“EXPRESSION OF P16 IN ENDOMETRIAL CRACINOMA- HOSPITAL BASED CROSS-SECTIONAL STUDY”

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LIST OF ABBREVIATIONS

1.	**CDK**	**Cyclin dependent kinase**
2.	**MTS1**	**Multiple tumor suppressor 1**
3.	**CDKN2A**	**Cyclin dependent kinase inhibitor 2A**
4.	**INK**	**Inhibitor of cyclin-dependent kinase**
5.	**SRY**	**Sex – determining region Y chromosome**
6.	**DAX1**	**Dosage sensitive sex reversal**
7.	**EH**	**Endometrial hyperplasia**
8.	**BMI**	**Body mass index**
9.	**WHO**	**World health organization**
10.	**ISGYP**	**Indian society of gynecologic pathologist**
11.	**AH**	**Atypical hyperplasia**
12.	**HPF**	**High power field**
13.	**EIN**	**Endometroid intraepithelial neoplasia**
14.	**VPS**	**Volume percentage of stroma**
15.	**EWG**	**European working group**
16.	**TCGA**	**The cancer genome atlas**
17.	**MMMT**	**Malignant mixed mullerian tumors**
18.	**HNPCC**	**Hereditary non-polyposis colorectal syndrome**
19.	**MMR**	**Mismatch repair**
20.	**CRC**	**Colorectal cancer**

21.	**OC**	**Ovarian cancer**
22.	**DNA**	**Deoxyribonucleic acid**
23.	**PTEN**	**Phosphatase and tensin homolog**
24.	**PCOS**	**Polycystic ovarian syndrome**
25.	**ECC**	**Endometroid endometrial carcinoma**
26.	**SC**	**Serous carcinoma**
27.	**CCC**	**Clear cell carcinoma**
28.	**MC**	**Mixed carcinoma**
29.	**UC**	**Undifferentiated carcinoma**
30.	**CS**	**Carcinosarcoma**
31.	**N/C ratio**	**Nucleus to cytoplasmic ratio**
32.	**ER**	**Estrogen receptor**
33.	**PR**	**Progesterone receptor**
34.	**NSMP**	**Non- specific molecular profile**
35.	**UDC**	**Undifferentiated carcinoma**
36.	**DDC**	**De-differentiated carcinoma**
37.	**MLC**	**Mesonephric like carcinoma**
38.	**GTEC**	**Gastric type endometrial carcinoma**
39.	**FIGO**	**International federation of gynecology and obstetrics**
40.	**ESGO**	**European society of gynecological oncology**
41.	**ESTRO**	**European society of radiotherapy and oncology**

42.	**ESP**	**European society of pathology**
43.	**MELF**	**Microcystic elongated fragmented pattern**
44.	**ICCR**	**International collaboration of cancer reporting**
45.	**LVSI**	**Lymphovascular space invasion**
46.	**MTS**	**Multiple tumor suppressor**
47.	**HPV**	**Human papillomatous virus**
48.	**H&E**	**Hematoxylin and eosin**
49.	**IHC**	**Immunohistochemistry**
50.	**DPX**	**Dibutylphthalate polystyrene xylene**
51.	**HRP**	**Horseradish peroxidase**
52.	**DAB**	**Diaminobenzidine**
53.	**TNM**	**Tumor, node, metastasis**
54.	**D&C**	**Dilatation and curettage**
55.	**PVB**	**Per vaginal bleeding**
56.	**HMB**	**Heavy menstrual bleeding**
57.	**AUB**	**Abnormal uterine bleeding**
58.	**PVS**	**Per vaginal spotting**
59.	**PVW**	**Per vaginal white discharge**
60.	**NA**	**Not applicable**
61.	**LVI**	**Lymphovascular invasion**
62.	**PNI**	**Perineural invasion**

ABSTRACT

STUDY OF EXPRESSION OF P16 IN ENDOMETRIAL CARCINOMA: HOSPITAL BASED CROSS- SECTIONAL STUDY

BACKGROUND:

Endometrial carcinoma is 4th most common cancer in females. Endometrial neoplasia incorporates a variety of lesions ranging from benign endometrial hyperplasia to endometrial carcinomas, collectively posing a substantial burden on global public health. P16 is the second most common tumor suppressor gene. Increased expression of p16 was observed in HPV-associated tumors, various high-grade carcinomas and lesions that affect female reproductive system including serous carcinomas of ovaries and endometrium. Squamous cell carcinoma of the uterine cervix, adenocarcinoma of the endocervix, and high-grade squamous intraepithelial lesions found in the vulva, vagina, and anogenital areas.

