of inflammatory pelvic diseases, uterine and ovarian pathological tumors, uterine endometriosis, severe somatic pathology, any form of endocrinopathy, and metabolic syndrome. The mean body mass index was 27.83±1.96 kg/sm^2.

For immunohistochemical (IHC) analysis, 40 endometrial samples were collected from women with AUB by endometrial scraping and subsequent histological examination of the endometrium. Another set of samples was collected from the same women after six months of therapy for endometrial hyperplasia without atypia, where a recurrent diagnosis of endometrial hyperplasia was made. All women received continuous oral administration of 100 mg micronized progesterone twice daily for 6 months. A follow-up biopsy, with a repeat determination of IHC markers, was performed after 6 months.

The control group comprised 20 women with secretory changes in the endometrium diagnosed in the initial diagnostic study, who did not undergo any therapy and were only observed.

For the research, the following methods were used:

Histological Examination Method: Fixed in formalin and paraffin-embedded tissue samples were obtained from the archive of Dnipro Clinical Hospital No. 9. Paraffin sections of 4-5μm were obtained using a Microtome Microm HM-340 and stained with standard hematoxylin and eosin. Microscopy was performed using a ZEISS "Primo Star" light microscope (objectives ×10, ×20, ×40). Microphotographs were obtained using a Zeiss Primo Star microscope camera - AxioCam ERC 5s with licensed software ZEN 2 blue edition.

Such markers as to ER, PgR, E-cadherin, β-catenin, p21, bcl- 2, BAX, Caspase 3, Cyclin D1, Ki-67, eNOS.

Biomarkers such as ER (estrogen receptors), PgR (progesterone receptors), E-cadherin, β-catenin, p21, bcl-2, BAX, caspase 3, cyclin D1, Ki-67, and eNOS are important in the diagnosis, treatment, and prognosis of cancer.

ER and PgR are receptors for the hormones estrogen and progesterone, respectively. Estrogen and progesterone are female sex hormones that play important roles in the development and function of the female body. ER and PgR are found in many types of tissues, including the breast, uterus, ovaries, and bones.

E-cadherin is a protein that binds cells together. It plays an important role in maintaining tissue structure. E-cadherin is often decreased in cancer cells, which can lead to their proliferation and spread.

β-catenin is a protein that is involved in signal transduction from ER and PgR. β-catenin is often increased in cancer cells, which can lead to their proliferation and spread.

p21 is a protein that inhibits the cell cycle. It plays an important role in preventing uncontrolled cell growth. p21 is often decreased in cancer cells, which can lead to their proliferation.

bcl-2 is a protein that protects cells from death. It plays an important role in regulating apoptosis, the process of cell death. bcl-2 is often increased in cancer cells, which can lead to their survival and spread.

BAX is a protein that promotes cell death. It plays an important role in regulating apoptosis. BAX is often decreased in cancer cells, which can lead to their survival and spread.

Caspase 3 is an enzyme that triggers the process of cell death. It plays an important role in regulating apoptosis. Caspase 3 is often decreased in cancer cells, which can lead to their survival and spread.

Cyclin D1 is a protein that regulates the cell cycle. It plays an important role in regulating cell growth. Cyclin D1 is often increased in cancer cells, which can lead to their proliferation.

Ki-67 is a protein that is expressed in cells that are dividing. It plays an important role in regulating cell growth. Ki-67 is often increased in cancer cells, which can lead to their proliferation.

eNOS is an enzyme that produces nitric oxide. Nitric oxide is a molecule that relaxes blood vessels and reduces inflammation. eNOS is often decreased in cancer cells, which can lead to their proliferation and spread.

These biomarkers are often used in the diagnosis and treatment of cancer. For example, ER and PgR can be used to determine whether cancer is estrogen-sensitive or progesterone-sensitive. E-cadherin and β-catenin can be used to predict the risk of cancer recurrence. p21, bcl-2, BAX, caspase 3, and cyclin D1 can be used to determine the stage of cancer and prognosis. Ki-67 and eNOS can be used to assess the activity of cancer.

Immunohistochemical Examination Method: Paraffin sections were mounted on adhesive slides SuperFrost Plus. After deparaffinization, rehydration, antigen retrieval, and endogenous peroxidase suppression, sections were incubated with primary antibodies in humid chambers. Primary monoclonal antibodies to ER (sp1, RTU), PgR (YR85, 1:200), E-cadherin (EP700Y, RTU), β-catenin (E247, RTU), p21 (sp1, RTU), bcl-2 (EP36, RTU), BAX (sp1, RTU), Caspase 3 (sp1, RTU), Cyclin D1 (EP12, RTU), Ki-67 (sp6, RTU), eNOS (sp1, RTU), and the UltraVision Quanto visualization system (LabVision) were used for the study. To identify the reaction, a solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen (LabVision) was applied under microscope control for 20 seconds to 3 minutes, resulting in brown staining, followed by additional counterstaining with Mayer's hematoxylin for 1-3 minutes. Subsequent dehydration and mounting were performed according to established methods [16].

