MEDICAL UNIVERSITY - PLOVDIV
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ANALYSIS OF HORMONAL RECEPTOR EXPRESSION AND THE BIOMARKERS P53 AND KI-67 IN ENDOMETRIAL POLYPS IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN

DISSERTATION ABSTRACT

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The dissertation comprises **210 pages**, illustrated with **56 figures** and **82 tables**. The bibliography includes **196 references**. The dissertation has been reviewed by the Extended Departmental Council at the **Department of Obstetrics and Gynecology, Medical University of Plovdiv**, and has been approved for public defense before a scientific jury endorsed by the Faculty Council.

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ABBREVIATIONS

Abbreviation	Full term
EP	Endometrial Polyp
EC	Endometrial Carcinoma
AE	Atrophic Endometrium
IHC	Immunohistochemistry / Immunohistochemical Examination
ER	Estrogen Receptor
PR	Progesterone Receptor
p53	Tumor Suppressor Protein p53
Ki67	Cellular Proliferation Marker (Nuclear Proliferation Antigen)
BMI	Body Mass Index
DM	Diabetes Mellitus
AH	Arterial Hypertension
E2	Estradiol (Serum Estradiol)

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I. Introduction

Endometrial polyps are among the most common benign neoplasms within the uterine cavity. The widespread use of transvaginal ultrasonography in routine gynecological practice has enabled their diagnosis as incidental findings in both premenopausal and postmenopausal women. However, the pathogenesis of endometrial polyps remains incompletely understood and continues to be a subject of discussion.

It is believed that the imbalance in the expression of steroid hormone receptors in the endometrium—estrogen and progesterone receptors—alongside elevated serum estradiol concentrations, represent key factors in the etiopathogenesis of polyps in premenopausal and postmenopausal women. Additionally, disturbances in cellular proliferation and apoptosis mechanisms, as well as local inflammatory factors, are also considered potential pathogenic contributors.

In recent years, associations have been established between endometrial polyp formation and conditions such as overweight, insulin resistance, diabetes mellitus, arterial hypertension, obesity, metabolic syndrome, and polycystic ovary syndrome. Furthermore, the use of tamoxifen, advanced age, early menarche, and late menopause are significant risk factors for the development of endometrial polyps, their transformation into precancerous lesions, and progression to type I endometrial carcinoma. According to data from the National Cancer Registry, endometrial carcinoma ranks second only to breast cancer among oncological diseases in women.

In contemporary research, certain biomarkers have been identified as predictors of the malignant potential of endometrial polyps—specifically, the tumor suppressor gene **p53** and the proliferation marker **Ki-67**. Examination of serum estradiol concentrations, along with the immunohistochemical expression of steroid hormone receptors and the biomarkers p53 and Ki-67 in endometrial polyps, could provide further insights into their etiology and pathogenesis, assess the risk of malignant transformation and progression to endometrial carcinoma, and determine the appropriateness of hormonal therapy in premenopausal and postmenopausal women with concomitant risk factors.

II. Aim and Objectives

1. Aim:

To analyse the expression of estrogen and progesterone steroid receptors, as well as the biomarkers p53 and Ki-67, in endometrial polyps in premenopausal and postmenopausal women.

2. Objectives:

1. To analyse the demographic characteristics of the studied cohort.
2. To perform a comparative analysis of serum estradiol levels among the three groups of women with endometrial polyps, endometrial carcinoma, and atrophic endometrium.
3. To conduct a comparative evaluation of the expression of estrogen and progesterone receptors in endometrial polyps, endometrial carcinoma, and atrophic endometrium.
4. To perform a comparative analysis of the expression of the biomarkers p53 and Ki-67 in the studied groups.
5. To develop a model based on the obtained results, analysing the interrelations among hormonal receptor expression, biomarker expression, serum estradiol concentrations, and demographic characteristics in premenopausal and postmenopausal women with endometrial polyps.

III. Materials and Methods

Materials

1. Study Design:

1.1. This is an applied, cross-sectional study, similar in design to that of the Brazilian researchers de Carvalho S et al., as presented in their publication: *Differential expression of estrogen and progesterone receptors in endometrial polyps and adjacent endometrium in postmenopausal women* (Anal Quant Cytol Histol. 2011 Apr;33(2):61-7).
1.2. The subject of investigation was the role of steroid receptors and their significance in the development of endometrial polyps (conventional and atrophic) in premenopausal and postmenopausal women, as well as their malignant potential assessed through the immunohistochemical expression of hormonal receptors—estrogen receptors (ER), progesterone receptors (PR), and the biomarkers p53 and Ki-67.

2. Study Population:

Hospitalised patients in the Department of Obstetrics and Gynecology, University Hospital "St. George"–Plovdiv. A cross-sectional study was conducted on **120 women**, divided into three groups (Fig.1), assessing parameters such as serum estradiol levels, menstrual (menarche, menopause) and reproductive factors (number of births, age at first birth), and the expression of ER, PR, p53, and Ki-67:

- **Group I:** 40 women with endometrial polyps (EP).
- **Group II:** 40 women matched by criteria to Group I with histologically confirmed endometrial carcinoma (EC).
- **Group III:** 40 women with histologically confirmed atrophic endometrium (AE).

Twenty-six patients were excluded for not meeting inclusion criteria (Table 1).

3. Units of Observation:

3.1. **Technical units:** Department of Obstetrics and Gynecology, Medical University of Plovdiv; Department of General and Clinical Pathology, Medical University of Plovdiv; Morphological Research Center, Medical University of Plovdiv; Central Clinical Laboratory, UniversityHospital"St.George",Plovdiv.

3.2. **Logical units:** Premenopausal and postmenopausal women with histologically confirmed EP and EC, and a control group with histologically confirmed AE.

4. Observed Indicators: 4.1. Demographic parameters.

4.2. Factorial parameters: age, risk factors (arterial hypertension, obesity, diabetes mellitus), use of combined oral contraceptives (COC), hormone replacement therapy (HRT), progestins, LNG-IUD, menstrual factors (age at menarche and menopause), reproductive history (number of births, age at first birth), smoking status, and histological results across groups. 4.3. **Outcome parameters:** abnormal uterine bleeding, postmenopausal bleeding, serum estradiol concentrations, and immunohistochemical expression of ER, PR, p53, and Ki-67 in EP, EC, and AE.

5. Study Location:Departments and clinics of Obstetrics and Gynecology and General and Clinical Pathology at the Medical University of Plovdiv and University Hospital "St. George", including the Morphological Research Center and Central Clinical Laboratory.

6. Study Period: December 2022 – December 2024.

7. Research Team:All studies were conducted with the direct participation and supervision of the doctoral candidate under the guidance of the scientific supervisors.

8. Ethics Approval: (Protocol № 8 / 10.10.2024 – attached "Extract-Copy") The Ethics Committee concluded that the research complies with standards of scientific and ethical conduct, aligned with the Declaration of Helsinki, Good Clinical Practice principles, and Bulgarian laws and regulations governing human research (P-KHE-14/31.12.2024).

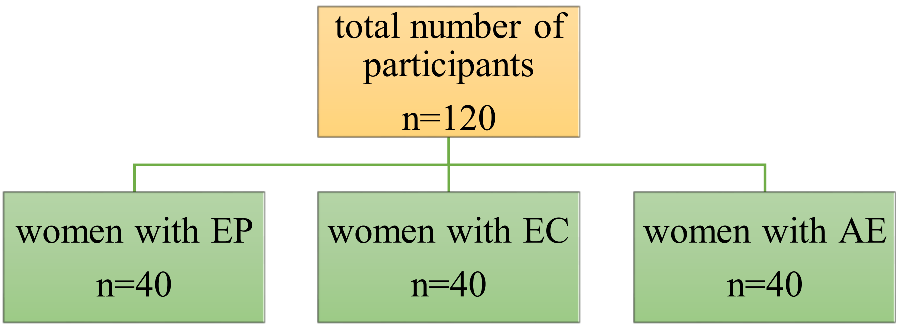

Figure 1. Distribution of study participants by group.

Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
1. Hospitalised patients in the clinic.	1. Patients under 40 years of age.
2. Premenopausal women aged ≥40 years in the follicular phase.	2. Pregnancy.
3. Postmenopausal women.	3. Histological results other than EP, EC, or AE.
4. Histologically confirmed EP.	4. Current hormonal therapy use.
5. Histologically confirmed EC.	5. Presence of an IUD, including LNG-IUD.
6. Histologically confirmed AE.	6. Autoimmune diseases.
7. No hormonal therapy for ≥6 months.	7. Breast cancer patients on aromatase inhibitors (e.g. tamoxifen) or other malignancies requiring hormonal therapy.
-	8. Women on HRT, GnRH agonists/antagonists, androgens, antiestrogens, or selective progesterone receptor modulators.
-	9. Progesterone implants as contraception.
-	10. Non-representative histological results.

Methods

1.Survey Method: A structured anonymous questionnaire was used to collect data on:

- Age
- Menarche and menopause age
- Number of births and age at first birth
- Smoking
- Use of COC and/or HRT
- Presence of IUD or implant
- Comorbidities (oncological, autoimmune, diabetes mellitus, arterial hypertension, obesity).

2. Clinical Methods:

2.1. Anamnesis Medical history taking included detailed information regarding:

- **Age** of the patients;
- **Menopausal status** (absence of menstruation for more than one year, presence of typical menopausal symptoms);
- **Presence of symptoms** such as postmenopausal bleeding or abnormal uterine bleeding in menstruating women.

Additionally, data on **height and weight** were obtained from the medical records and used to calculate the **Body Mass Index (BMI)** using the formula: BMI = weight (kg) / (height (m))^2.

Arterial hypertension and **diabetes mellitus** were identified based on anamnesis, medical documentation, and consultation with an internist. Hypertension was defined as blood pressure values >140/90 mmHg, while diabetes mellitus was determined based on fasting blood glucose levels meeting the diagnostic criteria. The type of diabetes and treatment regimen were not subject to analysis.

Breast cancer and **tamoxifen therapy** were exclusion criteria for participation in the study.

Parity was analysed and categorised into three groups:

- Nulliparous;
- One birth;
- Two or more births.

Use of **hormone replacement therapy (HRT)** and the presence of an **intrauterine device (IUD) or implant** were also assessed.

2.2. Gynecological Examination

The gynecological examination included:

- **Inspection and examination using a vaginal speculum** to assess the external genitalia, vagina, and cervix;

- **Vagino-abdominal bimanual palpation** to evaluate the position, size, and consistency of the uterus and adnexa.

2.3. Transvaginal Ultrasonography

All participants underwent transvaginal ultrasonography using a vaginal probe (Philips Healthcare Clear Vue 650) in two planes to:

- **Assess uterine size;**
- **Examine the uterine cavity;**
- **Measure endometrial thickness.**

In postmenopausal patients with an endometrial thickness greater than 4 mm, or when endometrial polyps were suspected (Fig. 2)—based on their size, the presence of a feeding vessel, or increased vascularisation—**diagnostic hysteroscopy with fractional curettage** was performed.

2.4. Diagnostic Hysteroscopy

Diagnostic hysteroscopy was performed using a flexible hysteroscope with camera (Karl Storz, model PENTAX FHY-10RBS) under short intravenous anesthesia, in accordance with established standards. This minimally invasive method allows for:

- **Visualization of intrauterine pathology;**
- **Diagnosis of endometrial polyps, endometrial carcinoma, or endometrial atrophy.**

Panoramic hysteroscopy enabled assessment of:

- **Polyp localisation;**
- **Number and type of polyps;**
- **Presence of a pedicle or broad base;**
- **Specific hysteroscopic features such as surface colour and the presence of atypical vessels (Fig. 3).**

Upon completion of diagnostic hysteroscopy, **fractional curettage** was routinely performed.

2.5. Fractional Curettage

Fractional curettage was performed as an invasive procedure under short intravenous anesthesia and included:

- **Dilation of the cervical canal;**
- **Curettage of the endocervical canal;**
- **Separate curettage of the uterine cavity using gynecological curettes (Fig. 4).**

This method was preferred in patients with heavier genital bleeding, where adequate visualization via hysteroscopy could not be achieved. **Fractional curettage allowed for the collection of sufficient tissue material for histological examination and establishing a definitive diagnosis.**

2.6. Abdominal Hysterectomy

Following **histological verification of endometrial carcinoma (EC)** and disease staging using imaging modalities—computed tomography (CT) or positron emission tomography (PET)—patients were presented to a multidisciplinary oncology board. After expert discussion and approval of the therapeutic plan, **surgical treatment involving total abdominal hysterectomy (TAH)** was performed.

TAH was carried out by removing the uterine body along with the cervix. Surgical access was achieved via **laparotomy**, either through a lower midline incision or Pfannenstiel incision, depending on the surgical team’s assessment and the patient's individual anatomical features.

In many cases, depending on the disease stage and risk factors, **bilateral adnexectomy** (removal of ovaries and fallopian tubes) was added to the procedure. Additionally, in the presence of indications for higher tumour aggressiveness or more advanced disease stage, **pelvic lymph node dissection** was performed.

All surgical interventions were carried out under **general endotracheal anesthesia** with continuous intraoperative monitoring of vital functions. All removed specimens (uterus, adnexa, lymph nodes) underwent subsequent **histopathological processing** to establish the definitive diagnosis, confirm disease staging, and plan further management.

2.7. Vaginal Hysterectomy

Women diagnosed with **total uterovaginal prolapse**, often combined with cystocele and rectocele, were hospitalised in the Department of Obstetrics and Gynecology. All patients underwent **preoperative transvaginal ultrasonography (TVUS)** to assess endometrial thickness, with measured values ranging from **2.2 mm to 4 mm**. These measurements are within the normal range for the postmenopausal period and indicative of the absence of hyperplastic or neoplastic processes.

In accordance with the diagnosis, all patients underwent **vaginal hysterectomy**, involving surgical removal of the uterus via the vaginal route. Additionally, during the same surgical intervention, **anterior and posterior colporrhaphy** was performed to correct the concomitant cystocele and rectocele.

The removed tissue specimens were subjected to **standard histological examination** in the pathology laboratory. In all analysed cases, histological results confirmed **the presence of atrophic endometrium without evidence of neoplasia or hyperplasia**. Women with histologically confirmed atrophic endometrium were used as the **control group** in the present study.

Figure 2. Transvaginal ultrasonography showing a hyperechoic area within the uterine cavity, suspicious for an endometrial polyp.

Figure 3. Flexible hysteroscope used for diagnostic panoramic hysteroscopy.

Figure 4. Instrument set for performing fractional curettage.

3. Classical Histological Method

After obtaining tissue material through surgical intervention, the next step involved **fixation in 10% buffered formalin** and submission to the Department of General and Clinical Pathology for processing. This method included:

1. Fixation in formalin;
2. Embedding in paraffin blocks;
3. Sectioning using a microtome to obtain **paraffin sections of 5 μm thickness**;
4. Deparaffinisation and rehydration through descending grades of alcohol;
5. Standard staining with **hematoxylin-eosin (H&E)**;
6. Microscopic examination of the H&E-stained slides.

4. Immunohistochemical Method

Immunohistochemical (IHC) analysis was conducted at the **Morphological Research Center of the Medical University – Plovdiv**, strictly following validated standard protocols provided by **DAKO (Denmark).**

From selected paraffin blocks containing representative tissue fragments, **serial 5 µm sections** were prepared and mounted on adhesive microscope slides. For the IHC reaction, **monoclonal antibodies** targeting specific markers—steroid hormone receptors (ER, PR), Ki-67, p53, among others—were used (Table 2).

A **control reaction** was performed on a HER2-positive breast adenocarcinoma verified by CISH (Chromogenic In Situ Hybridization), ensuring reliability and quality control of the IHC procedure.

The IHC reaction utilised a **visualisation system and "3-in-1" preparation protocol** with Dako PT Link, according to the manufacturer's instructions. The procedure included the following key steps:

1. Preparation of the working solution by diluting EnVision FLEX Target Retrieval Solution (50x) at a ratio of 1:50 with distilled or deionised water;
2. Loading the PT Link tank with 1.5 L of working solution;
3. Preheating the solution to **65°C**;
4. Immersing the formalin-fixed, paraffin-embedded tissue sections in the preheated buffer and incubating at **97°C for 20 minutes** (HIER – Heat-Induced Epitope Retrieval);
5. Cooling the slides to **65°C** within the PT Link;
6. Transferring the slides to a container with diluted EnVision FLEX Wash Buffer (20x) at room temperature;
7. Rinsing the slides for **1–5 minutes** in the same diluted buffer.

The IHC reaction was performed using the **Dako Autostainer Link 48 (Figure 5)**, which ensures a standardised and reproducible automated staining process.

Figure 5. Dako Autostainer Link 48 used for immunohistochemical analysis at the Morphological Research Center, Medical University of Plovdiv.

To ensure objectivity and quantitatively measure the expression of selected immunohistochemical markers, **morphometric analysis** was performed using the specialised digital microscopy and image analysis system **Quick PHOTO MICRO 23**. This is professional software designed for morphometric measurements in histological specimens. The software enables automated image processing, identification, and quantitative evaluation of stained tissue structures, and in the present study it was utilised for:

- **Calibration of images** against a scale grid to ensure measurement accuracy;
- **Automatic recognition of stained cell nuclei** based on predefined colour, saturation, and shape criteria;
- **Calculation of the area of positively stained nuclei** per unit area (0.02 mm^2);
- **Determination of the number of expressing cells** for each specific marker.

The procedure for morphometric analysis included the following steps:

1. Capturing microscopic fields at magnifications of ×**100 and** ×**200** using a digital camera mounted on an **Olympus BX 51 microscope**;
2. **Calibrating images** within the software for accurate measurement of area and objects;
3. Setting **threshold parameters** for automatic recognition—selecting colour thresholds corresponding to the specific **DAB (3,3'-diaminobenzidine) immunostaining**;
4. **Segmentation of nuclei**—automatic differentiation of positively stained from negative cells;
5. **Automatic counting** of the number of positive cells within the defined area.

Table 2. Description of the monoclonal antibodies used in the study.