Owing to tumor heterogenicity, a comprehensive understanding of the clinicopathological correlation of p16 expression in endometrial carcinoma is essential to unravel its true diagnostic and prognostic potential.

OBJECTIVES

The study is aimed to evaluate expression of P16 in endometrial carcinoma, to correlate p16 positivity scores with clinicopathological parameters and to compare p16 expression in endometrial carcinoma and hyperplasia

METHODS

A total of 40 endometrial carcinoma and 40 endometrial hyperplasia diagnosed during January 2022 to December 2024 were included in this study. Slides stained with H&E, and immunohistochemically for p16 were evaluated for histopathological examination. Results were subjected to appropriate statistical analysis.

RESULTS

The results of this study show an increased expression of p16 in endometrial carcinoma and hyperplasia. There was significant difference of p16 expression in different types and grades of endometrial carcinoma, between endometrial hyperplasia and carcinoma reinforcing p16 as a marker of tumor progression and differentiation.

P16 expression in endometrial carcinoma was significantly associated with myometrial invasion, lymphovascular and perineural invasion.

CONCLUSION

In conclusion, the findings of this study suggest a possible role of p16 in development and progression of endometrial carcinoma. Further research is needed to establish its precise role in tumor progression and potential clinical applications in colorectal cancer management.

KEYWORDS:

Endometrial neoplasia; p16; Endometrial carcinoma; Endometrial hyperplasia; prognosis

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INTRODUCTION

Endometrial cancer, the most prevalent malignancy affecting women in developed nations, has shown an increasing trend in recent decades. Between 1990 and 2017, the age-standardized incidence rate increased by 0.58% annually, while the prevalence rate increased by 0.89% per year. This steady growth in both new cases and overall occurrences underscores the significant public health challenge posed by this disease.[1]

Endometrial cancer is the 2nd most common malignancy of the female genital tract, after cervical cancer. While post-menopausal women are the primary demographic affected by endometrial cancer, it is important to note that women in the menopausal transition period are also at an elevated risk.[2]

The INK4 family of cyclin-dependent kinase (CDK) inhibitors includes p16 as a key component, which is located on the short arm of chromosome 9 (9p21.3). Also referred to as MTS-1, INK4a, and CDKN2A, p16 is the second most prevalent tumor suppressor gene. It plays a crucial role in the G1-to-S phase transition of the cell cycle by encoding a nuclear protein that regulates this process by inhibiting the interaction of CDK 4 and 6 with cyclin D1. In the absence of p16, CDK4/6 binds to cyclin D1, resulting in pRB phosphorylation. This leads to pRB deregulation, which halts the cell cycle in the G1/S phase and promotes cell proliferation. In HPV-associated tumors, increased expression of the p16 protein was observed. Significant p16 positivity has been observed in various high-grade carcinomas and lesions that affect the female reproductive system. These include serous carcinomas of the ovaries and endometrium, squamous cell carcinoma of the uterine cervix, adenocarcinoma of the endocervix, and high-grade squamous intraepithelial lesions found in the vulva, vagina, and anogenital areas.[3]

Diffuse immunoreactivity for p16 was observed in 90% of endometrial serous carcinoma cases and 38% of endometroid carcinoma cases but in many studies p16 alterations are rarely seen, none of these has focused on prognostic relevance of p16 alterations thus further studies are required.[4,5]

p16 can be considered a promising therapeutic option, offering a potential cure in cancer patients through the early detection of malignant potential. As an easily accessible marker, emphasizing the inclusion of p16 expression in histopathology reports may contribute to an improved understanding of tumor behavior and enhanced patient care.