Following the recommendations of Antunes A. et al. (2014), the expression of ER and PR was assessed in the stroma and glandular epithelium of endometrial tissues using a semi-quantitative method of nuclear reaction by analyzing the percentage of stained cells, the intensity of nuclear staining, and the final evaluation. The calculation was done in 100 cells from different fields of view under a ×40 objective. The percentage of stained cells was visually evaluated and classified as follows: grade 0, no staining; grade 1, <1% staining; grade 2, 1-10% staining; grade 3, 11-33% staining; grade 4, 34-66% staining; and grade 5, >66% staining [17].

Following the recommendations of Ahmed R. H. et al. (2014), the level of expression of E-cadherin and β-catenin was measured using a scale that combines the intensity of immunoreactions with the percentage of positive cells. Cells present in four high-power fields at ×400 magnification were counted and evaluated in each case. The intensity of immunoreactions was indicated as negative, weakly positive, moderately positive, or strongly positive. These four categories were weighted as 0, 1, 3, and 10, respectively. The final score was calculated by multiplying the intensity of immunoreaction by the percentage of positive cells [18].

According to the recommendations of Peiró G. et al. (2001), the expression of oncoproteins bcl- 2, BAX, and caspase-3 was assessed using a semi-quantitative evaluation based on intensity and staining degree: (0) - no immunostaining, (1+) for weak positive staining (low or faint), (2+) - moderate staining found in some cells, and (3+) - strong positive staining. Immunostaining results were evaluated with respect to the approximate percentage of positive tumor cells (<10 %, 10 % to 50 %, >50 %) and the relative intensity of immunostaining (0, 1+, 2+, 3+), which was present in most or all cells [19].

Following the recommendations of Brucka A. et al. (2009), the expression level of p21 and cyclin D1 was measured quantitatively as the percentage of immunopositive cells among 1000 glandular cells and 1000 stromal cells, expressing the index of immunoreactivity of cell cycle markers in percentages [20].

The Ki-67 proliferation index and eNOS were divided into three groups, including low (Ki-67 ≤ 15 %), moderate (Ki-67, 16-30 %), and high (Ki-67 > 30%) [20, 21].

Statistical analysis of the obtained results was conducted using Microsoft Office 365 A1 for faculty software (product key №1003BFFD8C8E8B0D). Parametric analysis was used. The calculations included the arithmetic mean (M) and standard error of the mean (m). The probability of differences was assessed using the Student's t-test. The chi-square (χ^2) test was employed to compare qualitative characteristics. Differences were considered statistically significant at $p < 0.05$ (95 % level of significance) [22].

Results of the study

Regardless of the initial response to therapy, all women in this study were under long-term observation with an average follow-up time of 12 ± 2 months.

Analyzing each biomarker's detailed relative changes in the glands and stroma of the endometrium in different groups (Figure 1 and Figure 2) before the start of therapy in comparison to the control group:

ER: The level of ER expression in the glands significantly increased by 20% after therapy compared to baseline values and the control group. This indicates a positive response to therapy in the glands, which may be associated with increased sensitivity of the glands to estrogen after treatment.

The level of ER increased by 63.3 % ($p < 0.05$) after therapy in the stroma, which may suggest a positive effect of therapy on the stroma and could be related to greater sensitivity of the stroma to the action of progesterone.

PgR: The level of PgR expression in the glands significantly decreased after therapy, indicating the suppression of the expression of this biomarker. This may be associated with the binding of receptors to progesterone and their inactivation, followed by an increase in their expression. The 85% decrease in PgR ($p<0.05$) may suggest reduced sensitivity of the glands to progesterone after therapy.

The level of PgR decreased to 15 % ($p < 0.05$) after therapy in the stroma, which may indicate changes in the progesterone response of the stroma to therapy.

p21: The level of p21 in the glands increased significantly after therapy compared to baseline values by 114 % and compared to the control group by 29.3 % ($p < 0.05$). This may indicate increased cell proliferation in the glands after treatment.

The level of p21 increased by 5 % ($p \leq 0.05$) after therapy in the stroma, which may suggest increased cell proliferation in the stroma after treatment, by 48 % ($p < 0.05$).

bcl-2: The level of bcl-2 significantly decreased after therapy, both in the glands by 80 % ($p < 0.05$) and in the stroma by 90 % ($p < 0.05$). This decrease in bcl-2 levels may indicate reduced antagonistic activity of this biomarker, which promotes apoptosis.

Ki-67: The level of Ki-67 significantly increased after therapy, both in the glands by 114 % ($p < 0.05$) and in the stroma by 67 % ($p < 0.05$). This suggests increased cellular proliferation after treatment. The level of Ki-67 significantly decreased in the stroma after therapy, indicating reduced cell proliferation in the stroma.

eNOS: The level of eNOS significantly increased by 69 % ($p < 0.05$) after therapy in the glands. This increase in eNOS may be associated with an elevated level of angiogenesis in the glands following treatment.