Type of Monoclonal Antibody	Supplied Reagent	Immunogen	Purpose
FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α Clone EP1	Ready-to-use monoclonal rabbit antibody in liquid form in buffered solution containing stabilising protein and 0.015 mol/L sodium azide. Clone: EP1.	Recombinant protein of ER α amino acids 1–300.	Semi-quantitative detection of human estrogen receptor α.
FLEX Monoclonal Mouse Anti-Human Progesterone Receptor Clone PgR 636	Ready-to-use monoclonal mouse antibody in liquid form in buffered solution containing stabilising protein and 0.015 mol/L NaN_3. Clone: PgR 636. Isotype: IgG1, kappa.	Formalin-fixed recombinant A-form of human progesterone receptor.	Detection of progesterone receptor expression.
FLEX Monoclonal Mouse Anti-Human Ki-67 Antigen Clone MIB-1	Ready-to-use monoclonal mouse antibody in liquid form in buffered solution containing stabilising protein and 0.015 mol/L sodium azide. Clone: MIB-1 (8). Isotype: IgG1, kappa.	Human recombinant peptide corresponding to 1002 bp Ki-67 cDNA fragment.	Detection of Ki-67 antigen expression in normal and neoplastic cells.
FLEX Monoclonal Mouse Anti-Human p53 Protein Clone DO-7	Ready-to-use monoclonal mouse antibody in liquid form in buffered solution containing stabilising protein and 0.015 mol/L sodium azide. Clone: DO-7. Isotype: IgG2b, kappa.	Recombinant wild-type human p53 protein.	Detection of wild-type and mutant p53 protein expression.

5. Clinical-Laboratory Method

All hospitalised women diagnosed with **EP (endometrial polyp), EC (endometrial carcinoma), or AE (atrophic endometrium)** underwent standard clinical and laboratory examinations. Additionally, serum estradiol levels were assessed. Venous blood samples were collected under standard conditions, ensuring absence of lipemia or haemolysis. In premenopausal women, **venipuncture was performed during the follicular phase (days 7–10) of the menstrual cycle**.

Serum estradiol (E2) concentrations were measured using a **chemiluminescent immunoassay**. Original kits by **Beckman Coulter, Inc., USA**, were used with an automated immunological system (Access 2). Reference ranges were method-dependent, with the following intervals applied:

1. **Follicular phase:** 24–114 pg/ml
2. **Menopause:** 20–88 pg/ml

6. Statistical Methods

Systematisation, processing, and analysis of primary data, presented as quantitative and qualitative variables, were performed using **IBM SPSS Statistics (version 26.0; SPSS, Inc., Chicago, IL, USA)**. A significance level of $p < 0.05$ was adopted for all analyses.

Descriptive statistical analysis included **variation analysis** with calculation of means, standard deviations, and dispersion indicators for quantitative variables. The **Kolmogorov-Smirnov test** was used to check for normality of data distribution. Where necessary, conversion from ordinal to interval variation series was performed, and interval widths were calculated using **Sturges' formula**.

To compare two means, the **Student's t-test** for independent or paired samples was used, with significance set at $\alpha = 0.05$. For comparisons involving more than two groups, **one-way and two-way ANOVA** were applied, with **Tukey's method** used for post hoc comparisons.

For qualitative variables, **non-parametric analysis** using the **Pearson chi-square (χ^2) test** was performed, as well as the **Wilcoxon signed-rank test** for assessing differences between two related samples. **Alternative analysis** was used to evaluate the distribution of dichotomous variables.

Relationships between quantitative variables were analysed using **Pearson's linear correlation coefficient** and **Spearman's rank correlation coefficient**, depending on the distribution type.

Graphical analysis was applied to visualise the main trends and results.

To assess the influence of molecular markers on diagnostic group classification (EP, EC, or AE), **multinomial logistic regression** was performed, enabling prediction of group membership probabilities based on individual marker values.

The **diagnostic effectiveness** of markers and their combinations was evaluated using **ROC curve analysis (Receiver Operating Characteristic curve analysis)**. For each marker and combined models, the **area under the ROC curve (AUC)** was calculated, determining the sensitivity and specificity of the respective diagnostic tests.

IV. Results

I. Analysis of the Demographic Characteristics of the Studied Cohort

Demographic indicators are an important component in elucidating the interrelationships and predisposing factors involved in the **etiopathogenesis of endometrial polyps and endometrial carcinoma**.

1. Age of the Cohort

The study included **120 women**, hospitalised at the Department of Obstetrics and Gynecology, University Hospital "St. George", Plovdiv, in the period **from December 2022 to December 2024**. Participants were divided into three groups as follows:

- **Group I:** women with endometrial polyps (EP)
- **Group II:** women with endometrial carcinoma (EC)
- **Group III:** women with atrophic endometrium (AE)

Each group comprised an equal number of participants (**n = 40; 33%**) (Figure 6).

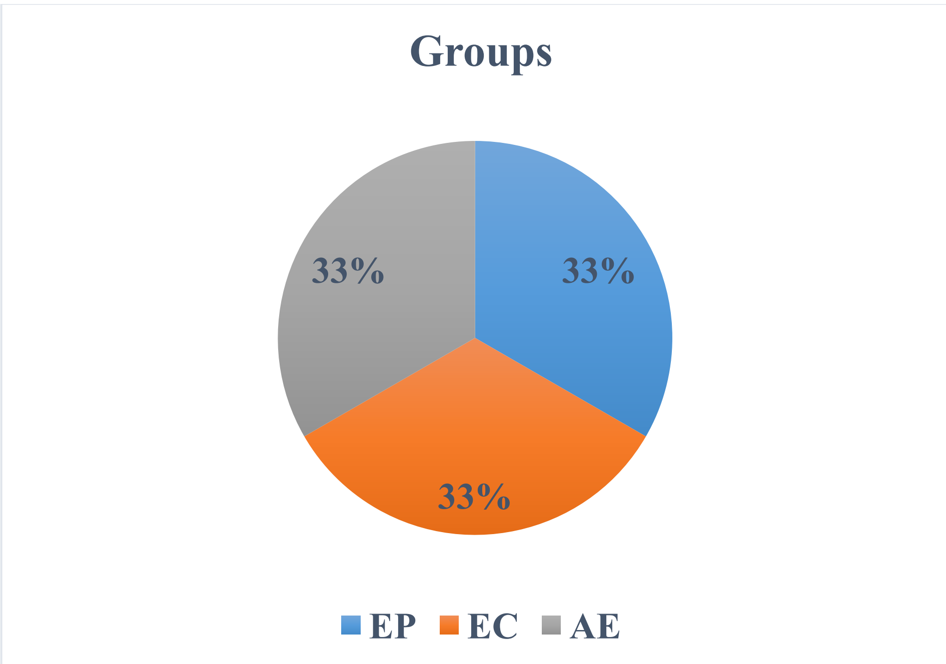

Figure 6. Diagram showing the participant groups in the study. EP – endometrial polyp; EC – endometrial carcinoma; AE – atrophic endometrium.

The **mean age** of the studied cohort was **62.1 ± 11.1 years (n = 120)**. The youngest participant was **41 years old**, and the oldest was **83 years old**.

All study participants were divided into four age groups as follows:

- **Group I:** 41–50 years inclusive
- **Group II:** 51–60 years inclusive
- **Group III:** 61–70 years
- **Group IV:** over 71 years

Patients aged **over 71 years** comprised **29.2%**, followed by Groups III and II (**27.5% and 24.2%**, respectively), while the lowest proportion was observed in women aged **41–50 years (19.2%)**.

The **percentage distribution** of participants by age group is shown in **Figure 7**.

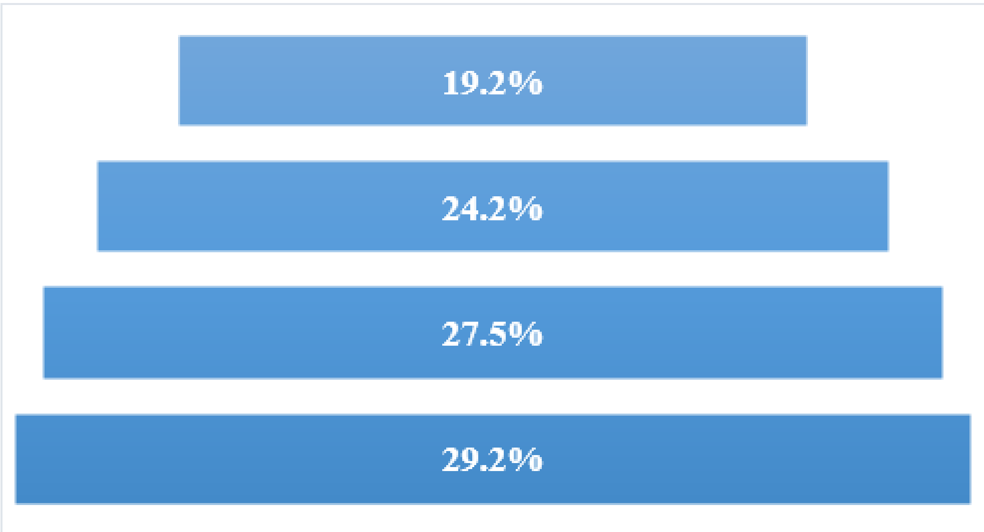

Figure 7. Percentage distribution of the cohort by age groups.

1.1.Age Distribution of Patients with Endometrial Polyps (EP)

The youngest woman in this group was **41 years old**, and the oldest was **80 years old**. The **mean age** of women in the EP group was **56.9 ± 12.2 years (n = 40)**.

The **highest relative proportion** was observed among women aged **41–50 years**, followed by those **over 71 years**. Participants in the remaining two age groups were **equally distributed** (Figure 8)

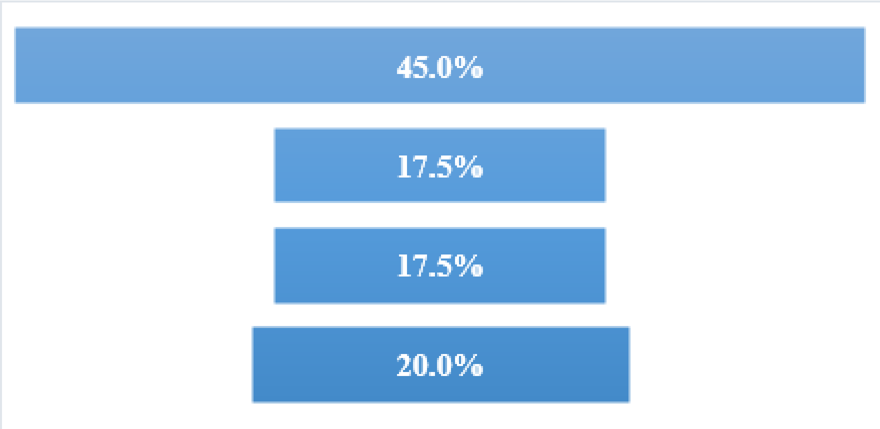

Figure 8. Percentage age distribution of women with endometrial polyps (EP).

1.2. Age Distribution of Participants with Endometrial Carcinoma (EC)

The **mean age** of women in the EC group was **63.2 ± 10.5 years (n = 40)**. The youngest woman diagnosed with EC was **42 years old**, and the oldest was **81 years old**.

The **highest relative proportion** of EC patients was observed in the **51–60 years** age group, followed by those **over 71 years**. The **lowest proportion** was in the youngest age group, with **3 cases (7.5%)** (Figure 9).

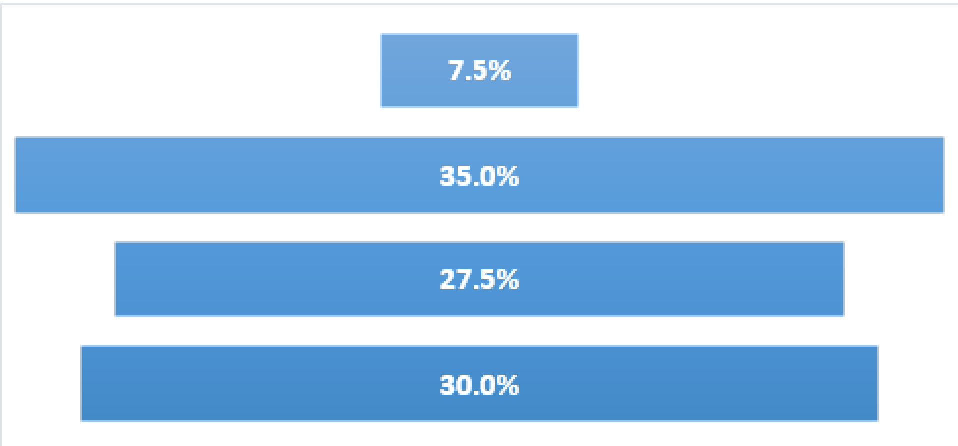

Figure 9. Percentage age distribution of women with endometrial carcinoma (EC).

1.3. Age Distribution of Participants with Atrophic Endometrium (AE)

The youngest woman in this group was **42 years old**, and the oldest was **83 years old**. The **mean age** of women in the AE group was **66.4 ± 8.3 years (n = 40)**.

The **highest relative proportion** of patients was observed among those **over 71 years**, followed by women aged **61–70 years**, with **equal distribution** between these two groups. The **lowest proportion** was among women aged **41–50 years** (Figure 10).

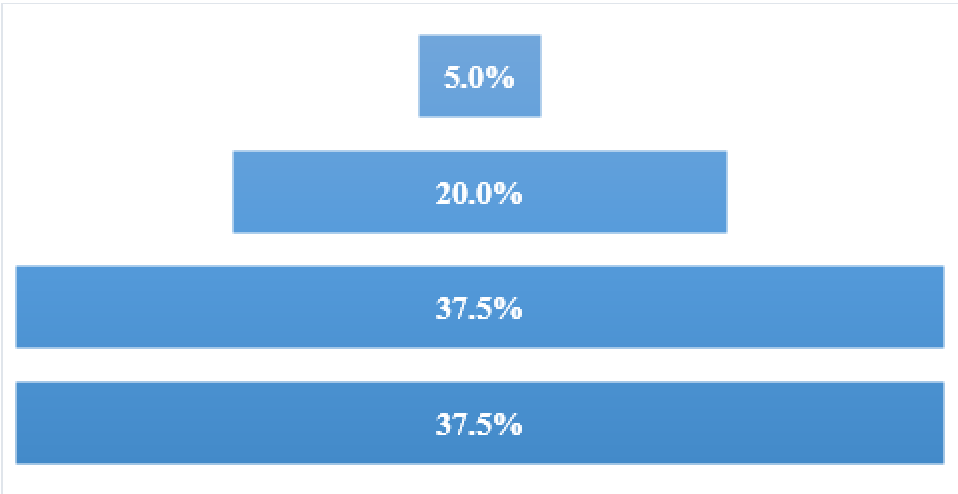

Figure 10. Percentage age distribution of women with atrophic endometrium (AE).

There was a **statistically significant difference** between the **mean age** and the type of gynecological condition (Table 3).

Table 3. Mean age of participants.

		n	$\bar{x}$	$S\bar{x}$	F	p
AGE	EP	40	56,9	12,2	8,551	0,000
	EC	40	63,2	10,5		
	AE	40	66,4	8,3		

From the analysis of the **frequency distribution** of participants by age group (across the three study groups), the following conclusions were drawn:

- **EP, EC, and AE** were diagnosed in **all age groups**.
- The **highest frequency of EP** was observed in women aged **41–50 years**.

- **Endometrial carcinoma (EC)** was most commonly diagnosed in the **51–60 years** age group within the studied cohort.
- In the **third and fourth age groups (61–70 years and over 71 years)**, the highest relative proportion was observed among women with **AE**, followed by **EC**, with the lowest proportion in participants with **EP**.
- **Age is a significant risk factor** for the occurrence of EP, EC, and AE (**F = 8.5; P = 0.000**).

2. Distribution of Participants According to Menstrual Factors

2.1. Distribution of Women in the Study According to Age at Menarche. The **mean age at menarche** for all women across the three groups was **13.2 ± 1.5 years (n = 120)**. The **lowest mean age** was observed in women with **EP (12.8 ± 1.4 years; n = 40)**, and the **highest** in the group with **atrophic endometrium (AE) (13.5 ± 1.4 years; n = 40)** (Table 4).

No **statistically significant association** was found between age at menarche among women in the **EP and EC groups** and those in the **AE group**.

Table 4. Mean age at menarche among women in the three study groups.

		n	$\bar{x}$	$S\bar{x}$	F	p
MENARCHE	EP	40	12,8	1,4	2,465	0,089
	EC	40	13,3	1,6		
	AE	40	13,5	1,4		

2.2. Summary Data on Menopausal Status by Groups

A **statistically significant association** was found between the **mean age at menopause** and the type of gynecological pathology (Table 5) (**F = 6.261; P = 0.003**).

Table 5. Mean age at menopause among women in the three study groups.

		n	$\bar{x}$	$S\bar{x}$	F	p
MENOPAUSE	EP	23	48,2	3,1	6,261	0,003
	EC	35	50,9	2,5		
	AE	38	48,7	3,7		

According to the **relative proportion of postmenopausal women** in the three groups, a **statistically significant association** was found between **menopausal status** and the development of **EP, EC, and AE (χ^2 = 19.56; p = 0.000)** (Figure 11).

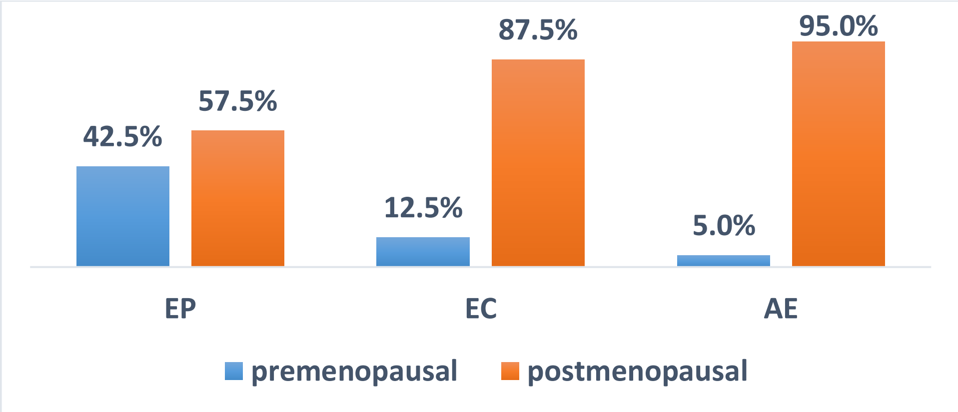

Figure 11. Relative proportion of premenopausal and postmenopausal women in the three groups.