AIMS AND OBJECTIVES

- Primary,

1. To evaluate expression of P16 in endometrial carcinoma.

- Secondary,

1. To correlate p16 positivity scores with clinicopathological parameters.
2. To compare p16 expression in endometrial carcinoma and hyperplasia

REVIEW OF LITERATURE

Embryogenesis-

Genetic sex determination of embryos occurs at the time of fertilization; however, morphological characteristics do not occur until the seventh week of development. The sex-determining region of the Y chromosome (SRY) is a testis-determining factor, in the absence of it female development occurs. Specific genes such as DAX1 induce ovarian development and differentiation. Estrogen stimulates the Müllerian (paramesonephric) ducts and the external genitalia. The paramesonephric ducts form the uterine tubes, uterus, cervix, and upper vagina. Labia minora, majora and clitoris constitute to form the external genitalia.[6]

The paramesonephric ducts have cranial and caudal parts; the cranial part remains unfused and forms uterine tubes, whereas the caudal part fuses to form the uterus and superior part of the vagina. Fusion of paramesonephric duct forms the peritoneal fold later known as broad ligament which divides anterior and\

posterior compartments of uterus into rectouterine pouch and vesicouterine pouch.[7]

The müllerian tubercle (uterovaginal plate) contains epithelial tissue that hollows out to create the lower section of the vagina, while the paramesonephric ducts fuse to form the upper part of vagina.[8]

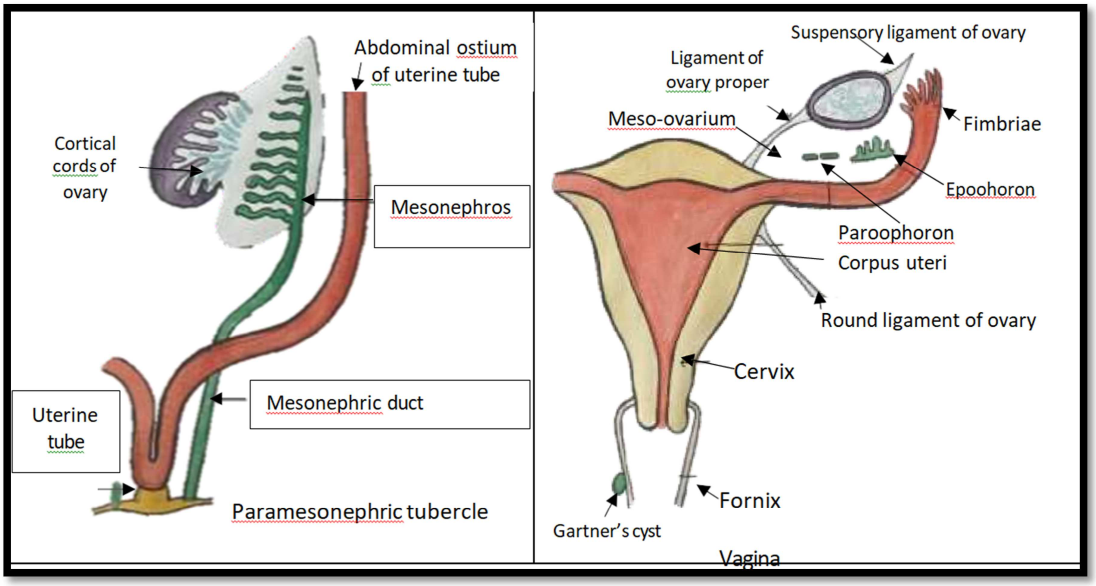


Figure 1 and 2: Embryology of female genital tract[6]

Anatomy-

The external and internal genitalia collectively constitute the female reproductive system. The vulva and vagina form the external genital tract, whereas the ovaries, fallopian tubes, uterus, and cervix form the internal genital tract. The uterus is located anterior to rectum and posterior to the bladder. The rectouterine pouch separates it from the rectum, while the vesicouterine pouch separates it from the bladder.