The level of eNOS significantly increased by 85 % ($p < 0.05$) after therapy in the stroma.

cycl D1: The level of cyclin D1 increased slightly after therapy in the glands by 15 % ($p < 0.05$) and also increased slightly in the stroma by 12 % ($p < 0.05$). These findings suggest changes in the regulation of the cell cycle, potentially indicating alterations in the cell cycle control mechanisms in response to therapy.

BAX: The level of BAX increased by 10 % ($p < 0.05$) after therapy both in the glands and in the strom. This increase in BAX may be associated with an enhancement of apoptosis (programmed cell death) in the glands.

b-cat: The level of β-catenin (b-cat) remained stable in both the glands and stroma after therapy ($p<0.01$).

E-cad: The level of E-cadherin (E-cad) increased after therapy, both in the glands by 50 % ($p < 0.05$) and in the stroma by 60 % ($p < 0.05$). This suggests changes in cellular adhesion in both the glands and stroma in response to therapy.

Caspasa3: The level of Caspase-3 significantly increased after therapy, both in the glands by 76% ($p<0.05$) and in the stroma by 80% ($p<0.05$). This suggests an increase in apoptosis (programmed cell death) processes in both the glands and stroma in response to therapy.

The overall significance of these results is that therapy has a different impact on the glands compared to the stroma of the endometrium. This difference may reflect the complex interaction between the cells of both components during treatment. These data emphasize the need for further study and monitoring of these changes after therapy, as well as the importance of an individualized approach to treatment. Understanding how therapy affects different components of the endometrium can lead to more tailored and effective treatment strategies.

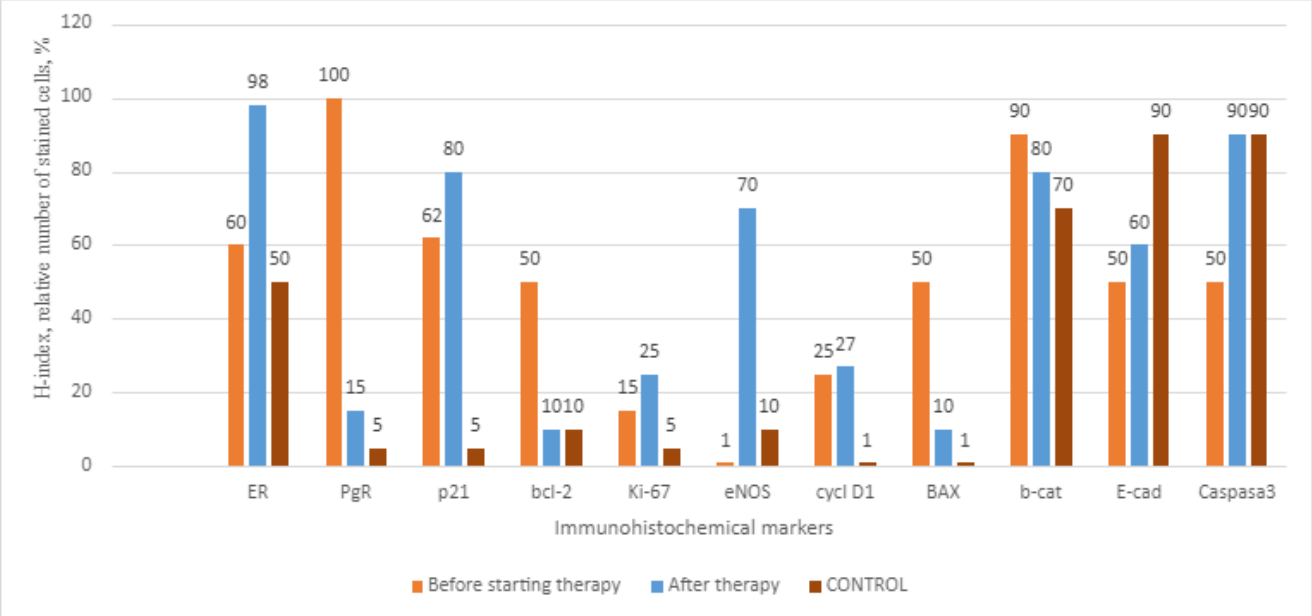

Fig. 1. The level of expression of immunohistochemical markers in endometrial glands Research group (EH) before therapy and after therapy (n=40), control group (n=20)