From the analysis of the data on **menstrual factors**, the following conclusions can be drawn:

- A **mean menarche age above 12.8 years** is **not a risk factor** for the development of endometrial pathology.
- **EPs** are diagnosed in both **premenopausal and postmenopausal women**.
- The **frequency of EP** is higher among **premenopausal women**.
- The **frequency of EC and AE** is higher among **postmenopausal women**.
- **Postmenopause** is associated with the occurrence of **both benign (EP) and malignant (EC) endometrial pathology**.
- **Menopause occurring at a mean age of 50.9 years** is a **risk factor for EC**.

3. Characteristics of the Cohort According to Reproductive Factors

3.1. Distribution of Participants According to Parity

Data showed that in the present study, the **relative proportion of women who had given birth was slightly over 90%**, while **nulliparous women accounted for 10.8%**. Based on the number of births, participants were divided into those with **one or two births**, and those with **more than two births** (Figure 12).

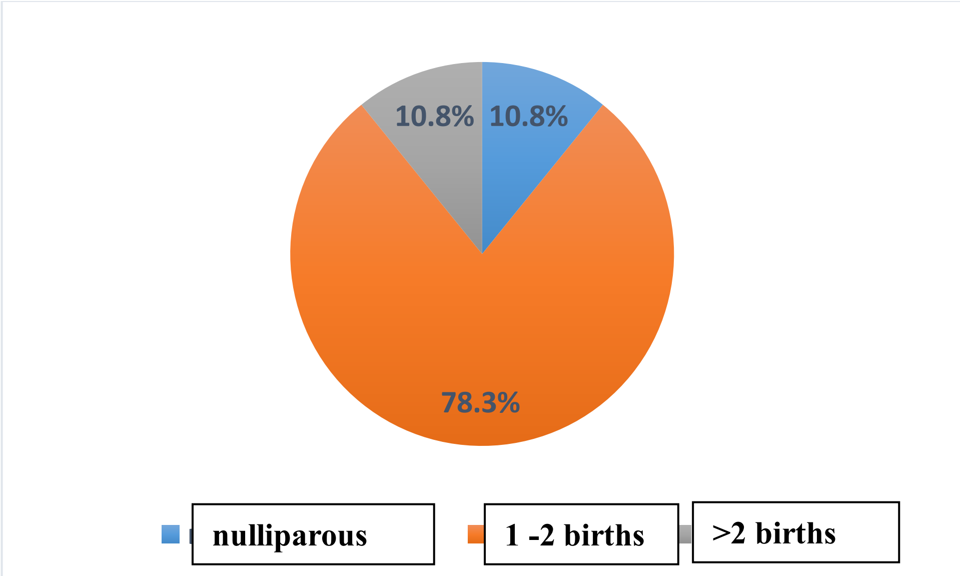

Figure 12. Distribution of participants according to parity.

The **highest proportion** was observed among women who had given birth to **one or two children**, compared to those with **more than two births**.

3.2. Distribution of Participants According to Age at First Birth

The **mean age at first childbirth** was analysed by group based on data from participant questionnaires. In all three groups, **no statistically significant association** was found between age at first birth and the type of intrauterine pathology (**Table 6**) (**F = 0.838; P = 0.435**).

Table 6. Relative proportion of participants according to age at first birth.

		n	$\bar{x}$	$S\bar{x}$	F	p
mean age at first childbirth	EP	35	22,6	4,9	0,838	0,435
	EC	34	21,6	4,4		
	AE	38	21,3	4,2		

The **mean age at first childbirth** among women with **EP** was **22.6 ± 4.9 years (n = 35)**, which is **one year later** compared to patients with **EC (21.6 ± 4.4 years; n = 34)** and **AE (21.3 ± 4.2 years; n = 38)**. Between the latter two groups, the difference in mean age was approximately **three months**.

Analysis of the data showed **no statistically significant association** between **parity** and **participant age**.

Conclusions:

- In our study, the **proportion of women who had given birth** was **higher** compared to nulliparous women. Based on these findings, **nulliparity is not a risk factor** for EP or EC.
- A **mean age at first childbirth** ranging from **21.6 ± 4.4 years to 22.6 ± 4.9 years** is **not a statistically significant risk factor** for the development of EC or EP.

4. Assessment of Risk Factors Associated with the Etiology of EP and EC

4.1. Analysis of Overweight and Obesity as Risk Factors

In this study involving **120 women**, BMI (Body Mass Index) was calculated based on **questionnaire data and measurements** of body weight (kg) and height (m), using the formula:**BMI = kg/m²** (Table 7).

Table 7. Relative proportion of participants with obesity.

		n	%
obesity	without	82	68,3
	with	38	31,7

The **mean BMI** for the entire cohort was **29.8 ± 7.4 kg/m² (n = 120)**, which falls into the **overweight category** according to WHO classification.

Analysis of the **mean BMI values** in the **EP, EC, and AE groups** showed that **none of the groups were characterised by normal body weight**.

In women with **EP**, the mean BMI was **28.7 ± 6.6 kg/m² (n = 40)**, which is **higher than that in the AE group (26.2 ± 3.7 kg/m²; n = 40)**. With **F = 15.940 and P = 0.000**, it can be concluded that **overweight is a risk factor for the development of EP**.

In women with **EC**, the mean BMI was **34.4 ± 8.6 kg/m² (n = 40)**, indicating **Class I obesity**.

A **statistically significant association** was found between **obesity and endometrial cancer** (Table 8).

Table 8. Mean BMI values by group.

		n	$\bar{x}$	$S\bar{x}$	F	P
BMI kg/m2	EP	40	28,7	6,6	15,940	0,000
	EC	40	34,4	8,6		
	AE	40	26,2	3,7		

4.1. Relative Proportion of Patients with Obesity in the Three Groups

Analysis of the results showed a **statistically significant association** between **obesity** and the presence of **endometrial polyps** and **endometrial carcinoma** (**χ^2 = 23.18; p = 0.000**) (Figure 13).

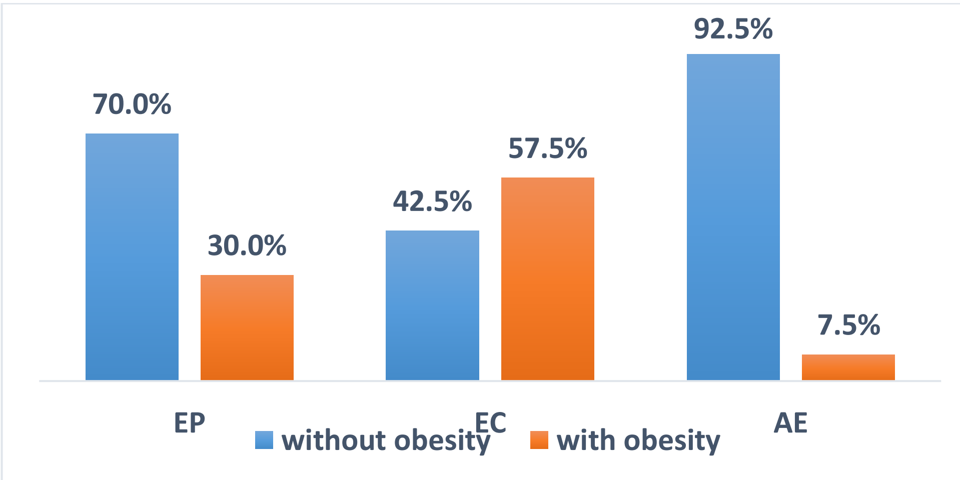

Figure 13. Percentage distribution of women with obesity across the three groups.

From the summarised data, the following conclusions can be drawn:

- **Overweight** is a **risk factor** for the development of endometrial polyps.
- **Obesity** is a **risk factor** for endometrial carcinoma.
- **Normal body weight** within the respective age group is associated with **atrophic endometrium**.

4.2. Assessment of Arterial Hypertension (AH) as a Risk Factor

Using the **questionnaire**, women indicated whether they had been previously diagnosed with **arterial hypertension (AH)**. During hospitalisation, each patient was also consulted by an **internal medicine specialist**.

Blood pressure was measured **twice using a sphygmomanometer**, recording **systolic and diastolic values** in millimetres of mercury (mmHg). The obtained data were classified according to the **International Hypertension Society classification** for individuals aged **18 years and above**.

The data showed that a **large proportion of women had Stage I AH**, defined as **systolic blood pressure between 140–159 mmHg** and **diastolic blood pressure between 95–99 mmHg**.

Compared to obesity, **arterial hypertension was present as a comorbidity in approximately two-thirds of cases**. Hypertension was more frequently observed as an accompanying condition compared to obesity (Table 9).

Table 9. Relative proportion of women with arterial hypertension (AH).

		n	%
AH	without	41	34,2
	with	79	65,8

4.2.4. Summary Analysis of the Frequency Distribution of Participants According to the Presence of Arterial Hypertension (AH)

The results indicate that **AH is a common comorbidity**, with the **highest proportion among women with EC**, followed by those with **EP**. In the **control group**, the proportion of participants with hypertension was **the lowest** (Figure 14).

Figure 14. Percentage distribution of women with arterial hypertension (AH) in the three groups.

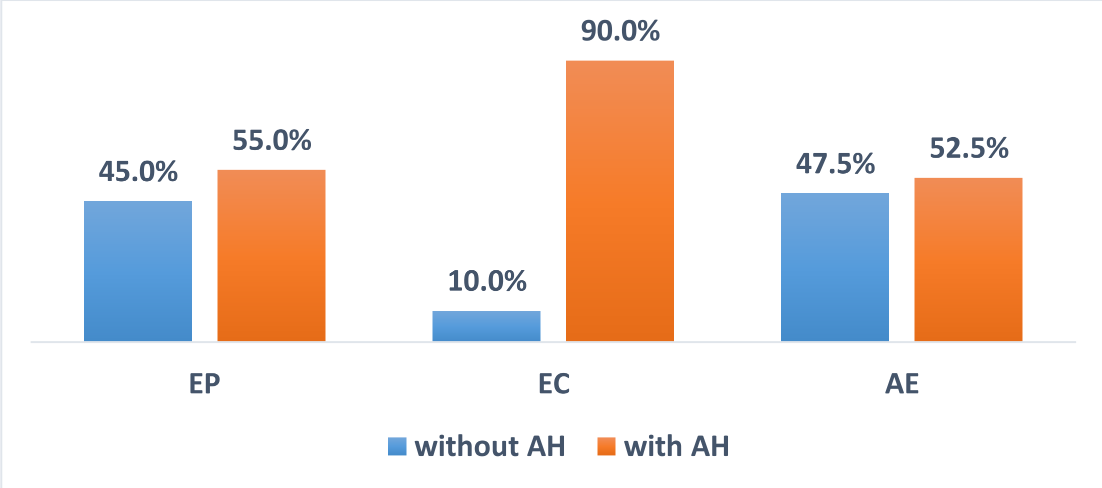

Figure 14. Relative proportion of women with arterial hypertension (AH) in the case groups (EP and EC) and the control group (AE).

Analysis of the data showed a **statistically significant association** between the presence of **arterial hypertension as a comorbidity** and gynecological pathology (**$\chi^2 = 15.63$; p = 0.000**).

Conclusions:

- **Arterial hypertension** is more common than obesity as a **component of metabolic syndrome** among women with endometrial pathology.
- **Arterial hypertension** is a **risk factor** for the development of **endometrial polyps** and **endometrial carcinoma**.

4.3. Analysis of Diabetes Mellitus (DM) as a Risk Factor

Diabetes mellitus is a metabolic disease characterised by absolute or relative insulin deficiency or impaired insulin action. Using the questionnaire, each participant indicated whether they had such a comorbidity. **Fasting blood glucose levels** were measured in all women in the morning. In some participants, fasting serum glucose levels were **above 7.0 mmol/L**, and although newly diagnosed, these women were also included in the analysis.

The **relative proportion of women diagnosed with DM** was approximately **one-quarter of the total cohort** (Table 10).

Table 10. Relative proportion of patients with diabetes mellitus (DM) in the studied cohort.

		n	%
DM	without	89	74,2
	with	31	25,8

4.3.1. Summary Analysis

The **percentage distribution** of participants with **diabetes mellitus (DM)** in the **EP and EC groups** showed that the **frequency of DM was higher among women with endometrial carcinoma** compared to those with endometrial polyps.

Statistical analysis confirmed a **statistically significant association** between **DM as a risk factor** and the presence of **intrauterine pathology** (**χ^2 = 11.57; p = 0.003**) (Figure 15).

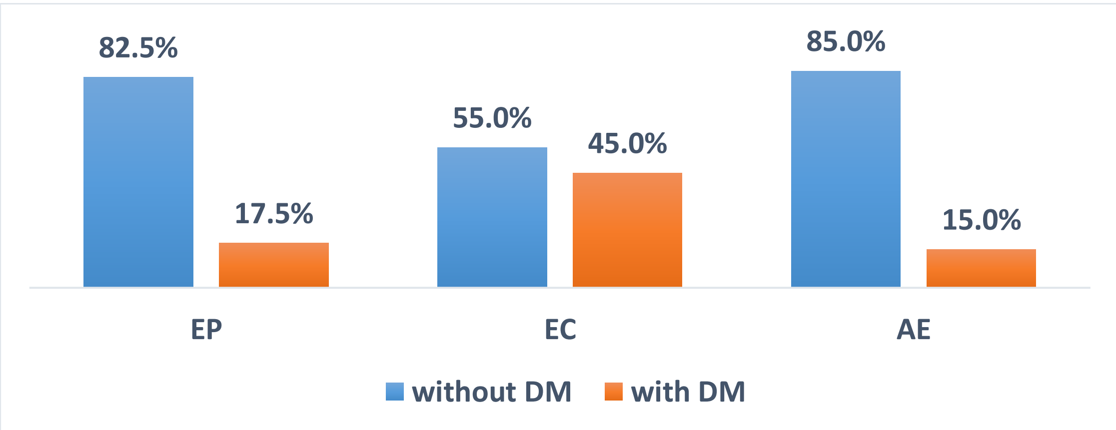

Figure 15. Percentage distribution of women with diabetes mellitus (DM) in the EP and EC groups.

From the obtained data, the following **conclusions** can be drawn:

- **Diabetes mellitus (DM)** ranks **third in frequency** among components of **metabolic syndrome** in women with endometrial pathology.
- **DM is a risk factor** for the development of **endometrial polyps**.
- **DM is a risk factor** for the development of **endometrial carcinoma**.

4.4. Assessment of the Influence of Hormone Replacement Therapy (HRT) on the Formation of EP and EC

In our study, only **5 out of 120 surveyed women** reported having used **HRT in the past** (Figure 16).

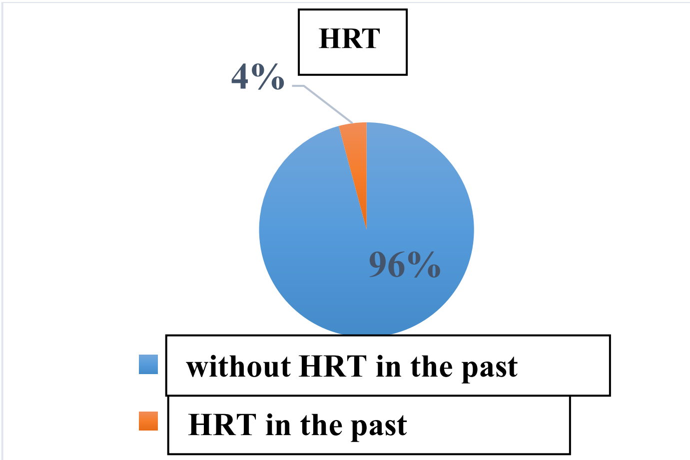

Figure 16. Questionnaire data on the use of hormone replacement therapy (HRT) in the studied cohort.

Two of these patients were hospitalised with **EP** and **AE**, respectively, and one with **EC**. **Statistical analysis** of the obtained results showed **no significant association** between past HRT use and the development of intrauterine pathology. **Conclusions:** The **use of HRT** among the patient cohort was **relatively low**, and therefore its influence as a risk factor cannot be assessed.

4.5. Assessment of Combined Oral Hormonal Contraceptive (COHC) Use in the Studied Cohort

Participants were asked retrospectively about **COHC use more than 6 months prior**. The data showed that the number of patients who had ever used COHC was **extremely low (7.5%; n = 120)**. The conclusion is that **no statistically significant association** was found between past COHC use and **endometrial impact at the time of the study**.

4.6. Assessment of Smoking as a Risk Factor

Data analysis from the questionnaire showed that **13.3% (n = 120)** reported **smoking** as a harmful accompanying habit. Interestingly, the **lowest proportion of smokers** was in the **EP**

group (10.0%; n = 40), while the **highest** was in the **EC group (17.5%; n = 40)**. However, **no statistically significant association** was established.

5. Assessment of Genital Bleeding as a Clinical Sign

In the studied cohort, **47 women** reported experiencing **genital bleeding** in the questionnaire. During the gynecological examination, the bleeding was determined to be **of uterine origin**. The **majority of women did not report bleeding** (Table 19).

Table 19. Frequency of genital bleeding in the studied cohort.

		n	%
genital bleeding	without	73	60,8
	with	47	39,2

5.1. Summary Result for the Frequency of Genital Bleeding

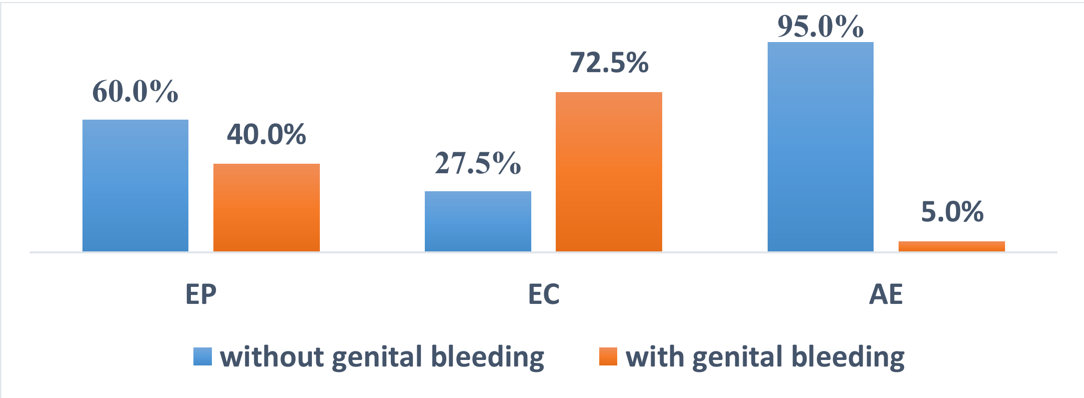

Figure 17. Summary frequency of genital bleeding in the studied cohort.