The upper two-thirds of the uterus is made up of a muscular body, while the lower third is made up of a fibrous cervix. Both divisions have different anatomical and functional characteristics: the cervix is tilted forward in relation to the vaginal axis (anteversion) and the uterine body is tilted forward in relation to the cervix (anteflexion) in nulliparous women. The isthmus separates the body and the cervix.[9]

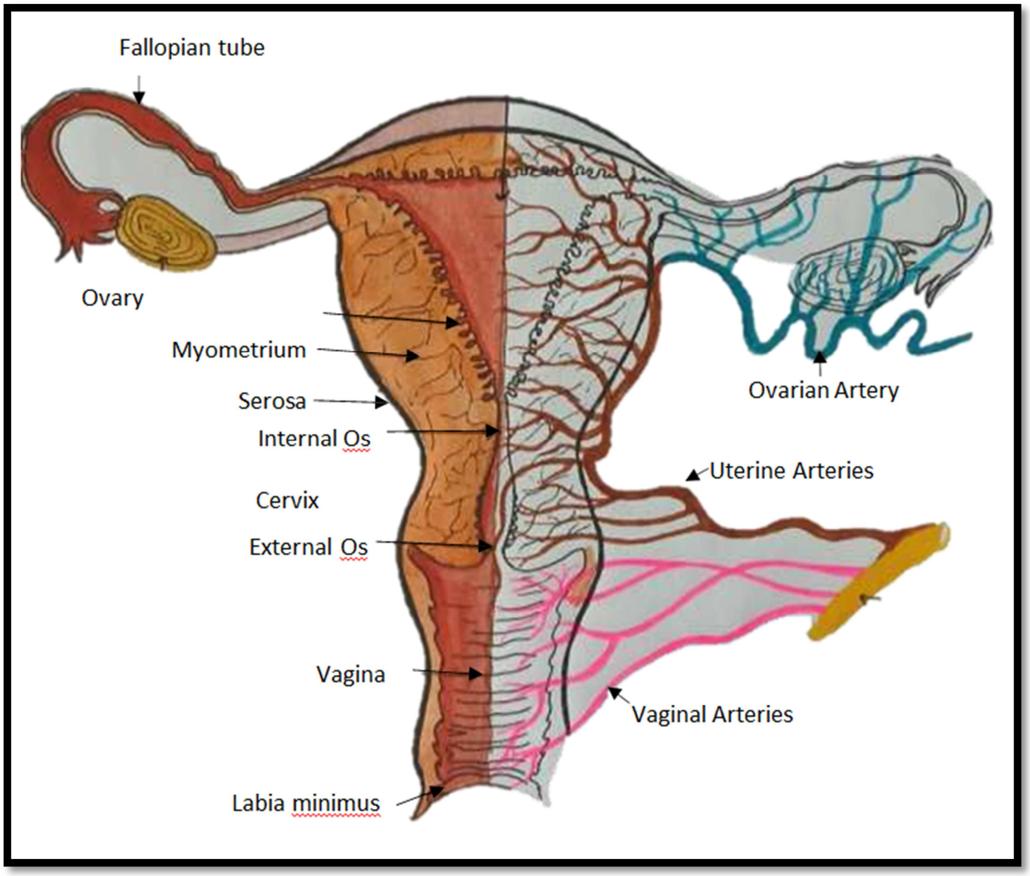


Figure 3: Anatomy of uterus[9]

The lymphatic drainage of the uterus varies by region. Fundal lymph vessels accompany the ovarian vessels and empty into the aortic lymph nodes. The body of the uterus has lymphatic vessels that drain into the external iliac lymph nodes. Cervical lymphatics follow the uterine artery's path and empty into the internal iliac and sacral ganglia.

Uterine nerves come from the uterovaginal plexus. The nerves form two distinct bundles that supply the uterine body and the cervical isthmus.[9]

Histology of uterus-

The uterus is divided into three histological layers:[10]

S.N.	Layers
	innermost mucosal layer- **Endometrium**
2	middle muscular layer- **Myometrium**
3	outermost layer- **Parametrium** or **Serosa**

Table 1: Histological layers of uterus

The endometrium consists of two primary layers.