ISSN 2617-409X Actual problems of modern medicine. Issue 12, 2023

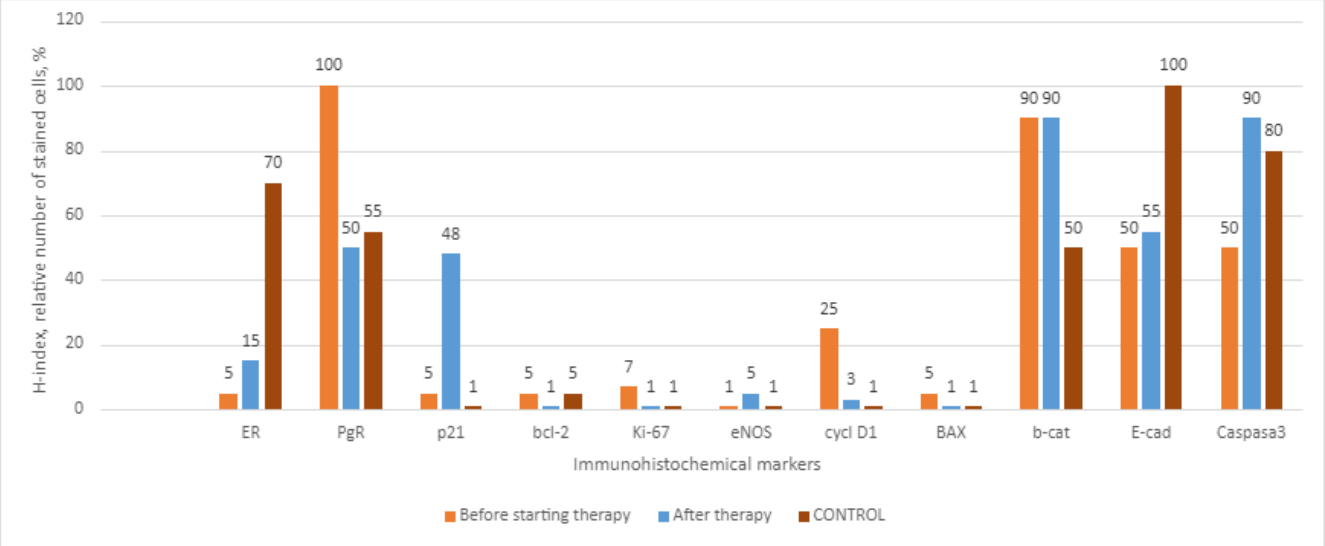

Fig. 2. The level of expression of immunohistochemical markers in the stroma in the absence of an effect from EH progestin therapy Research group (EH) before therapy and after therapy (n=40), control group (n=20)

Based on the analysis conducted, there were observed certain changes in the expression of immunohistochemical markers both in the glands and stroma of the endometrium in the group after therapy compared to the group before therapy.

Overall, the results indicate an impact on the level of biomarkers associated with cell growth, apoptosis, and cell adhesion. These changes may be of interest as indicators of therapy effectiveness and may reflect various factors influencing the cells. Understanding these changes in biomarker expression can provide insights into the response to treatment and potentially guide further therapeutic strategies.

Discussion

In recent years, a large number of immunohistochemical markers have been evaluated in both normal and hyperplastic endometrium. However, their utility for the diagnosis and prediction of EH recurrence remains unclear. The expression of receptors for some markers typically varies in different forms of EH with atypia/EC compared to EH without atypia [13-15]. The expression levels of receptors for PR, ER, and markers such as p21, bcl-2, KI-67, eNOS, cyclin D1, BAX, b-catenin, E-cadherin, and Caspase3 were predominantly investigated in women with neoplastic lesions of the endometrium (EH with atypia and carcinoma) and may be of greater significance in women with EH without atypia, for predicting the risk of progression and recurrence in the next 5 years.

In summary, the role of the expression of various biomarkers in endometrial hyperplasia can lead to the following conclusions:

ER and PgR are steroid hormone receptors that play an important role in regulating the functions of the endometrium. Reduced expression of ER and PgR can contribute to the development of endometrial hyperplasia and endometrial cancer [23]. In our study, EH without atypia and secretory endometrium had a relatively high expression of ER, indicating their ability to respond to hormonal therapy. However, atypical EH partially lost ER expression in the stroma, which may be a sign of changes in estrogen responsiveness and suggest a potential risk of developing endometrial cancer. Regarding PgR, a similar pattern was observed, with secretory endometrium and EH without atypia showing high PgR expression, indicating their ability to respond to progestin therapy. However, non-secretory EH lost PgR expression, especially in the stroma, which may indicate a disruption in progesterone responsiveness and an increased risk of endometrial cancer.

E-cadherin and b-catenin are cell adhesion molecules that play an important role in regulating cell adhesion and invasion. Reduced expression of E-cadherin and increased expression of b-catenin are associated with an increased risk of developing endometrial hyperplasia and endometrial cancer [24]. In our study, secretory endometrium and EH without atypia showed positive membranous expression of E-cadherin in the glands. However, atypical

EH reduced the expression of E-cadherin in both glands and stroma, which may indicate disturbances in cell adhesion and potential tissue instability.

Regarding the b-catenin marker, aberrant expression was observed in EH samples with membranous-cytoplasmic reactivity and excess expression, which may suggest disruptions in the Wnt/b-catenin signaling pathway and potential activation of non-canonical signaling mechanisms.

p21 is an inhibitor of cyclin-dependent kinases and plays an important role in regulating the cell cycle. Reduced expression of p21 is known to be associated with an increased risk of developing endometrial hyperplasia and endometrial cancer [24].

bcl-2 and BAX are proteins that play a crucial role in the regulation of cellular apoptosis. Reduced expression of BAX and increased expression of bcl-2 can contribute to the development of endometrial hyperplasia and endometrial cancer [25].