The **summary analysis** of results regarding the presence of bleeding in the case groups showed that in **EC**, this clinical sign was **most frequent**, compared to **EP** and the control group. The **lowest frequency** of bleeding was observed in the **control group**.

This indicates a **statistically significant association** between the presence of **genital bleeding** and the occurrence of **intrauterine pathology** (**χ^2 = 38.26; p = 0.000**).**Conclusions:**

- The frequency of **asymptomatic EPs** is **higher** compared to EPs causing genital bleeding.
- **Abnormal genital bleeding** is a pathological sign associated with an **increased frequency of EC and EP** in both premenopausal and postmenopausal women.

6. Analysis of the Type of Surgical Intervention in the Studied Cohort

At the **Department of Obstetrics and Gynecology**, routine procedures include **diagnostic hysteroscopy, resectoscopy, fractional curettage, abdominal and vaginal hysterectomy** (brief descriptions of these surgical interventions are provided in the **Methods section**).

In the **EP group**, **resectoscopy was not performed** due to **preoperative ultrasound findings** indicating polyps of **small size (up to 10 mm)**, which were removed by **dilation and curettage** (Figure 18).

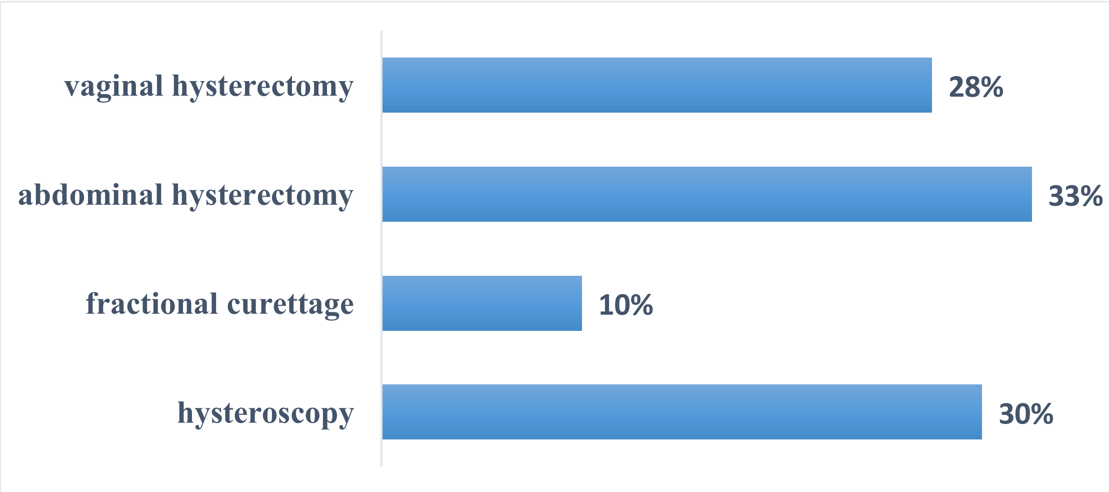

Figure 18. Types of surgical interventions performed in the studied cohort.

Our results showed that, in relation to the entire cohort, the **most common gynecological intervention was hysterectomy**, with **abdominal hysterectomy** performed more frequently than vaginal hysterectomy.

The **second most preferred method** was **hysteroscopy**, followed by **fractional curettage**. Nowadays, **fractional curettage alone ("blind" curettage)** is performed **very rarely**, only in cases of **profuse genital bleeding**, which prevents adequate hysteroscopic visualisation.

6.1. Frequency Distribution of Types of Surgical Interventions in Patients with EP, EC, and AE.

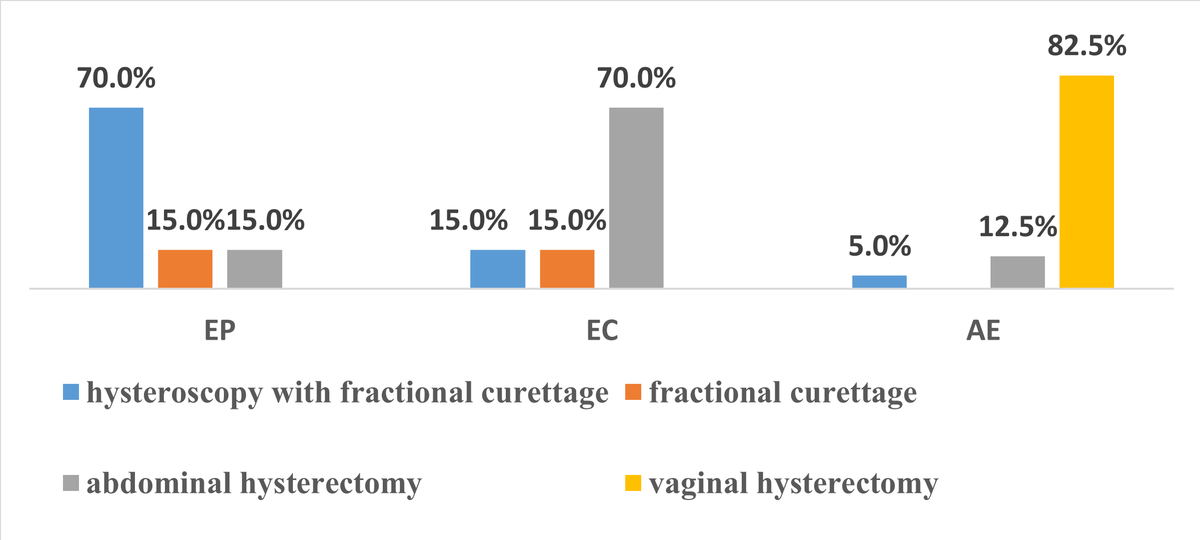

Figure 18. Frequency distribution of surgical interventions by diagnosis (EP, EC, AE).

Analysis of the results regarding the **frequency distribution of different types of surgical interventions** showed that in the **case groups**, the **lowest relative proportion** was for **fractional curettage alone**.

For the **EP group**, the **leading diagnostic and therapeutic method** was **diagnostic hysteroscopy with fractional curettage**, while in the **EC group**, **abdominal hysterectomy** predominated.

Atrophic endometrium in the control group was most frequently obtained through **vaginal hysterectomy**, with very low percentages obtained via hysteroscopy and curettage.

The analysis demonstrated a **statistically significant association** between the **type of surgical intervention** and the **type of intrauterine pathology** (**$\chi^2 = 130.67$; $p = 0.000$**).**Conclusions:**

- **Diagnostic panoramic hysteroscopy with fractional curettage** is the **method of choice for EPs < 10 mm**.
- **Abdominal hysterectomy** is the **leading intervention for EC treatment**.
- **Fractional curettage alone** is the **least frequently performed** in all three groups, illustrating the **modern trend to avoid it** due to its **low diagnostic value**.
- Following **vaginal hysterectomy** performed for genital prolapse, the **most common histological finding** was **atrophic endometrium**. **7. Analysis of Histological Results in the Studied Cohort**

7.1. Histological Types of Endometrial Polyps (EP)

Following the surgical interventions analysed above, tissue samples were **fixed in 10% formalin solution** and sent for **routine histological examination and immunohistochemistry** (as described in the Materials and Methods section).

A **high proportion** of histopathology reports issued by pathologists listed the diagnosis of **"endometrial polyp"**. In some cases, the **specific types of polyps** were indicated, with the **most common type** being **EPs with atrophic and cystic-atrophic changes** (Figure 19).

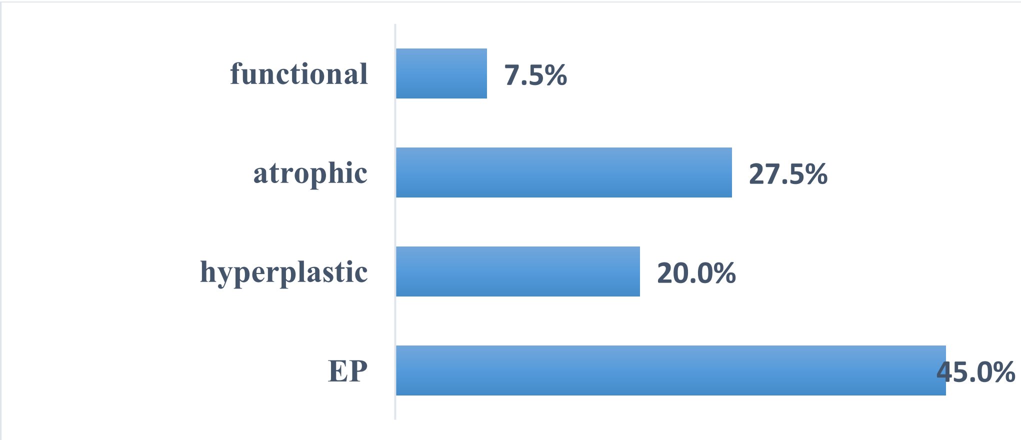

Figure 19. Histological variants of endometrial polyps (EP) identified in the present study.

The **second most frequent type** was **hyperplastic EPs**, while **functional EPs** were the **least common**. Importantly, **all polyps were benign** (Figure 20).

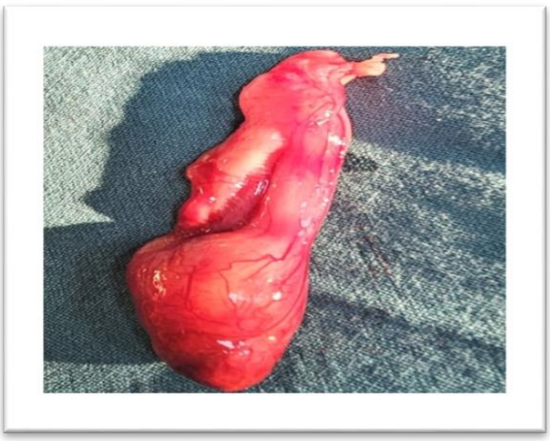

Figure 20. Pedunculated endometrial polyp.

7.2. Histological Types of Endometrial Carcinoma (EC)

After obtaining the results from the **routine histopathological examination**, we compared and grouped the different types of **endometrial carcinoma** according to **Bokhman's dualistic model**, which classifies EC into **Type I and Type II**.

- **Type I** included **endometrioid adenocarcinoma**.
- **Type II** included **serous papillary carcinoma** and **poorly differentiated squamous cell carcinoma**. Additionally, **one case** of **highly differentiated mixed type carcinoma** was registered (Figure 21).

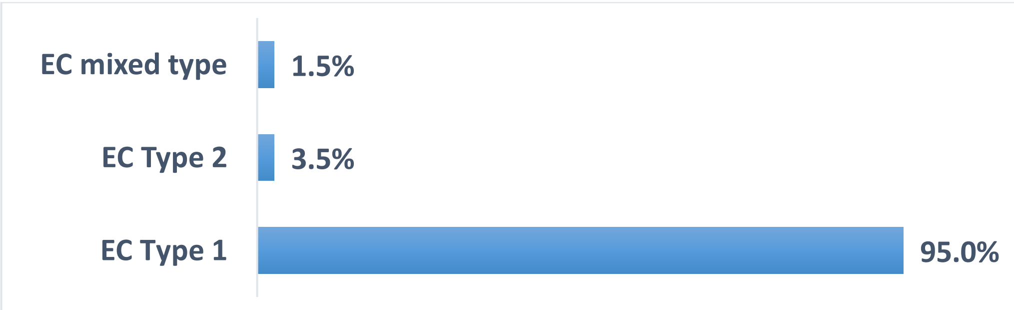

Figure 21. Frequency of different types of endometrial carcinoma (EC).

Our results confirmed a **higher frequency of Type I EC**. The **most common histological subtype**, according to the degree of differentiation, was **moderately differentiated endometrioid adenocarcinoma**, followed by **well-differentiated**, and in third place, **poorly differentiated adenocarcinoma**, based on the analysed results (Figure 22).

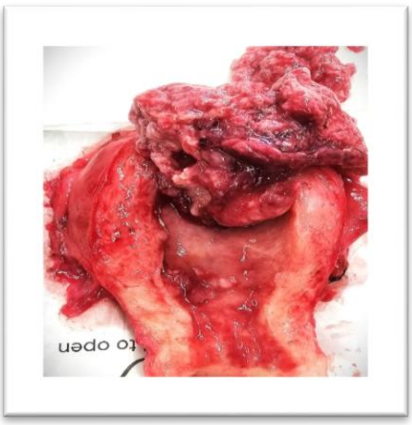

Figure 22. Macroscopic specimen of the uterus following hysterectomy showing the presence of an invasive tumour process in the fundal region.

7.3. Evaluation of Histological Results in the AE Group

In women with **atrophic endometrium (AE)**, the results showed that in **30% (n = 40)** of cases, **cystic changes** were also observed against the background of atrophic changes (Figure 23).

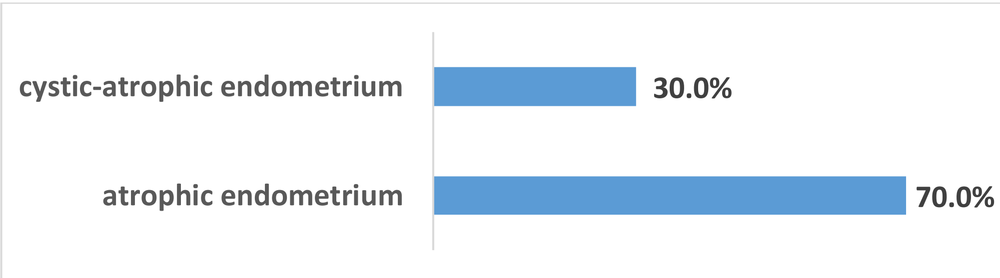

Figure 23. Histological results in the control group.

Nevertheless, the **relative proportion** of cases with **pure atrophic changes** was higher (Figure 24).

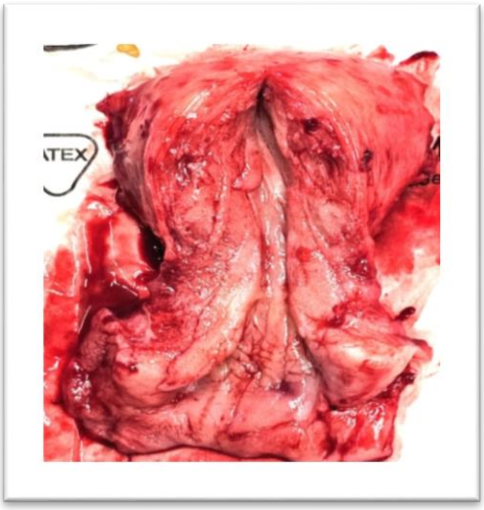

Figure 24. Macroscopic specimen of the uterus following hysterectomy showing atrophic endometrium.

Conclusions:

- The **most common histological type** in premenopausal and postmenopausal women was **EP with atrophic and cystic-atrophic changes**, all of which were **benign**.
- The **most common histological type of EC** was **Type I**.
- In **atrophic endometrium**, **cystic changes** can occur **without being associated with malignant processes**.

II. Comparative Analysis of Serum Estradiol Levels in the Three Groups of Women with EP, EC, and AE

In **premenopausal women**, **venepuncture** was performed during the **follicular phase of the menstrual cycle (days 7–10)**. Serum **estradiol (E2) concentration** was measured in **pg/ml**, with reference intervals of **24–114 pg/ml for the follicular phase** and **20–88 pg/ml for postmenopausal women**.

1. Comparative Analysis of Mean Estradiol Levels in the Three Groups According to Menopausal Status

The study included **24 premenopausal women**, divided into three groups based on their endometrial pathology: **EP group (n = 17)**, **EC group (n = 5)**, and **AE group (n = 2)** (Table 20).

Table 20. Comparative analysis of serum estradiol concentrations in different endometrial pathologies (premenopausal women).

GROUP	**n**	$\bar{x}$	$S\bar{x}$	**Min.**	**Max.**	**F**	**p**
EP	17	61,98	49,89	6,7	196,2	0,589	0,564
EC	5	46,18	45,10	16,4	118,6		
AE	2	27,49	24,49	10,2	44,8		

The **mean estradiol values** for the three groups were as follows: **EP group – 61.98 ± 49.89 pg/mL (n = 17)**, **EC group – 46.18 ± 45.10 pg/mL (n = 5)**, and **AE group – 27.49 ± 24.49 pg/mL (n = 2)**.

Estradiol levels **varied substantially** within each group, with **minimum values** of **6.7 pg/mL (EP)**, **16.4 pg/mL (EC)**, and **10.2 pg/mL (AE)**, while **maximum values** reached **196.2 pg/mL (EP)**, **118.6 pg/mL (EC)**, and **44.8 pg/mL (AE)**.

Results from the **ANOVA test** indicated **no statistically significant difference** between the mean estradiol levels among the groups (**F = 0.589, p = 0.564**). This suggests that despite observed differences in estradiol levels, these differences were **not statistically significant**.

From **Table 21**, it is evident that a total of **96 postmenopausal women** were divided into three groups based on **histopathologically confirmed diagnosis**.

GROUPS	n	$\bar{x}$	$S\bar{x}$	Min.	Max.	F	p
EP	23	245,16	1051,70	6,26	5068,00	1,707	0,187
EC	35	23,83	15,22	0,48	77,14		
AE	38	15,00	18,39	2,26	118,80		

The **mean serum estradiol level** was **significantly higher** in the **endometrial polyp (EP) group – 245.16 ± 1051.70 pg/mL (n = 23)** compared to the **endometrial carcinoma (EC) group – 23.83 ± 15.22 pg/mL (n = 35)** and the **atrophic endometrium (AE) group – 15.00 ± 18.39 pg/mL (n = 38)**.

However, the **high standard deviation** in the EP group (**1051.70**) indicates **substantial variability** in measured values.

Statistical analysis showed **no significant differences** between the groups (**F = 1.707; p = 0.187**), suggesting that the observed differences were **not sufficiently pronounced** to be considered statistically significant.

Although the **EP group** demonstrated a **tendency towards higher estradiol levels**, the lack of statistical significance prevents a **definitive conclusion** regarding serum estradiol as a **diagnostic or predictive biomarker** for differentiating endometrial pathologies in postmenopausal women.

In conclusion, among **pre- and postmenopausal women**, **no statistically significant association** was established between **mean estradiol levels** and the **type of endometrial pathology**, despite observed variations in the samples.

Table 22. Relationship between mean estradiol concentrations and obesity in premenopausal women with EP.

Compared pairs	MD	$S\bar{x}$	T	df	p
BMI (kg/m²) – estradiol (postmenopausal women)	-212,81	1052,43	-0,970	22	0,343
Estradiol (premenopausal women) – BMI (kg/m²)	38,30	51,35	3,075	16	0,007

The results of the analysis showed that in **premenopausal women**, there was a **statistically significant association** between **estradiol levels** and **obesity**, suggesting that obesity is an **independent risk factor** in the **pathogenesis of endometrial polyps**.

On the other hand, in **postmenopausal women**, **no such association** was observed. This may be explained by the **reduced production of estrogens after menopause**.

The **highest mean estradiol level** was observed in the patient with **mixed-type carcinoma (66.20 pg/mL)**, but due to the presence of only **one case** in this subgroup, statistical analysis was **not applicable**.

The **lowest mean values** were recorded in patients with **Type II EC (18.73 ± 10.30 pg/mL)**, while in **Type I (endometrioid carcinoma)** the mean value was **23.25 ± 14.04 pg/mL** (Table 21).

ANOVA statistical analysis (**F = 5.209; p = 0.011**) indicated that the differences between the subgroups were **statistically significant (p < 0.05)**, suggesting that **estradiol levels can vary substantially depending on the histological subtype of EC** in postmenopausal women.

Table 23. Relationship between estradiol levels, menopausal status, and BMI in women with EC.

Compared Pairs	**MD**	$S\bar{x}$	**t**	**df**	**p**
BMI (kg/m²) – estradiol (postmenopausal women)	9.32	13.60	4.055	34	0.000
Estradiol (premenopausal women) – BMI (kg/m²)	3.05	32.45	0.211	4	0.843

Results from the **t-test** showed a **statistically significant positive association** between **BMI** and **estradiol levels** in **postmenopausal women with endometrial carcinoma** (**t(34) = 4.055, p < 0.001**). This indicates that in this subgroup, **higher BMI is associated with elevated estradiol levels**.

In **premenopausal women**, **no statistically significant relationship** was found between estradiol levels and BMI (**t(4) = 0.211, p = 0.843**).

The data from our study demonstrate a **strong correlation between obesity and increased estradiol levels** in the postmenopausal group, which may explain the **higher risk of endometrial carcinoma** in obese women during this phase.

Analysis of the **relationship between age and estradiol levels** in women with endometrial carcinoma in **both premenopausal and postmenopausal stages** is presented in **Table 24**.

Table 24. Relationship between estradiol levels, age, and menopausal status in women with endometrial carcinoma.

Compared Pairs	**MD**	$S\bar{x}$	**t**	**df**	**p**
Age – estradiol (postmenopausal women)	41,65	18,58	13,263	34	0,000
Estradiol (premenopausal women) – age	-1,01	47,02	-0,048	4	0,964

The results showed that in **postmenopausal women**, there was a **statistically significant relationship** between **age and estradiol levels** (**mean difference = 41.65; t(34) = 13.263; p < 0.001**). In contrast, in **premenopausal women**, **no statistically significant relationship** was observed between age and estradiol levels (**mean difference = -1.01; t(4) = -0.048; p = 0.964**).

Based on these results, it can be concluded that **menopausal status is a key moderating factor** in the interaction between **age and endocrine profile** in women with endometrial carcinoma.

Conclusions:

- **Estradiol levels vary** depending on the **histological subtype of EC** in postmenopausal women.
- **Obesity is associated with elevated estradiol levels** and a **higher risk of endometrial carcinoma** in postmenopausal women.

III. Comparative Evaluation of Estrogen Receptor (ER) and Progesterone Receptor (PR) Expression in EP, EC, and AE

1. Analysis of Immunoexpression in EP

1.1. Analysis of Estrogen and Progesterone Receptor Immunoexpression in EP Figure 29.

In the present study, a **quantitative analysis** of **estrogen receptor (ER) expression** was performed using **immunohistochemical staining** at different structural levels of endometrial polyps:

- **First level:** glandular component
- **Second level:** surface epithelium
- **Third level:** stromal component

The study included **40 histological samples** (Table 25).

Table 25. Analysis of ER expression in EP at levels I, II, and III.

	n	$\bar{x}$	$S\bar{x}$	Min	Max
ER- I	40,0	82,15	55,96	21,0	243,0
ER- II	40,0	75,2	46,26	14,0	197,0
ER- III	40,0	65,65	66,90	0,0	214,0

At the **first level** – **glands of the polyp (ER I)** – the **highest mean expression of estrogen receptors** was observed (**82.15 ± 55.96; n = 40**), with values ranging from **21.0 to 243.0**. These results indicate a **strong hormonal sensitivity** of the glandular epithelium within the polyp structures. The **high variability (large SD)** may reflect **heterogeneity** in the degree of differentiation and functionality of glandular cells across different samples.

At the **second level** – **surface/remaining endometrial epithelium (ER II)** – a **moderate reduction** in mean expression was observed (**75.2 ± 46.26; n = 40**), with measured values ranging from **14.0 to 197.0**. Although decreased compared to the glandular component, this value still reflects **preserved hormonal activity** typical of endometrial epithelium, suggesting that epithelial structures of the polyp maintain some degree of endocrine dependence.

At the **third level** – **stroma of the polyp (ER III)** – the **lowest mean expression** of estrogen receptors was recorded (**65.65 ± 66.90; n = 40**), with significant data dispersion (**0.0 – 214.0**). This indicates **lower and highly variable hormonal sensitivity** in the stromal component. The presence of **zero values** in some cases may be interpreted as **absence of receptor expression**, reflecting possible **functional inertness** of the stroma regarding estrogen signalling in certain polypoid formations.

Analysis of the **mean values of progesterone receptors (PR)** in EP across the different levels (**Table 26, Figure 25**) – first (I), second (II), and third (III) – showed **significant differences** in PR expression.

At the **first level (PR I)**, the **highest mean value** of receptors was observed (**94.0 ± 66.78; n = 40**), indicating **greater receptor activity** in the glands of EP. However, PR values were characterised by **substantial variability (SD = 66.776)**, and the **range of measured values was wide** (**Min = 14.0; Max = 304.0**), demonstrating large **individual differences**.

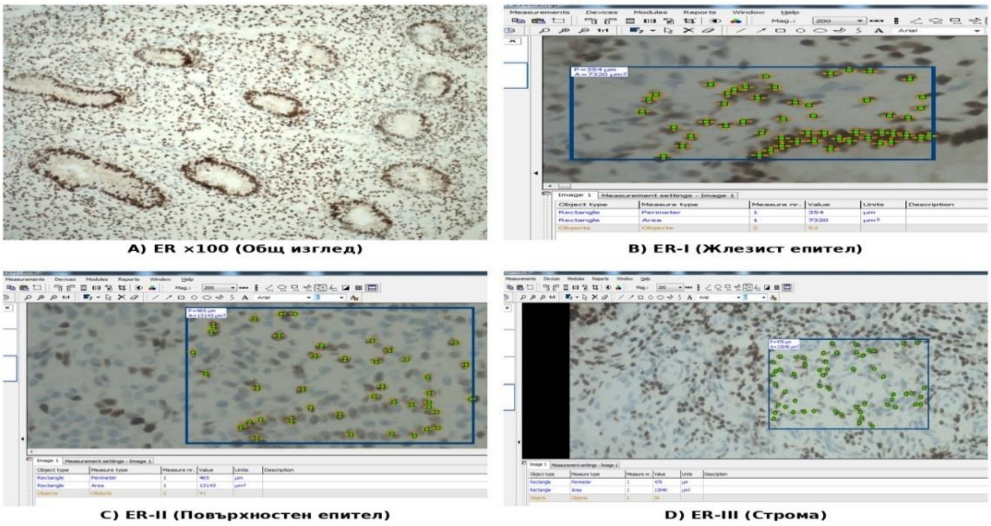

Figure 25. Immunoexpression and morphometric analysis of estrogen receptors (ER) in endometrial polyps (magnification ×100 and ×200).

At the **second level (PR II)**, the **mean receptor value** slightly decreased to **90.3 ± 69.08 (n = 40)**, with variability (**SD = 69.08**) remaining substantial. The **range of values** also remained wide (**Min = 13.0; Max = 279.0**), suggesting a **partial reduction** in receptor activity compared to the first level, while **large differences between samples persisted**.

At the **third level (PR III)**, the **lowest mean value** of progesterone receptors was observed (**67.78 ± 69.02; n = 40**), indicating a **significant decrease** in receptor activity compared to the first and second levels. However, variability remained high (**SD = 69.023**), with the **minimum value reaching 0.0**, indicating **complete absence** of progesterone receptors in some samples. The **maximum value (265.0)** demonstrated a **wide range of measured values**, despite the reduced mean.

Table 27. Analysis of PR expression in EP at levels I, II, and III.

Променлива	n	$\bar{x}$	$S\bar{x}$	Min	Max
PR - I	40,0	94,0	66,78	14,0	304,0
PR - II	40,0	90,3	69,08	13,0	279,0
PR - III	40,0	67,78	69,02	0,0	265,0

The results of the analysis showed that **progesterone receptor (PR) expression** in EP **varied significantly** across the different levels examined. The **first level** demonstrated the **highest**

receptor activity, while the **third level** exhibited the **lowest**, with **substantial individual differences** between samples.

Despite the decrease in mean values at the third level, **variability of measured values remained high** across all levels, suggesting differences in the **expression of progesterone receptors** among individual samples.

These findings may have **significant clinical and physiological implications** related to the **dynamics of progesterone regulation** in the endometrium during different stages of physiological processes.

In summary, the following conclusions can be drawn:

- **Progesterone receptors** are predominantly expressed at the **superficial level (I)**, with a **progressive decrease** towards deeper layers (III), including **complete absence** in some cases.
- **Estrogen receptors** maintain a **more stable expression**, but a **decrease is observed** from the glandular component towards the stroma, reflecting **tissue differentiation**.
- **Both types of receptors** exhibit **selectivity in their expression**.
- **Progesterone expression** shows a **more pronounced dynamic**, making it a **more sensitive marker** for disturbances in hormonal regulation.
- **Estrogen heterogeneity** indicates **morphofunctional differences** within the polyps.

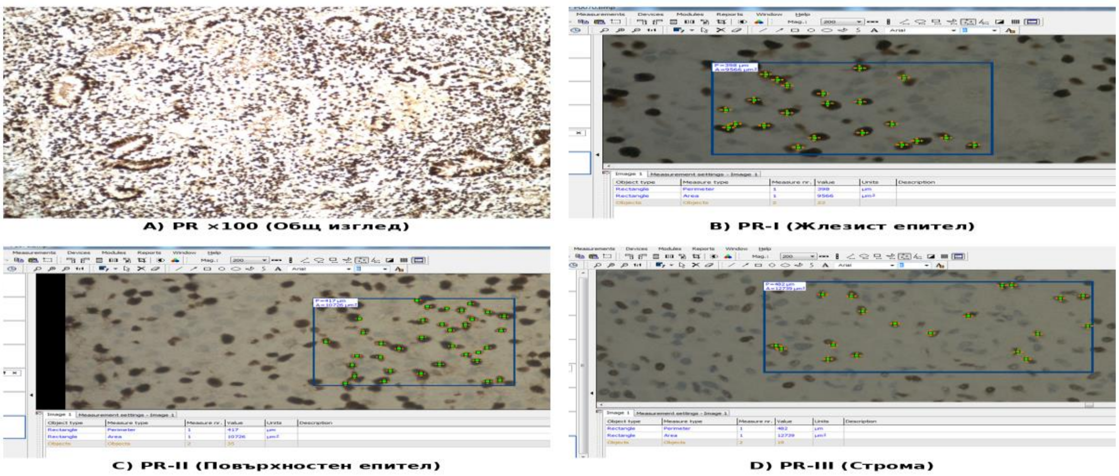

Figure 25. Immunoexpression and morphometric analysis of progesterone receptors (PR) in endometrial polyps (magnification ×100 and ×200).

2.1. Analysis of Estrogen Receptor (ER) Immunoexpression in EC

An **assessment of estrogen receptor (ER) expression** was performed in **three tissue components** of women diagnosed with **endometrial carcinoma (EC) (n = 40)**.

The **minimum expression values** varied significantly across the different tissues. In the **glands (6.0)** and **epithelium (16.0)**, expression was detected in **all samples**, whereas in the **stroma**, cases of **complete absence of estrogen receptors (0.0)** were recorded.

The **maximum expression values** also varied, with the **highest value observed in the epithelium (234.0)**, highlighting the **potential role of epithelial cells** in the **hormonally driven pathogenesis** of endometrial carcinoma (**Table 26**).

Table 26. Analysis of estrogen receptor expression in women with endometrial carcinoma.

	n	$\bar{x}$	$S\bar{x}$	Min	Max
ERI	40,0	75,2	42,72	6,0	196,0
ERII	40,0	75,08	51,47	16,0	234,0
ERIII	40,0	71,25	48,93	0,0	169,0

The results indicate that **estrogen receptor expression** is **relatively evenly distributed** between the **glandular** and **epithelial tissues**, whereas in the **stroma**, **lower mean levels** and **cases of absent expression** were observed. These data suggest a **potential dependence** of tumor biology on the **degree of hormonal regulation**.

2.2. Analysis of Progesterone Receptor (PR) Immunoexpression in EC

The results showed that the **highest expression of progesterone receptors** was observed in the **glandular component (PR I)**, with a **mean value of 77.78**. The **epithelial component (PR II)** demonstrated a slightly lower mean value (**70.3**), while the **lowest expression** was found in the **stromal component (PR III)** with a **mean value of 59.53**.

Table 27. Progesterone receptor expression in different tissue components of endometrial carcinoma.

	n	$\bar{x}$	$S\bar{x}$	Min	Max
PRI	40,0	77,78	39,07	26,0	161,0
PRII	40,0	70,38	43,14	8,0	219,0
PRIII	40,0	59,53	39,84	0,0	171,0

Based on the obtained data, several key conclusions can be drawn:

- The **glandular component** demonstrates the **highest expression of progesterone receptors (PR)** in endometrial carcinoma.
- The **epithelial component** also maintains **high levels of receptor expression**, albeit with **greater variability**.
- The **stromal component** shows the **lowest expression**, with **complete absence of PR** in some cases.
- **PR expression** plays a **crucial role** in determining prognosis and therapeutic management in patients.
- **High levels of PR** are associated with a **more favorable prognosis**, **lower tumor aggressiveness**, and **better response** to **progestin therapy**.
- **Reduced expression**, particularly in the stroma, may be an indicator of **more advanced and aggressive neoplastic transformation**.
- Assessment of **progesterone receptors across all tissue components** of endometrial carcinoma is **essential for accurate risk stratification** and for **optimising therapeutic planning** in affected patients.

3.1. Distribution of Estrogen and Progesterone Receptors in Different Components of Atrophic Endometrium

Table 28 clearly demonstrates a **pronounced gradient** in the expression of **steroid receptors**. The **highest mean values** were observed in the **glandular epithelium**, highlighting its **relative hormonal activity**, which is **preserved even under conditions of atrophy**.

At the **second level**, a **moderate reduction** in receptor expression was noted. The **stroma** demonstrated the **lowest values**, indicating a **markedly reduced sensitivity** to **estrogenic and progestogenic influences**.

The presence of **minimum values reaching zero** in some cases is indicative of a **complete absence of receptor expression** in part of the examined samples.

Table 28. Distribution of estrogen and progesterone receptors in different components of atrophic endometrium.

	n	$\bar{x}$	$S\bar{x}$	Min	Max
ERI	40,0	79,17	62,29	0,0	250,0
ERII	40,0	68,9	47,57	0,0	224,0
ERIII	40,0	53,2	44,39	0,0	185,0
PRI	40,0	83,02	68,90	0,0	277,0
PRII	40,0	70,12	54,71	6,0	200,0
PRIII	40,0	50,9	50,37	7,0	213,0

The conclusions that can be drawn are: In **atrophic endometrium**, there is a characteristic **decrease in hormonal sensitivity** from the **glandular to the stromal component**.The **glands remain the primary site of preserved hormonal regulation**, which has **potential significance for therapeutic approaches** in **estrogen-deficient conditions**.

IV. Comparative Analysis of p53 and Ki-67 Biomarker Expression in the Examined Groups: EP, EC, and AE

1. Analysis of p53 and Ki-67 Expression in EP. The obtained results emphasize that **proliferative activity** and **cell cycle regulation** in endometrial polyps are **mainly concentrated** within the **epithelial and glandular elements**. The **absence of p53 expression** in the **stromal component** suggests that this area does **not actively participate in potential neoplastic processes** within the polyps. The **low proliferative activity** of the stroma, as reflected by **Ki-67**, further supports this concept. The **immunoexpression profile** of p53 and Ki-67 in the examined samples confirms the **predominantly benign nature** of endometrial polyps, while also providing additional information regarding **cellular heterogeneity** within the individual structural components.

Table 29. Immunoexpression of p53 and Ki-67 biomarkers in different structural components of endometrial polyps.

	n	$\bar{x}$	$S\bar{x}$	**Min**	**Max**
p53I	**40,0**	**8,17**	**12,07**	**0,0**	**52,0**
p53II	**40,0**	**9,1**	**17,13**	**0,0**	**68,0**
p53III	**40,0**	**0,0**	**0,0**	**0,0**	**0,0**
Ki67I	**40,0**	**21,02**	**19,37**	**0,0**	**90,0**
Ki67II	**40,0**	**14,92**	**16,21**	**0,0**	**63,0**
Ki67III	**40,0**	**0,17**	**0,67**	**0,0**	**3,0**

The conclusion that can be drawn is that the **expression of p53 and Ki-67** in **endometrial polyps** is **predominantly confined** to the **glandular and epithelial components**, while in the **stroma**, it is either **absent or minimal** (**Figures 26 and 27**).