Furthermore, an increase in the expression level of Ki-67 indicates an increase in the proliferative activity of the endometrium, which may be associated with the development of hyperplasia. Animal studies have shown that estrogen inhibitors can reduce the expression level of Ki-67 and prevent the development of endometrial hyperplasia [26]. Caspase 3 is a key factor in cellular apoptosis, or programmed cell death. This protein promotes the breakdown of cellular proteins, leading to cell death. In the endometrium, reduced expression of Caspase 3 can contribute to excessive proliferation and hyperplasia.

The change in the expression of markers such as p21, Bcl-2 (except for individual cells), BAX (weak expression), Caspase 3 (excessive expression in atypical EH), and eNOS (excessive expression in atypical EH) indicates disruptions in various cellular processes, including regulation of the cell cycle, apoptosis, and vascularization. Moreover, changes in the expression of immunohistochemical markers can influence the development of EH by altering the balance between proliferative and apoptotic cell activity, disrupting the β-catenin signaling pathway, and causing endothelial dysfunction due to eNOS dysfunction. However, research on the mechanisms of endometrial hyperplasia development and the role of these markers in this process is still ongoing and requires further detailed investigation [27].

Immunohistochemical markers such as ER, PgR, E-cadherin, β-catenin, p21, Caspase 3, Cyclin D1, Ki-67, and eNOS can be valuable tools for studying the development of endometrial hyperplasia and endometriosis. Combining these markers can provide more precise information about changes in endometrial cells and their relationship with the development of hyperplastic processes in the endometrium [28].

The study of immunohistochemical markers in the endometrium indicates a complex network of interrelationships among them, which may reflect various mechanisms involved in the development of endometrial hyperplasia and its progression to cancer. Therefore, for a more detailed understanding of these mechanisms, further research involving a larger number of patients and a wider range of markers is needed.

Summarizing the analysis of biomarker changes when using progestagens, the following conclusions can be made:

ER, PgR, p21, Ki-67, cyclin D1, E-cadherin, Caspase3: Provided that these biomarkers increase in response to the positive effect of therapy, it is expected that their values in the "After Therapy" group will be higher compared to the "Before Therapy" group. The control group may have values close to the baseline since they did not receive therapy.

bcl-2, b-cat: If these biomarkers decrease due to therapy, it is expected that their values in the "After Therapy" group will be lower compared to the "Before Therapy" group. The control group may have values similar to the baseline.

eNOS, BAX: The values of these biomarkers may change due to therapy, but the direction of these changes may vary for different individuals or groups. Comparing the "After Therapy" group with the "Before Therapy" group can determine the overall health of the group after therapy.

Ki-67: This is a biomarker of cell proliferation. Typically, lower Ki-67 levels indicate reduced cell division activity after therapy.

Caspase3: This is a biomarker of apoptosis, or programmed cell death. Increased Caspase3 levels may indicate increased cell death as a result of therapy.

Summarizing the data from the conducted study, it can be concluded that therapy has different effects on the glands compared to the stroma of the endometrium, which may reflect a complex interaction between the cells of both components during treatment.

These data emphasize the need for further study and monitoring of these changes after therapy, as well as an individualized approach to treatment.

Conclusions

1. Markers ER, PgR, b-catenin, p21, cyclin D1, Ki-67, and Caspase-3 (all $p<0.05$) demonstrated differences between the non-secretory endometrium group and the control group in the glandular component, as well as ER, PgR, and b-catenin (all $p<0.05$) in the stromal component, providing a basis for their use as primary diagnostic markers.
2. Markers ER, b-catenin, p21, cyclin D1, Ki-67, and eNOS (all $p<0.05$) showed differences between the NSE group after treatment and the control group in the glandular component, and ER, b-catenin, and eNOS (all $p<0.05$) in the stromal component, supporting their use as primary diagnostic markers.
3. Markers PgR, Ki-67, Caspase-3, and eNOS (all $p<0.05$) demonstrated differences between the NSE group before therapy and the control group in the glandular component, and eNOS ($p<0.05$) in the stromal component, indicating their potential as primary diagnostic and prognostic markers.
4. Bcl-2 and BAX markers did not show statistically significant differences between the study groups, suggesting their inability to be used individually as diagnostic or prognostic markers for endometrial hyperplastic processes. Interpretation of the expression results of these markers should consider them in conjunction with other indicators.

Funding Sources: The research was conducted using the authors' own funds.