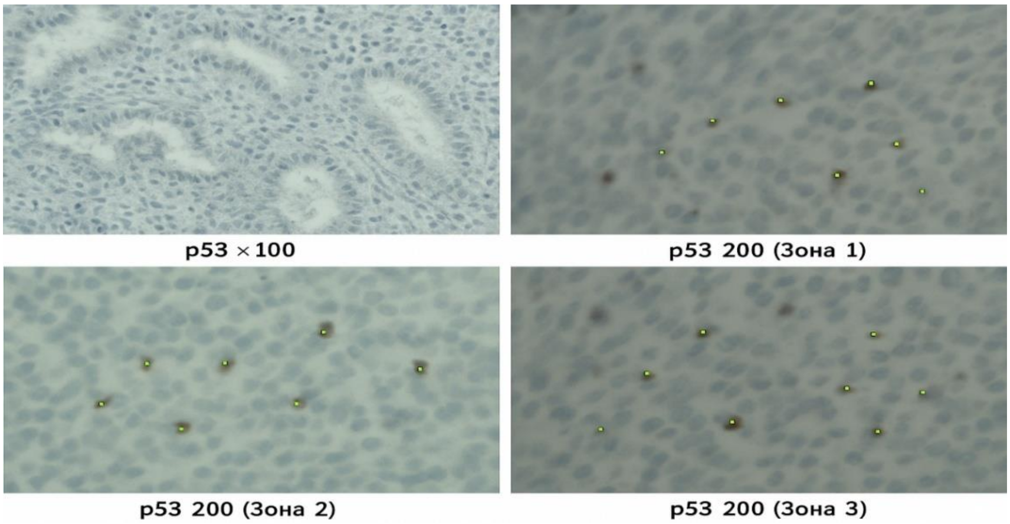

Figure 26. Immunohistochemical expression and morphometric analysis of p53 at ×100 and ×200 magnification in endometrial polyps (EP).
Zone 1 – glands; Zone 2 – epithelium; Zone 3 – stroma.

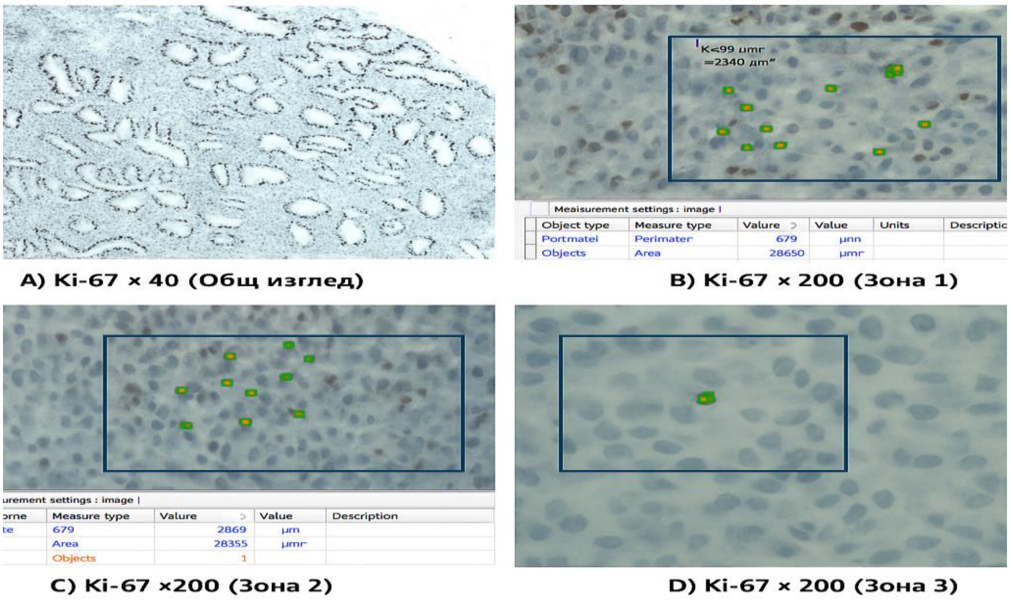

Figure 27. Immunohistochemical expression of Ki-67 at ×40 and ×200 magnification in endometrial polyps (EP).

Zone 1 – glands; Zone 2 – epithelium; Zone 3 – stroma.

2. Evaluation of Menopausal Status and Expression of p53 and Ki-67 Biomarkers in Glands, Epithelium, and Stroma of EP

The expression of **p53 and Ki-67 biomarkers** was analyzed in **three levels** of endometrial polyps – the **glandular component (I)**, **epithelial component (II)**, and **stromal component (III)** – in **premenopausal and postmenopausal women** (**Table 30**).

Table 30. Expression of p53 and Ki-67 in three levels of EP in premenopausal and postmenopausal women.

Група	Има/Няма	n	$\bar{x}$	$S\bar{x}$	F	p
p53-I	няма	17	11,77	13,35	2,728	0,107
p53-I	има	23	5,52	10,56		
p53-I	Total	40	8,18	12,08		
p53-II	няма	17	11,53	17,46	0,588	0,448
p53-II	има	23	7,30	17,05		
p53-II	Total	40	9,1	17,13		
p53-III	няма	17	0	0	-	-
p53-III	има	23	0	0	-	-
p53-III	Total	40	0	0	-	-
Ki67-I	няма	17	33,18	21,98	16,138	0
Ki67-I	има	23	12,04	10,76		
Ki67-I	Total	40	21,03	19,38		
Ki67-II	няма	17	23,59	20,02	10,495	0,002
Ki67-II	има	23	8,52	8,59		
Ki67-II	Total	40	14,93	16,2		
Ki67-III	няма	17	0,41	1,0	3,907	0,055
Ki67-III	има	23	0	0		
Ki67-III	Total	40	0,18	0,68		

From **Table 30**, it can be seen that **p53 expression in glands (p53-I)** shows a **higher mean value** in **premenopausal women** compared to **postmenopausal women**, although the difference does not reach statistical significance (**F = 2.728; p = 0.107**). A similar trend is observed at the **epithelial level (p53-II)**, where the mean expression in premenopausal women is **11.53**, compared to **7.30** in postmenopausal women, but again **without a statistically significant difference** (**F = 0.588; p = 0.448**).

In the **stroma (p53-III)**, **no p53 expression** is observed in either group, regardless of menopausal status, indicating **no role of this biomarker** in the stromal component of endometrial polyps.

The **proliferative marker Ki-67** shows **significant differences** according to menopausal status. At the **glandular level (Ki67-I)**, premenopausal women demonstrate **significantly higher Ki-67 expression (33.18)** compared to postmenopausal women (**12.04**), with this difference being **statistically significant** (**F = 16.138; p < 0.001**). This suggests **more active proliferative activity in glands** of premenopausal women.

Similarly, in the **epithelial component (Ki67-II)**, Ki-67 expression is significantly higher in the premenopausal group compared to the postmenopausal group (**F = 10.495; p = 0.002**). At the **stromal level (Ki67-III)**, expression is **minimal to absent** in both groups, with only a slightly higher activity observed in premenopausal women compared to postmenopausal women (**F = 3.907; p = 0.055**), a difference that is **at the threshold of statistical significance**.

The conclusions that follow are:

- **Menopausal status** has a **significant impact** on **proliferative activity** (as measured by Ki-67) in both the **glandular and epithelial components** of endometrial polyps, with activity being **higher in premenopausal women**.
- **p53 expression** does **not show a statistically significant dependence** on menopausal status.
- In the **stromal component**, both biomarkers show **minimal to absent expression**.

2. Analysis of Biomarker Distribution in EC

This study analyzed the **expression of p53 and Ki-67 biomarkers** in patients diagnosed with **endometrial carcinoma (EC)**.

In the **glandular component**, the **mean p53 expression** is **26.52**, with large variability between individual cases (range **0 to 112**). In the **epithelial component**, the mean expression is **24.42%**, also with a wide range (**0%–94%**). In the **stromal component (p53-III)**, **no p53 expression** was detected.**Conclusion:** p53 shows **moderate to high expression** in the **glands and epithelium** of endometrial carcinoma, but **not in the stroma**. The high variability suggests that p53 expression is associated with **heterogeneity in the molecular profile of the tumor**.

Table 31. Expression of p53 and Ki-67 biomarkers in EC.

	n	$\bar{x}$	$S\bar{x}$	**Min**	**Max**
p53-I	40.0	26,52	27,05	0,0	112,0
p53-II	40.0	24,42	27,25	0,0	94,0
p53-III	40.0	0,0	0,0	0,0	0,0
Ki-67-I	40.0	32,65	25,48	0,0	138,0
Ki-67-II	40.0	30,12	20,33	0,0	74,0
Ki-67-III	40.0	0,0	0,0	0,0	0,0

For **Ki-67 expression levels** in the **glandular component (Ki-67-I)**, the **mean expression** is **32.65**, ranging from **0 to 138**. In the **epithelial component (Ki-67-II)**, the mean expression is **30.12**, with a range between **0 and 74**. In the **stromal component (Ki-67-III)**, **no expression** was detected.**The conclusion that can be drawn is that:**

- **Ki-67 demonstrates high proliferative activity** in the **glandular and epithelial components** of **endometrial carcinoma**, while **no proliferative activity** is observed in the **stroma**.
- These data highlight that **tumor cells** have an **active cell cycle** and **high mitotic activity**.

3. **Analysis of Biomarkers in Different Components of Atrophic Endometrium.** The **atrophic endometrium** is characterized by **low proliferative and malignant potential** (**Table 32**).

Table 32. p53 and Ki-67 biomarkers in the three levels of atrophic endometrium.

	n	$\bar{x}$	$S\bar{x}$	Min	Max
p53-I	40.0	4,85	10,62	0,0	56,0
p53-II	40.0	0,55	2,20	0,0	12,0
p53-III	40.0	0,0	0,0	0,0	0,0
Ki67-I	40.0	6,65	5,89	0,0	27,0
Ki67-II	40.0	3,77	5,92	0,0	26,0
Ki67-III	40.0	0,6	2,90	0,0	18,0

Analysis of p53 and Ki-67 Expression in Atrophic Endometrium

Analysis of the expression levels of the two key molecular markers – **p53 and Ki-67** – confirms the **profile of stability and low proliferative activity** in atrophic endometrium.

- **p53 expression** in the **glandular component** has a mean value of **4.85**, ranging from **0 to 56**. At the **second level**, expression is **dozens of times lower**, while at the **third level**, it is **absent**. This clearly indicates the **absence of active genetic instability processes in the stroma**, confirming that **potential molecular alterations are focused in the epithelial-glandular component**.
- Parallel analysis of **Ki-67**, a marker of cell proliferation, also reveals **limited activity**. In **glandular structures**, the mean expression is **6.65**, while in the **epithelial layer**, it is **3.77**. The presence of Ki-67 positive cells, even at minimal percentages, suggests that **atrophic endometrium may still contain small foci of reactive regeneration**.
  Conclusion:
  The **atrophic endometrium** is a tissue with **low malignant transformation potential**.

V. ANALYSIS OF THE RELATIONSHIPS BETWEEN HORMONAL RECEPTOR EXPRESSION, BIOMARKERS, SERUM ESTRADIOL CONCENTRATIONS, AND THE INCIDENCE OF ENDOMETRIAL POLYPS

1. Analysis of Hormonal Receptors and Biomarkers in the Three Groups

In all three groups (**endometrial polyps (EP), endometrial carcinoma (EC), and atrophic endometrium (AE)**), **high expression of estrogen (ER) and progesterone receptors (PR)** is observed in the **glandular and epithelial components**.

- In the **EP group**, the **highest PR and ER values** are recorded in both **glandular and epithelial components**.
- In **EC**, there is a **slight reduction in hormonal receptor expression**, reflecting **retained but partially lost hormonal sensitivity** of the tumor tissue.
- In **AE**, **hormonal receptor levels remain high**.

Regarding **p53 expression** as a **key indicator of genomic instability and tumorigenesis**:

- In **EP**, **p53 expression is low at all tissue levels**, confirming the **benign nature of these lesions**.
- In **EC**, there is a **substantial increase in p53 expression**, especially in the **glandular and epithelial components**, consistent with **TP53 gene mutations characteristic of malignant neoplasms**.
- In **AE**, **p53 expression is minimal to absent**, reflecting **genetic stability**.

Analysis of **Ki-67 expression** as a classic marker of **cell proliferation** shows:

- In **EP**, Ki-67 expression is **moderate**.
- In **EC**, **significantly higher Ki-67 expression** is recorded, particularly in the **glands**, indicating **increased mitotic activity**.
- In **AE**, Ki-67 expression is **minimal**.

These dependencies are presented in **Figures 28, 29, and 30**.

The discussed results delineate the **molecular profiles** characteristic of each of the three groups.

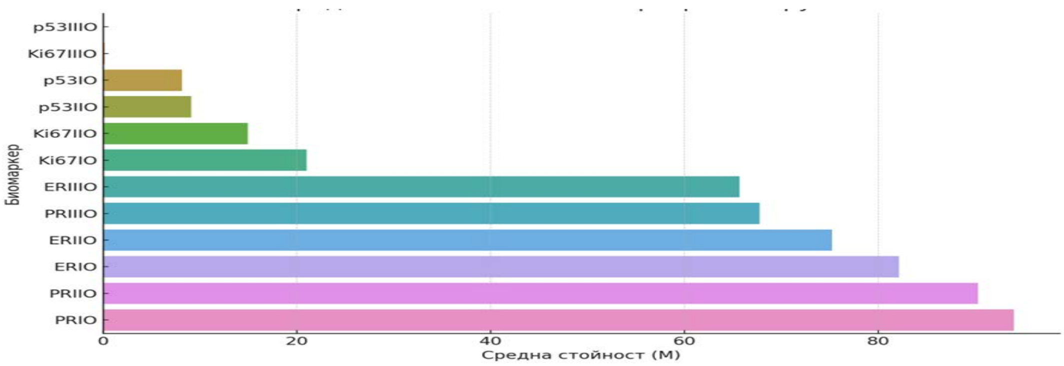

Figure 28. Mean values of hormonal receptors and biomarkers in endometrial polyps (EP).

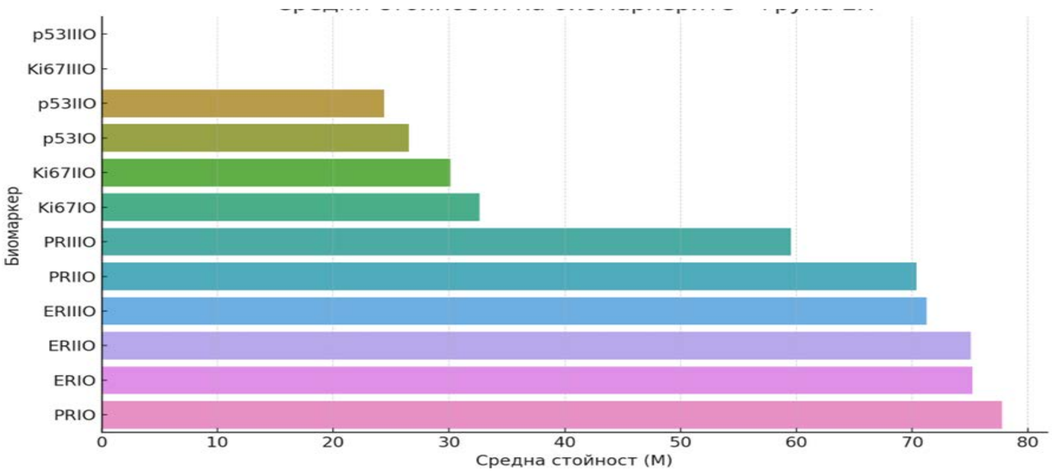

Figure 29. Mean values of hormonal receptors and biomarkers in endometrial carcinoma (EC).

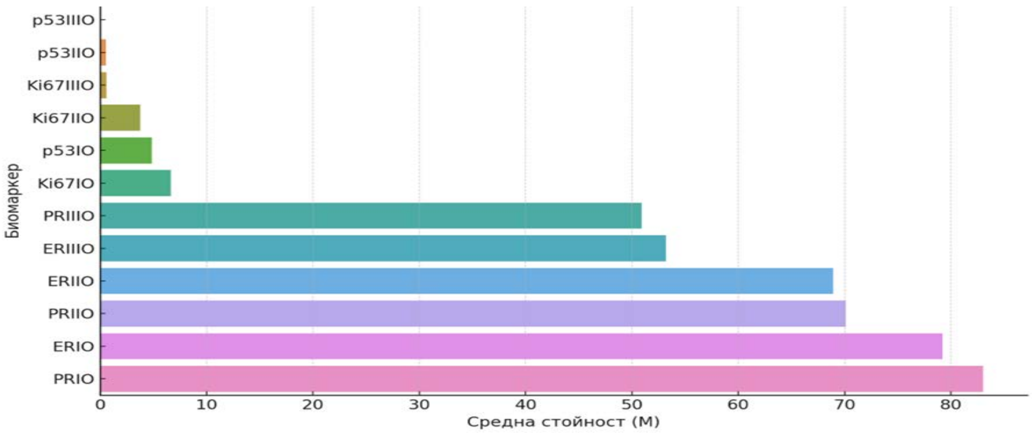

Figure 30. Mean values of hormonal receptors and biomarkers in atrophic endometrium (AE).

Conclusions:

- **Endometrial polyps (EP):** demonstrate hormonal dependence, low proliferative activity, and genomic stability, supporting their benign nature.
- **Endometrial carcinoma (EC):** shows reduced hormonal sensitivity, high proliferative activity, and increased genetic instability, consistent with its malignant nature.
- **Atrophic endometrium (AE):** retains high hormonal sensitivity with low cellular activity.

2. Evaluation of BMI and expression of hormonal receptors and biomarkers in different endometrial tissues using correlation analysis by groups. Correlation analysis between body mass index (BMI) and the expression of biomarkers and hormonal receptors revealed significant differences among the three histological groups. In endometrial polyps, a negative correlation was observed between BMI and cellular proliferation (Figure 31).

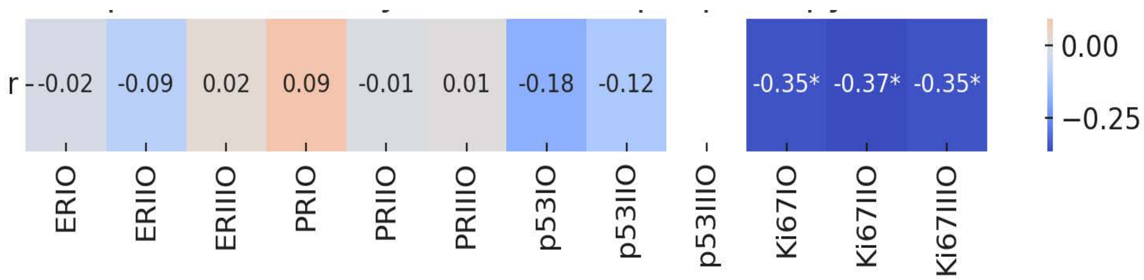

Figure 31. Heatmap – correlations (r) between BMI and biomarkers (EP group).