Conflict of Interest: The authors declare no conflicts of interest**.**

References

1. Ring KL, Mills AM, Modesitt SC. Endometrial Hyperplasia. Obstetrics and gynecology. 2022;140(6):1061-1075. doi:10.1097/AOG.0000000000004989.
2. De Silva PM, Gallos ID. Predicting risk of relapse in endometrial hyperplasia. BJOG: an international journal of obstetrics and gynaecology. 2019;126(7):944. doi:10.1111/1471-0528.15671.
3. Sanderson PA, Critchley HO, Williams AR, Arends MJ, Saunders PT. New concepts for an old problem: the diagnosis of endometrial hyperplasia. Hum Reprod Update. 2017;23(2):232-254. doi:10.1093/humupd/dmw042.
4. Khaskhachykh D, Potapov V. Molecular mechanisms of endometrial hyperplasia and therapy based on the study of receptor expression, cell markers of proliferation, differentiation, and apoptosis of endometrial cells in the hormone-dependent signal path. O Grail of Science. 2022;12-13:620-623. doi:10.36074/grail-of-science.29.04.2022.109.
5. Khaskhachykh D, Potapov V, Poslavskaya O. Molecular criteria for the diagnosis of hormone-resistant forms of endometrial hyperplasia without atypia in women of reproductive age]. Morphologia. 2022;16(3):118- 126. [Ukrainian]. DOI: https://doi.org/10.26641/1997-9665.2022.3.118-126
6. Khaskhachykh D, Potapov V, Kukina G, Garagulya I. Prospective study of the effectiveness of differentiated therapy of endometrium hyperplasia without atypia in women in reproductive age. The Grail of Science. 2021;(9):406-412. https://doi.org/10.36074/grail-of-science.22.10.2021.73
7. Chandra V, Kim JJ, Benbrook DM, Dwivedi A, Rai RJ. Therapeutic options for management of endometrial hyperplasia. Gynecol Oncol. 2016;27(1):88-98. doi:10.3802/jgo.2016.27.e8.
8. McKinnon B, Mueller M, Montgomery G. Progesterone Resistance in Endometriosis: An Acquired Property? Trends Endocrinol Metab. 2018;29:535-548. doi:10.1016/j.tem.2018.05.006.
9. Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: Origins, consequences and interventions. Acta Obstet Gynecol Scand. 2017;96:623-632. doi:10.1111/aogs.13156.
10. Li X, Feng Y, Lin JF, Billig H, Shao R. Endometrial progesterone resistance and PCOS. J Biomed Sci. 2014;21:2. doi:10.1186/1423-0127-21-2.
11. Gromova OL, Potapov VO, Khaskhachykh DA, Finkova OP, Gaponova OV, Kukina GO, Penner KV. Epigenetic profile of endometrial proliferation in different morphotypes of endometrial hyperplasia. Reproductive Endocrinology. 2021;57:68-78.
12. Singh G, Puckett Y. Endometrial Hyperplasia. StatPearls; 2021. PMID: 32809528.
13. Laas E, Ballester M, Cortez A, Gonin J, Canlorbe G, Daraï E, Graesslin O. Supervised clustering of immunohistochemical markers to distinguish atypical and non-atypical endometrial hyperplasia. Gynecol Endocrinol. 2015;31(4):282-285. doi:10.3109/09513590.2014.989981.
14. Singh G, Puckett Y. Endometrial Hyperplasia. StatPearls; 2023. doi:10.2139/ssrn.3835254.
15. Nguyen T. Immunohistochemistry: A Technical Guide to Current Practices. Cambridge: Cambridge University Press; 2022.

16. Antunes A, Vassallo J, Pinheiro A, Leao R, Pinto Neto AM, Costa-Paiva L. Immunohistochemical expression of estrogen and progesterone receptors in endometrial polyps: A comparison between benign and malignant polyps in postmenopausal patients. Oncol Lett. 2014;7(6):1944-1950. doi:10.3892/ol.2014.2004.
17. Ahmed RH, Ahme E, Muhammad MS. E-cadherin and CD10 expression in atypical hyperplastic and malignant endometrial lesions. Journal of the Egyptian National Cancer Institute. 2014;26(4):211-217. doi:10.1016/j.jnci.2014.08.002.
18. Peiró G, Diebold J, Baretton GB, Kimmig R, Löhrs U. Cellular apoptosis susceptibility gene expression in endometrial carcinoma: correlation with Bcl-2, Bax, and caspase-3 expression and outcome. Int J Gynecol Pathol. 2001;20(4):359-367. doi:10.1097/00004347-200110000-00008.
19. Brucka A, Bartczak P, Ratyńska M, Sporny S. Immunohistochemical pattern of protein P21, cyclin D1 and cyclin E in endometrial hyperplasia. Pol J Pathol. 2009;60(1):19-25.
20. Shevra CR, Ghosh A, Kumar M. Cyclin D1 and Ki-67 expression in normal, hyperplastic and neoplastic endometrium. J Postgrad Med. 2015;61(1):15-20. doi:10.4103/0022-3859.147025.
21. Najafi T, Ghaffari Novin M, Pakravesh J, Foghi K, Fadayi F, Rahimi G. Immunohistochemical localization of endothelial nitric oxide synthase in endometrial tissue of women with unexplained infertility. IJRM. 2012;10(2):121-126. URL: http://ijrm.ir/article-1-263-en.html.
22. Khaskhachykh DA, Potapov VO, Kukina GA, Garagulya IS. Prospective study of the effectiveness of differentiated therapy of endometrial hyperplasia without atypia in women in reproductive age. Grail Of Science. 2021;3:4.
23. Berx G, Van Roy F. The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. Breast Cancer Res. 2001;3(5):289-293.
24. Khaskhachikh DA, Potapov VO, Poslavska OV. Factors of resistance to progestin therapy in endometrial hyperplasia in women. Morphologia. 2023;17(1):56-62. doi:https://doi.org/10.26641/1997-9665.2023.1.56-62.
25. Kim J, Cho K, Lee Y, Kim J, Kim Y. Endometrial hyperplasia: molecular pathogenesis and new therapeutic opportunities. Korean J Med Sci. 2020;35(4):e37. doi:10.3346/jkms.2020.35.e37.
26. Sakai K, Yoshida T. Mechanisms of progesterone resistance in endometrial hyperplasia and their impact on pathogenesis and treatment. Biomed Pharmacother. 2020;1(3):44-53. doi:10.1016/j.biopha.2019.109204.
27. Xu L, Hu C, Qiu C, Yang Z, Xu Q. Progesterone resistance and endometrial hyperplasia: latest research and treatment prospects. J Med Sci. 2021;41(2):41-47. doi:10.3779/j.issn.1009-6574.2021.02.01.
28. Zhang Y, Guo H, Cui X, Song X, Wang Y. Molecular mechanisms of progesterone resistance and their clinical significance in endometrial hyperplasia. J Gynecol Obstet. 2022;1(1):15-22. doi:10.31083/j.jgo.2022.01.003