The heatmap indicates a weak negative correlation between BMI and Ki67 expression in the glandular component (r = –0.35), epithelial component (r = –0.37), and stromal component (r = –0.35), with statistical significance in two of these correlations (*). No significant associations were observed between BMI and the expression of p53, ER, and PR.

In endometrial carcinoma, higher BMI is associated with increased hormonal receptor expression (ER/PR), reflecting a potential dependence of tumour growth on metabolic and endocrine factors. The atrophic endometrium demonstrates a stable molecular profile, independent of the patient's body mass. **(Figures 32 and 33).**

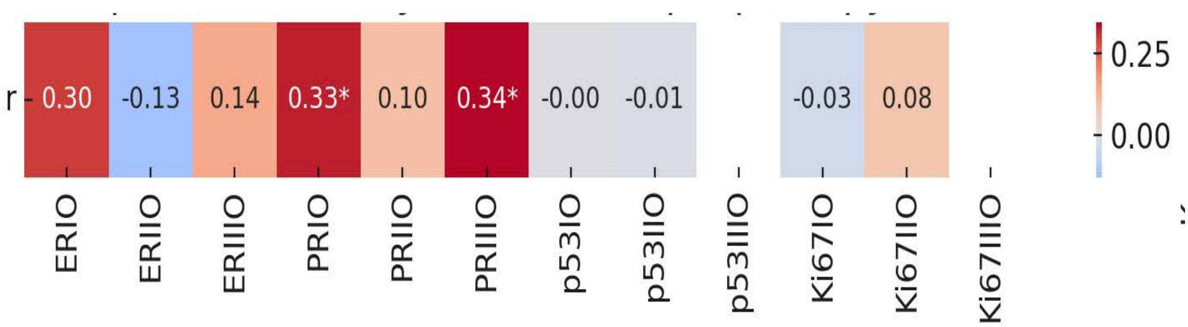

Figure 32. Heatmap – correlations between BMI, hormonal receptors, and biomarkers (EC group).

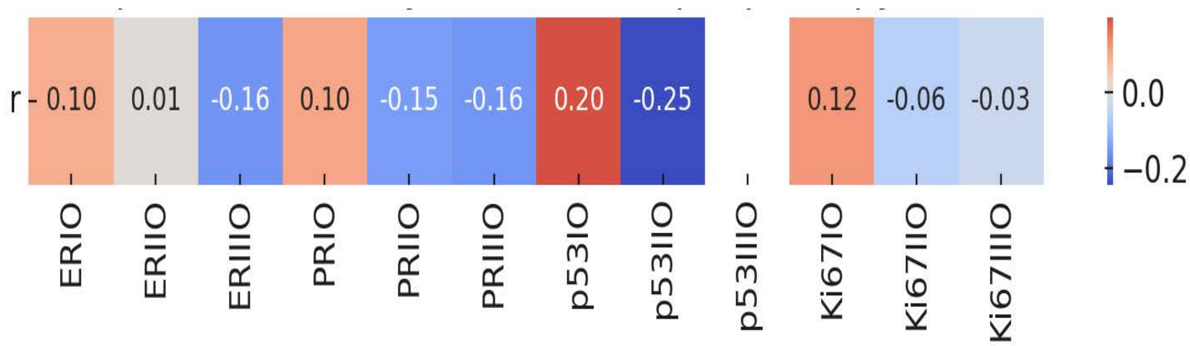

Figure 33. Heatmap – correlations between BMI, hormonal receptors, and biomarkers (AE group).

From Figure 33, it is evident that there are moderately positive correlations between BMI and PR expression in the glandular (r = 0.33*) and epithelial components (r = 0.34*), as well as a weak positive correlation with ER in the glandular component (r = 0.30). Notably, no significant correlations are observed between BMI and the expression of p53 and Ki67.

Conclusion: In patients with endometrial carcinoma, higher BMI is associated with increased expression of progesterone and estrogen receptors, particularly in the glandular and epithelial components.

3. **Assessment of the relationship between obesity and biomarkers and hormonal receptors in the EP and EC groups using Spearman correlation analysis.**

Figure 34 illustrates the graphical output of the Spearman correlation analysis, allowing assessment of the strength and direction of associations between BMI, as an indicator of metabolic factors, and the expression of key biomarkers (ER, PR, Ki67) in endometrial polyps (EP) and endometrial carcinoma (EC).

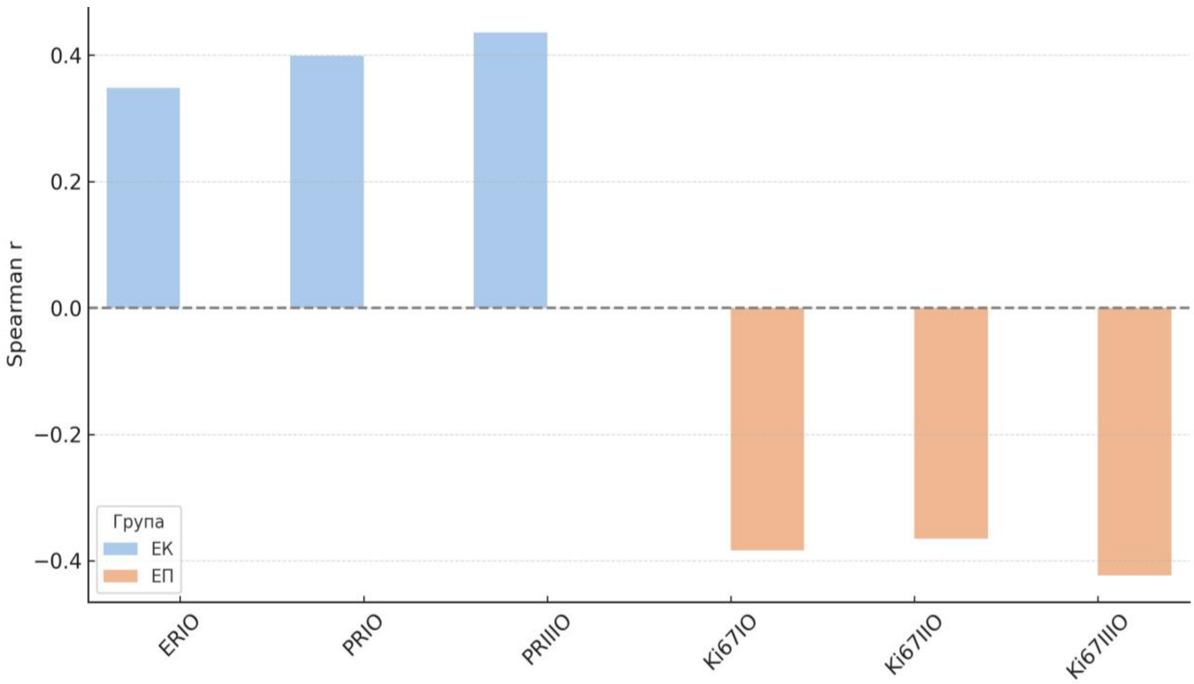

Figure 34. Correlation dependencies in the EP and EC groups.

In patients with endometrial carcinoma, positive and statistically significant correlations were established between BMI and the expression of hormonal receptors and Ki-67. In the group with endometrial polyps, negative significant correlations were identified between BMI and the expression of the proliferative marker Ki-67.

Conclusions:

- The obtained data underscore the substantial role of metabolic status in both benign and malignant endometrial lesions.
- In endometrial carcinoma, obesity is associated with enhanced hormonal receptor expression, whereas in endometrial polyps, higher BMI correlates with reduced cellular proliferation.

4. **Analysis of statistically significant correlations between biomarkers in the three groups.**

A correlation analysis was performed using Spearman coefficients to evaluate the internal interactions among the three conditions – atrophic endometrium (AE), endometrial carcinoma (EC), and endometrial polyps (EP). Figures 35, 36, and 37 illustrate these relationships.

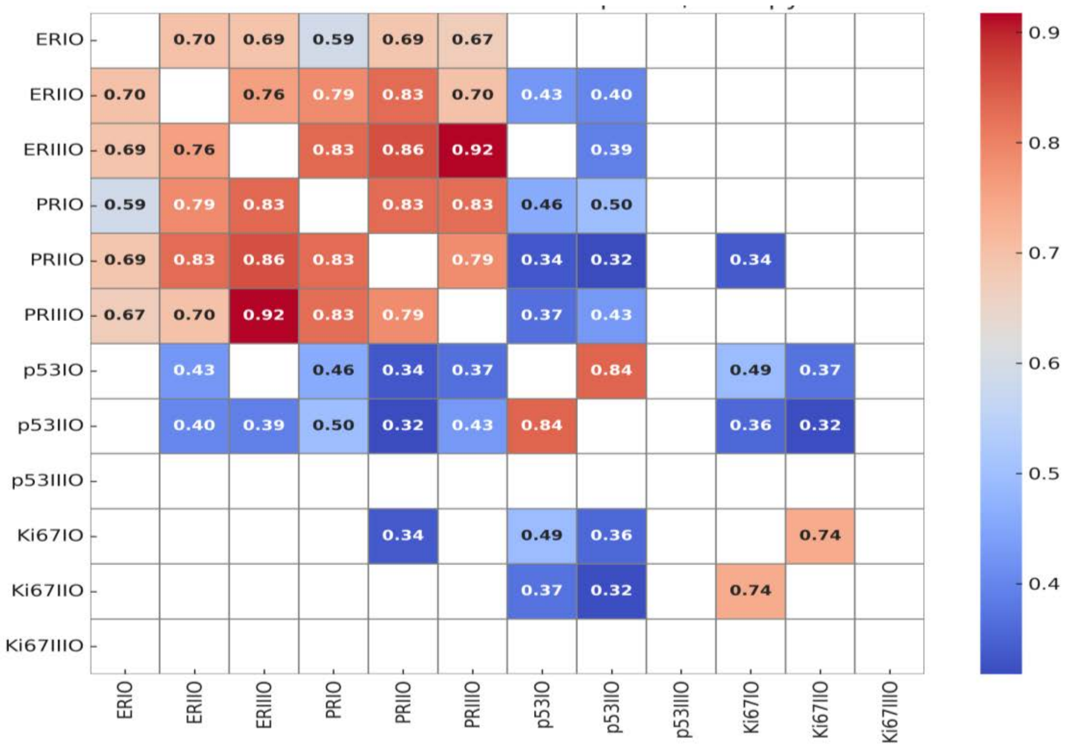

Figure 35. EP – significant correlations

From the figure, it is evident that in the EP group there are exceptionally strong interrelationships between ER and PR at all tissue levels (r = 0.67–0.92). Notably, there is also a high degree of correlation between ER/PR and p53 expression (r = 0.32–0.50). Significant correlations are observed between Ki67 and hormonal receptors (r = 0.32–0.49), as well as internally among the different Ki67 levels (r = 0.74).

Conclusion:

Endometrial polyps demonstrate a highly coordinated molecular hormonal regulation, corresponding to their biological profile as benign yet hormonally active lesions.

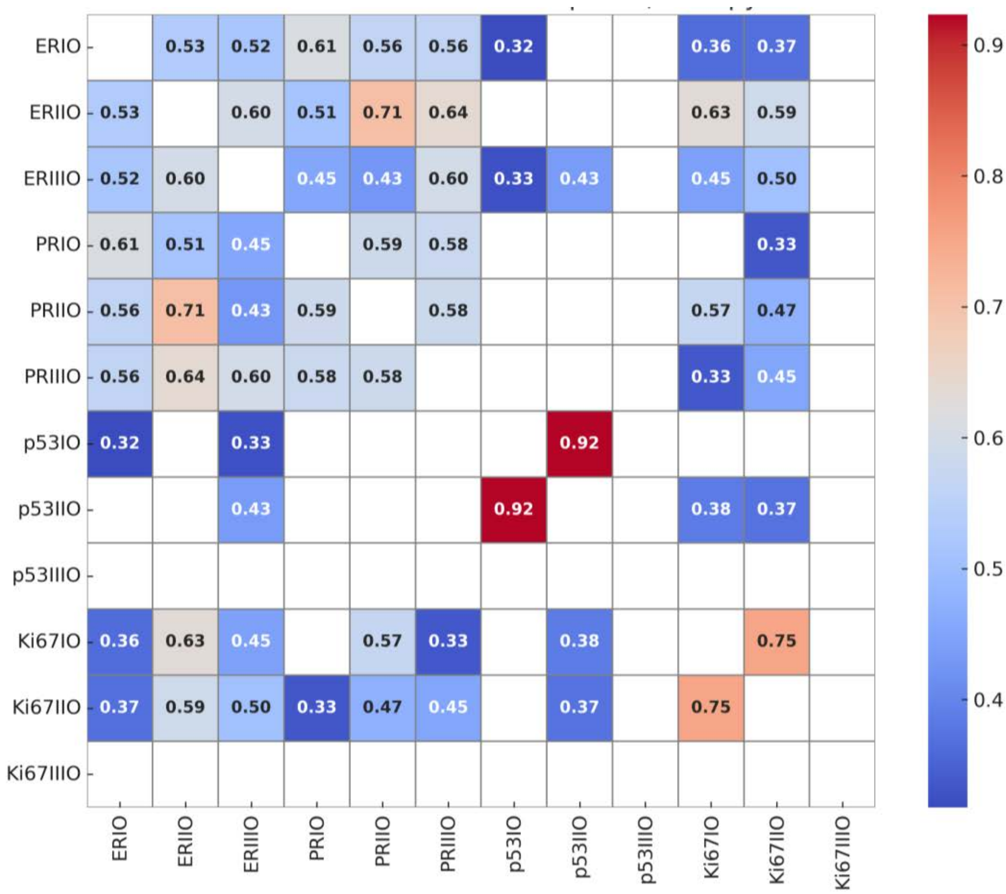

Figure 36. EC – significant correlations

From Figure 36, it can be concluded that there are strong correlations between ER and PR across the different tissue levels (r = 0.50–0.71). There is an exceptionally strong correlation between p53 expression in the glandular and epithelial components (r = 0.92), indicating genetic instability in these areas. Moderate correlations are observed between Ki67 and ER/PR (r = 0.33–0.63), with the highest correlations found between Ki67 and ERIII, as well as ERII. A strong interrelationship exists between Ki67 expression at different tissue levels (r = 0.75 between Ki67-I and Ki67-II).

Conclusion:

In endometrial carcinoma, genetic instability and high proliferative activity are present, particularly in regions with increased estrogen sensitivity.

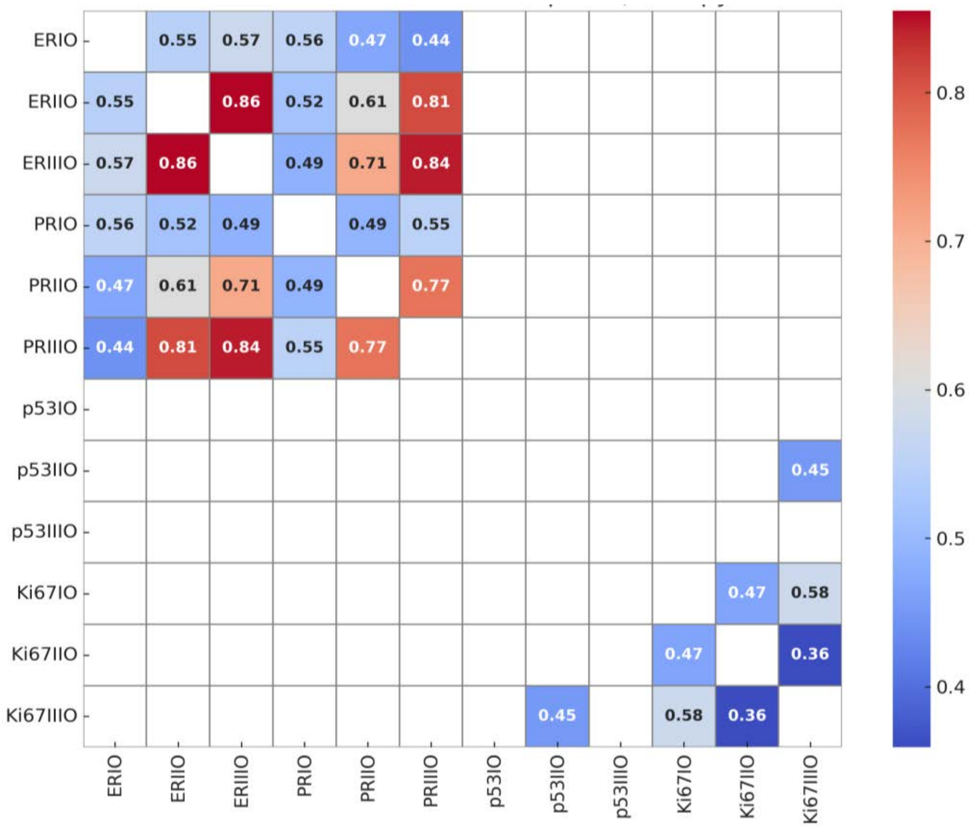

Figure 37. AE – significant correlations

From Figure 37, it is notable that there is a strong interrelationship between ER and PR expression across all tissue levels (glandular, epithelial, and stromal components), with coefficients r = 0.47–0.86. Moderate positive correlations are observed between the different levels of Ki67 expression (r = 0.36–0.58), indicating residual proliferative activity. There are no significant correlations between p53 and the other markers.

Conclusion:

The atrophic endometrium demonstrates a high hormonal receptor profile, characteristic of stable and hormonally sensitive tissue. The absence of interactions with p53 confirms its genetic stability.

5. Analysis of the relationships between serum estradiol concentrations, hormonal receptors, and biomarkers in the EP, EC, and AE groups.

Figures 38, 39, and 40 present the correlation dependencies between the mean serum estradiol levels in pre- and postmenopausal women from the three groups: endometrial carcinoma (EC), endometrial polyps (EP), and atrophic endometrium (AE).

In endometrial carcinoma, no strong or significant correlations are identified between serum estradiol levels and the expression of estrogen receptors (ER), progesterone receptors (PR), the proliferative marker Ki67, or the tumor suppressor gene p53. Although weaker correlations are observed between certain markers, estradiol does not appear to be a leading factor in defining the molecular profile of malignant tissue. This supports the hypothesis that, following malignant transformation, endometrial tissue acquires relative autonomy from the systemic hormonal milieu, with the main mechanisms of progression being linked rather to intracellular molecular alterations, including p53 mutations and increased cellular proliferation.