Received: 11.09.2023
Accepted: 09.11.2023

Д. А. Хасхачих [A, B, C, D], **В. О. Потапов** [E, F]
О. В. Послaвська [B,C] **І. С. Гарагуля** [C]
docdhas@gmail.com

ДОСЛІДЖЕННЯ ІМУНОГІСТОХІМІЧНИХ МАРКЕРІВ ПРИ РЕЦИДИВІ ГІПЕРПЛАЗІЇ ЕНДОМЕТРІЮ БЕЗ АТИПІЇ У ЖІНОК РЕПРОДУКТИВНОГО ВІКУ ПІСЛЯ ЛІКУВАННЯ ПРОГЕСТІНАМИ

A – концепція та дизайн дослідження; B – збір та/або збірка даних; C – аналіз та інтерпретація даних; D – написання статті; E – критична доопрацювання статті; F – остаточне затвердження статті

Анотація. Висока частота гіперплазії ендометрію, відсутність належної ефективності від гормональної терапії, а також ймовірність їх озлоякісності ставить гіперплазії ендометрію в ряд найбільш актуальних проблем сучасної медицини. Важливе клінічне значення ГЕ полягає в тому, що вони є однією з найчастіших причин маткових кровотеч та госпіталізації жінок до стаціонару. Відомо, що істотна роль у формуванні ГЕ, поряд з гормональними порушеннями, приділяється іншим активаторам проліферативної активності - факторам росту, маркерам проліферації та апоптозу, компонентам екстрацелюлярного матриксу.

Проведено дослідження імуногістохімічних маркерів в тканині ендометрія у жінок репродуктивноого віку з ГЕ без атипії у яких після проведенної терапії з використанням прогестинів на протязі 6 місяців в безперервному режимі в дозі 200 мг на добу та була знов діагностована ГЕ без атипії. Для дослідження були обрані такі маркери як: PR, ER, p21, dcl-2, KI-67, eNOS, cycl-D1, BAX, b-catenin, E-cadgerin і Caspasa3, експресію яких досліджували імуногістохімічним методом до початку і після проведенної терапії. Контрольну групу склали жінки з секреторними змінами ендометрю. Дослідження експресії рецепторів до PR, ER, p21, dcl-2, KI-67, eNOS, cycl-D1, BAX, b-catenin, E-cadgerin і Caspasa3 досліджувалися в основній мірі у жінок з неопластичними ураженнями ендометрія (ГЕ з атипією і раком ендометрію) і можуть бути цікавими і більш значущими у жінок

з ГЕ без атипії, для прогнозування ризику прогресування і прогнозування рецидивів.

Мета. Метою дослідження стало визначення змін у виявленні імуногістохімічних маркерів в ендометрії при ГЕ без атипії до та після застосування прогестинів, у яких через 6 міс. терапії з застосуванням прогестинів був діагностований рецидив захворювання, для вивлення найбільш прогностичних маркерів щодо прогнозування відповіді на терапію з застосуванням прогестинів.