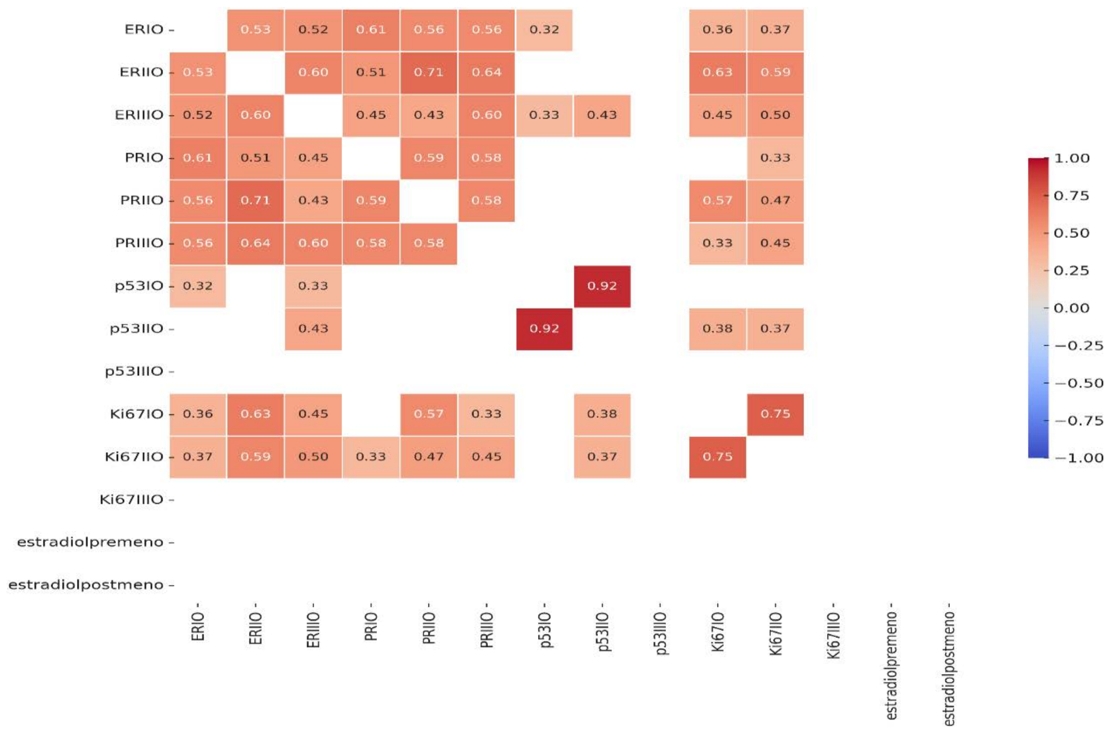

Figure 38. EC – correlation of serum estradiol with steroid receptors and biomarkers

In contrast, within the group of endometrial polyps, significant positive correlations are observed between serum estradiol—particularly in postmenopausal women—and the expression of ER in the stromal component (ER-III) and PR in the epithelial component (PR-II), with correlation coefficients of approximately 0.51 and 0.65, respectively.

These results indicate that even minimal postmenopausal estradiol levels continue to exert a significant influence on hormonal regulation within polyp tissue. This finding supports the concept that endometrial polyps are hormonally dependent lesions, wherein the systemic estrogenic environment directly affects their biological activity, proliferation potential, and persistence.

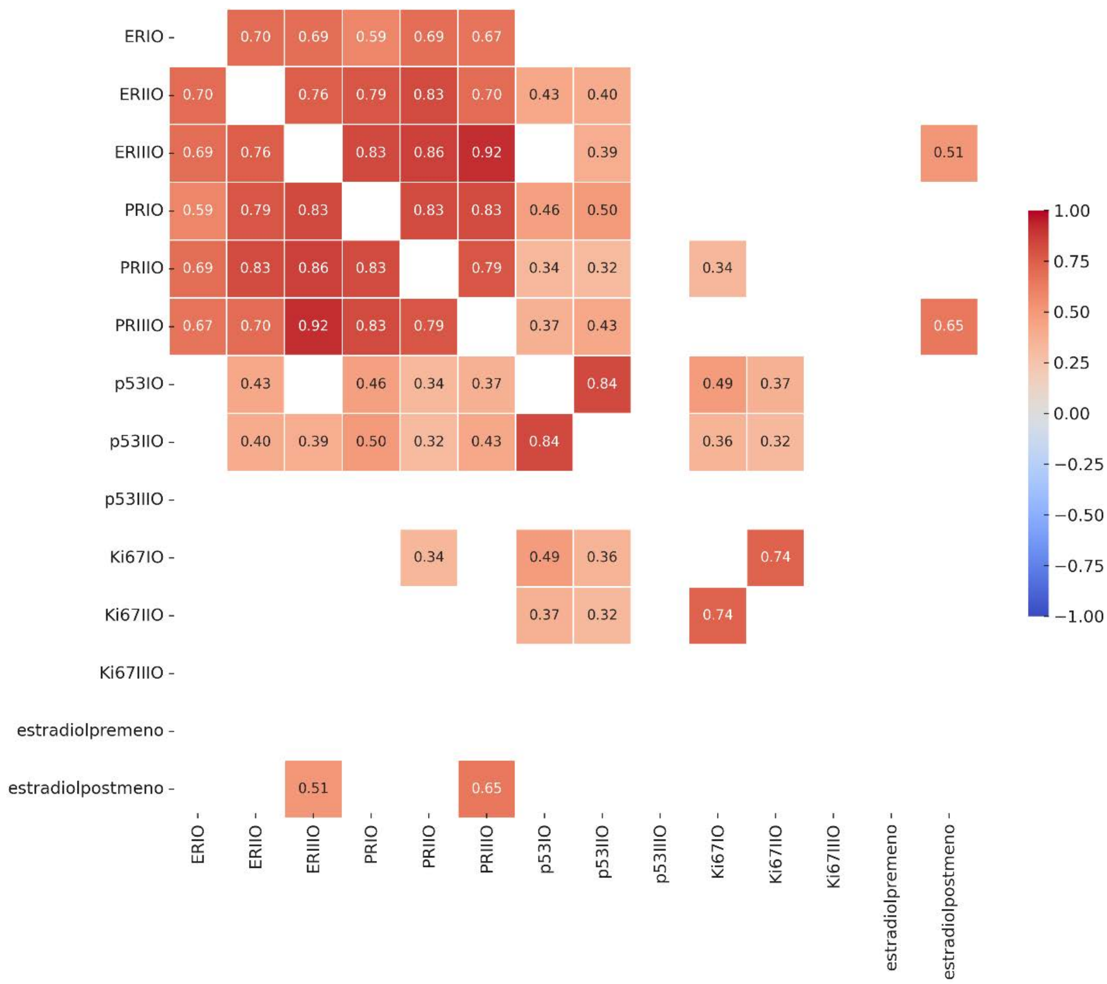

Figure 39. EP – correlation of serum estradiol with steroid receptors and biomarkers.

In the group with atrophic endometrium, statistically significant correlations were observed between postmenopausal serum estradiol levels and the expression of the proliferative marker Ki67 in the second and third tissue levels (Ki67-II and Ki67-III), with correlation coefficients of approximately 0.36 and 0.45, respectively.

This indicates that even the low estradiol levels characteristic of the postmenopausal period can induce residual proliferative activity in the endometrial epithelium and stroma. Although this activity is minimal, it carries clinical significance, as chronic hormonal stimulation in predisposed patients may increase the risk of developing endometrial hyperplasia or malignant transformation.

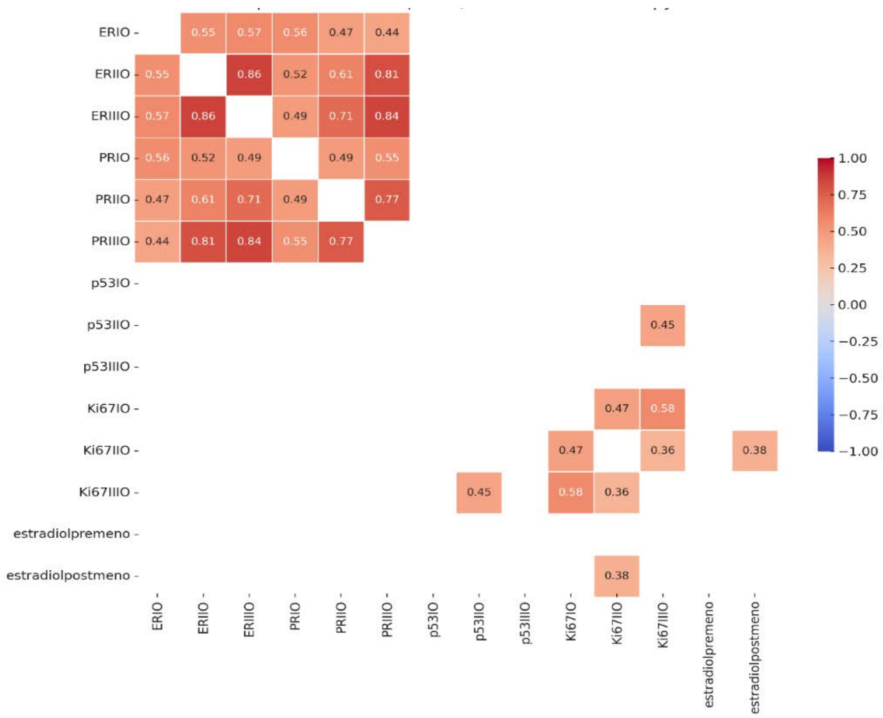

Figure 40. AE – correlation of serum estradiol with steroid receptors and biomarkers.

These observations underscore the importance of serum estradiol as a modulator of endometrial biology in benign lesions and atrophic changes, while simultaneously highlighting its limited influence in advanced neoplastic processes such as endometrial carcinoma.

V. CONCLUSIONS

1. Endometrial polyps (EP) are most common in premenopausal women aged between 41 and 50 years with overweight, whereas endometrial carcinoma (EC) is most frequently diagnosed in postmenopausal women aged between 51 and 60 years with obesity.
2. Serum estradiol in premenopausal women shows a statistically significant positive correlation with increased BMI and is associated with the risk of EP, while its levels in postmenopausal women are in a statistically significant positive correlation with obesity and the risk of EC.
3. EPs are characterized by high expression of estrogen and progesterone receptors in all tissue components, without statistically significant differences between premenopausal and postmenopausal women, underscoring the role of local hormonal regulation in their pathogenesis.
4. Menopausal status influences the proliferative potential of endometrial polyps, with Ki-67 demonstrating statistically significantly higher expression in the glandular and epithelial components in premenopausal women, whereas p53 remains unchanged.
5. The expression of p53 and Ki-67 in endometrial polyps shows significant positive correlations with hormonal receptors (ER and PR), indicating hormonally driven proliferative regulation.
6. In women with EP, assessment of age, BMI, serum estradiol levels, tissue Ki-67 expression, and steroid receptors allows risk stratification aimed at preventing malignant transformation.

VI. RISK STRATIFICATION MODEL FOR WOMEN WITH ENDOMETRIAL POLYPS (EP)

Criteria	Low Risk	Moderate Risk	High Risk
Age	< 50 years	50–60 years	> 60 years
Menopausal status	Premenopausal	Early postmenopausal	Late postmenopausal
Bleeding	Absent	Sporadic	Postmenopausal/recurrent
Histological subtype	Atrophic, functional	Hyperplastic without atypia	Suspicion of atypia
BMI, Hypertension, Diabetes Mellitus	Normal weight, no comorbidities	Overweight, single metabolic abnormality	Obesity, metabolic syndrome
IHC – ER/PR expression (morphometry)	Limited to superficial epithelium	Widespread but non-homogeneous	Diffuse and extensive expression in glands and stroma
IHC – Ki-67 (morphometry)	Isolated nuclei in epithelium	Clustered expression in basal areas	Diffuse across multiple levels (basal, intermediate, superficial)
IHC – p53 (morphometry)	Absent	Focal in glandular epithelium	Diffuse in multiple tissue zones
Previous therapy	No previous intervention	Single hysteroscopy, responsive to progestins	Multiple recurrences, unresponsive to therapy

Interpretation:

1. **Low risk** → Observation, possible hormonal therapy (progestins).
2. **Moderate risk** → Hormonal therapy plus monitoring; IHC if suspicious features are present.
3. **High risk** → Diagnostic hysteroscopy and mandatory IHC; consideration of surgical treatment.

VII. Hypotheses Regarding the Etiology and Pathogenesis of Endometrial Polyps (EP)

Main hypotheses:

1. **Hormone-mediated proliferation.** Elevated estrogen levels without sufficient progesterone counterbalance in postmenopausal women lead to local endometrial proliferation and polyp formation.
   Supporting evidence: High ER/PR expression in polyps; correlation with serum estradiol levels.
2. **Local genetic instability with low proliferative activity.** In a subset of polyps, p53 expression is detected without significant proliferative activity (low Ki67), suggesting an initial stage of genetic alterations that do not progress to malignancy.
   Supporting evidence: p53-high / Ki67-low profile observed in some polyps.
3. **Impact of obesity.** Increased adiposity creates a pro-proliferative endocrine environment that favors polyp development in postmenopausal women.
   Supporting evidence: Higher prevalence of polyps in women with elevated BMI.

Conclusion: Molecular profiling of endometrial lesions through analysis of p53, Ki67, ER, and PR expression provides a valuable basis for **differential diagnosis**, **assessment of malignant transformation risk**, and the **selection of individualized therapeutic strategies**.

VIII. Contributions

I. Theoretical contributions of original character:

1. For the first time in Bulgaria, a comprehensive analysis was performed examining **age, reproductive and menstrual factors, serum estradiol levels, steroid receptor expression, and cellular biomarkers (Ki-67, p53)** in women with endometrial polyps, endometrial carcinoma, and atrophic endometrium.
2. Based on the results, **hypotheses regarding the etiology and pathogenesis of endometrial polyps in pre- and postmenopausal women** have been formulated.
3. The **biological inertness of the stromal component** has been demonstrated through the absence of p53 and Ki-67 expression across all three endometrial conditions.

II. Scientific-applied contributions of original character:

1. This study represents the **first documented application in Bulgaria** of the combined evaluation of **Ki-67, p53, and ER/PR correlations at different tissue levels** to assess the biological potential of endometrial lesions.
2. **A pioneering integration of serum estradiol measurements** with tissue markers and clinical factors for comprehensive interpretation was conducted.
3. A **practical risk stratification model for endometrial polyps** was proposed, incorporating clinical, hormonal, metabolic, and immunohistochemical parameters.

III. Confirmatory contribution:

- Analysis confirming the role of **obesity, advanced age, and postmenopause in endometrial carcinogenesis**.

IX. PUBLICATIONS RELATED TO THE DISSERTATION WORK

1. Krum Vladov, Ekaterina Uchikova, Kamen Yamakov, Veselin Belovezhdov, Gita Yamakova-Vladova, Eleonora Hristova-Atanasova. *Etiology and pathogenesis of endometrial polyps: a review of current literature and clinical studies.* Rare Diseases and Orphan Drugs. 2025;16(2):3-7 – in press.
2. Krum Vladov, Ekaterina Uchikova, Kamen Yamakov, Veselin Belovezhdov, Gita Yamakova-Vladova, Eleonora Hristova-Atanasova. *Endometrial polyps: prevalence and clinical characteristics.* Rare Diseases and Orphan Drugs. 2025;16(1):45-48.
3. Vladov K, Uchikova E, Koleva-Ivanova M, Yamakov K, Belovezhdov V, Yamakova-Vladova G, Hristova-Atanasova E. *A case report of the synchronous occurrence of ovarian granulosa cell tumour and malignant endometrial polyp with immunohistochemical expression of hormone receptors and biomarkers p-53 and Ki-67.* Reports [Internet]. 2024;7(4):103.
4. Krum Vladov, Ekaterina Uchikova, Veselin Belovezhdov, Gita Yamakova-Vladova, Kamen Yamakov, Maria Koleva-Ivanova, Nikoleta Parahuleva-Rogacheva. *Expression of estrogen and progesterone hormone receptors in endometrial polyps in pre- and postmenopausal women – a literature review.* Scientific Works of the Union of Scientists in Bulgaria-Plovdiv, Series G. Medicine, Pharmacy and Dental Medicine. 2024; XXXI(2534–9392):130-132.
5. Krum Vladov, Gita Yamakova-Vladova, Ekaterina Uchikova, Kamen Yamakov. *Current aspects in the pathogenesis of endometrial polyps – a literature review.* In: University Publishing Centre: Science and Youth Conference 2021, Scientific Reports, Medical University of Plovdiv; 2021. p.194-200.
6. Kamen Yamakov, Gita Yamakova-Vladova, Eleonora Hristova, Ekaterina Uchikova, Krum Vladov, Radoslav Terzhumanov. *Assessment of endometrial polyps malignancy by conventional 2D transvaginal ultrasonography and color Doppler.* Scientific Works of the Union of Scientists in Bulgaria-Plovdiv, Series G. Medicine, Pharmacy and Dental Medicine. 2023; XXIX:46-50.

X. PARTICIPATION IN SCIENTIFIC FORUMS

1. **Oral presentation.** Immunohistochemical analysis of estrogen and progesterone receptors, biomarkers p53 and Ki-67 in a malignant endometrial polyp in a 67-year-old woman with granulosa cell ovarian carcinoma. Vladov K., Uchikova E., Koleva-Ivanova M., Yamakov K., Belovezhdov V., Yamakova-Vladova G., Hristova-Atanasova E. XXVI National Conference on Oncogynecology, 17–20 October 2024, Razlog, Bulgaria.
2. **Plenary lecture.** Metabolic syndrome and malignant endometrial pathology. The role of general practitioners in the prevention of endometrial carcinoma. Vladov K. XVI Scientific Meeting – Problem-based training of SOIBOM, Pamporovo, 24–26 March 2023, Bulgaria.
3. **Oral presentation.** Evaluation of clinical and hysteroscopic signs of malignancy of endometrial polyps – literature review. Kamen Y., Uchikova E., Vladova-Yamakova G., Vladov K. IX International Conference of Young Scientists, 2022, Plovdiv, Bulgaria – Online. *Awarded first place for best presentation in the "Clinical Medicine" section.*