Результати. За результатами гістологічного обстеження виявлено важливі зміни в біомаркерах ендометрія після терапії у жінок з відсутністю ефекту від проведенної терапії. Експресія рецепторів в ендометрії після проведенної терапії показала наступні показники: **ER:** в залозах відбулося зростання на 20% порівняно зі стартовими значеннями та було збільшене на 63,3% порівняно з групою контролю. В стооЗростання на 63,3% порівняно зі стартовими значеннями в стромі. **PgR**: Зниження на 85% порівняно зі стартовими значеннями. Зниження на 85% порівняно з групою контролю. **p21**: Зростання на 114% порівняно зі стартовими значеннями в залозах та зростання на 5% порівняно з значеннями в стромі. Загальне зростання експресії на 29,3% порівняно з групою контролю. **bcl-2**: зменшення на 80% порівняно зі стартовими значеннями в залозах та зменшення на 90% порівняно зі початковими значеннями в стромі. **Ki-67**: зростання на 114% порівняно з початковими значеннями в залозах та експресія більше на 67% порівняно з групою контролю. **eNOS**: зростання на 69% порівняно з початковим рівнем в залозах та зростання на 85% порівняно з початковими значеннями в стромі. **cycl D1**: Зростання на 15% порівняно зі початковими значеннями як в залозах так і в стромі. **BAX**: Зростання на 10% порівняно з початковими значеннями як в залозах так і в стромі. **b-катенін**: залишився стабільним порівняно з початковими значеннями в залозах і стромі. **E-cad**: зростання на 50% порівняно з початковими значенням в залозах, та зростання на 60% порівняно з початковими значеннями в стромі. **Caspasa3**: виявилося зростання на 76% порівняно з початковими значеннями та 80% після терапії в стромі, що може бути пов'язано зі збільшенням процесів апоптозу.

Висновки. 1. Різницю між групою НГЕ та контрольною групою секреторного ендометрія в залозистом компоненті продемонстрували маркери ER, PgR, b-catenin, p21, cyclin D1, Ki-67, Caspasa-3, а в стромальному компоненті - ER, PgR, b-catenin (всі $p<0,05$), що дає підставу використовувати їх в якості основних діагностичних маркерів. 2. Різницю між групою НГЕ після проведенного лікування та контрольною групою секреторного ендометрія в залозистом компоненті продемонстрували маркери ER, b-catenin, p21, cyclin D1, Ki-67, eNOS, а в стромальному компоненті - ER, b-catenin та eNOS, що дає підставу використовувати їх в якості основних діагностичних маркерів.3. Різницю між групою НГЕ до проведенної терапії та групою та контрольною групою в залозистом компоненті продемонстрували маркери PgR, Ki-67, Caspasa-3 eNOS, а в стромальному компоненті - eNOS, що дає підставу використовувати їх в якості основних діагностичних і прогностичних маркерів. 4. Маркери Bcl-2 та BAX не показали статистично достовірної різниці в групах дослідження, що говорить про неможливість використання їх окремо в якості діагностичних або прогностичних маркерів для гіперпластичних процесів ендометрія, а інтерпритацію результатів експресії цих маркерів необхідно враховувати в сукупності з іншими показниками.

Ключові слова: *ендометрій, рецептори, гіперплазія ендометрія, імуногістохімія, рецептори, резистентність до прогестерону, рецидив*

Для цитування: Хасхачих ДА, отапов ВО, Пославська ОВ, Гарагуля ІС. ДОСЛІДЖЕННЯ ІМУНОГІСТОХІМІЧНИХ МАРКЕРІВ ПРИ РЕЦИДИВІ ГІПЕРПЛАЗІЇ ЕНДОМЕТРІЮ БЕЗ АТИПІЇ У ЖІНОК РЕПРОДУКТИВНОГО ВІКУ ПІСЛЯ ЛІКУВАННЯ ПРОГЕСТІНАМИ. Актуальні проблеми сучасної медицини. 2023;12:3-13. DOI: https://doi.org/10.26565/2617-409X-2023-12-01

ІНФОРМАЦІЯ ПРО АВТОРІВ

Дмитро Хасхачих, к. мед. н., доцент кафедри акушерства та гінекології Дніпровського державного медичного університету, Дніпровський державний медичний університет, вул. Вернадського, 9, м. Дніпро, Україна, 49044. docdhas@gmail.com, https://orcid.org/0000-0001-5097-6667

Валентин Потапов, д-р мед. н., професор, завідувач кафедри акушерства та гінекології, Дніпровський державний медичний університет, вул. Вернадського, 9, м. Дніпро, Україна, 49044, e-mail: potapov250352@gmail.com, ORCID: https://orcid.org/0000-0001-7498-7416

Олександра Пославська, д-р мед. н., доцент кафедри патологічної анатомії, судової медицини та патологічної фізіології, Дніпровський державний медичний університет, вул. Вернадського, 9, м. Дніпро, Україна, 49044, e-mail: alexandraposlavskaya@gmail.com, ORCID: https://orcid.org/0000-0002-3133-8413

Ірина Гарагуля, к. мед. н., доцент кафедри акушерства та гінекології, Дніпровськи1 державний медичний університет, вул. Вернадського, 9, м. Дніпро, Україна, 49044, e-mail: nataliyadubenchuk@gmail.com, ORCID ID: https://orcid.org/0009-0000-0256-7658

Отримано: 11.09.2023 року
Прийнято до друку: 09.11.2023 року

Conflicts of interest: author has no conflict of interest to declare.
Конфлікт інтересів: відсутній.