1. Functional layer, and
2. Basal layer.

The highly dynamic functional layer undergoes periodic alterations in response to fluctuations of hormones during menstrual cycle, whereas the basal layer remains relatively unchanged, providing regenerative cells for the functional layer after menstruation.[11]

Histologically, the epithelium of the endometrium is composed of columnar cells, some of which are ciliated and secretory. The endometrial glands vary in shape and size according to the phase of menstrual cycle. The endometrial stroma is rich in fibroblasts, immune cells, and vascular structures providing structural support.[12]

During the menstrual phase, if implantation does not occur, the functional layer undergoes shedding and is lost in menstruation.[13]

Uterine cycle-

The uterine cycle is characterized by cyclical changes in the endometrial lining in response to fluctuating hormonal levels.

1. Proliferative phase
2. Secretory phase
3. Menstrual phase

These phases are associated with specific histological alterations in endometrial tissue, reflecting the cellular and molecular responses necessary for implantation and potential pregnancy. [14, 15]

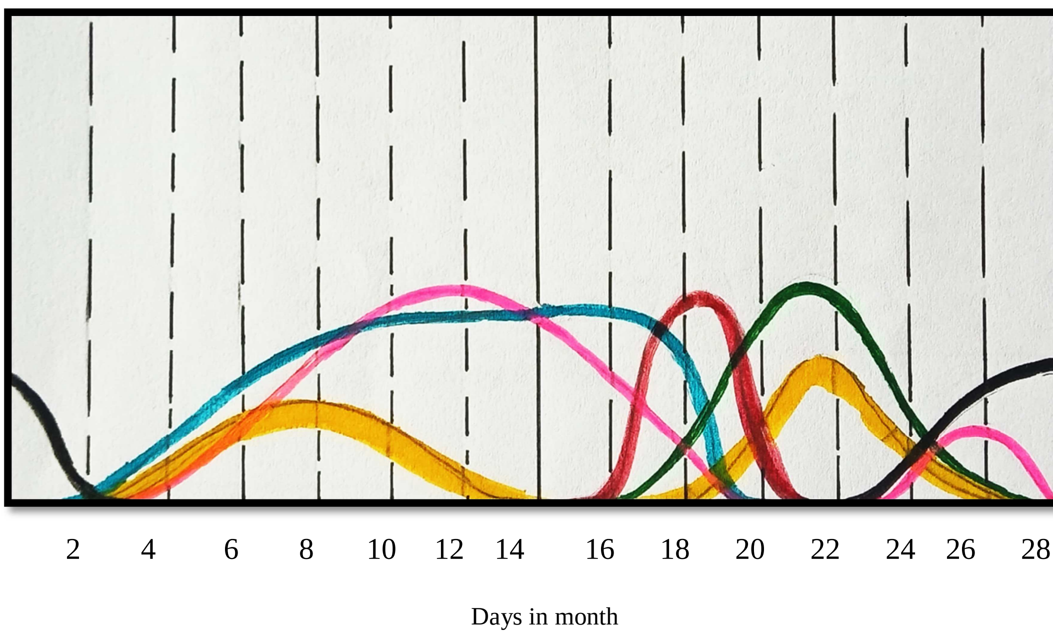


Figure 4: Cyclic changes in endometrium[16]

Color representation in above diagram:

- Blue- Gland mitosis and pseudostratification of the nuclei
- Red- Subnuclear vacuoles
- Black- Decidual reaction in stroma and leukocyte infiltration
- Yellow- Edema
- Pink- Stromal mitosis
- Green- Secretions

1. Proliferative Phase

The proliferative phase lasts approximately two weeks, with the endometrium initially thin and containing sparse, non-budding glands and a loose stromal matrix. As the phase progresses, stromal edema increases endometrial thickness, peaking around day 10. During preovulatory period, as the estrogen level declines, edema subsides and glands grow, developing a lining of tall columnar cells. Stroma becomes densely cellular and there is increase in mitotic activity, indicating ongoing proliferation and preparation for possible implantation. [14,15]

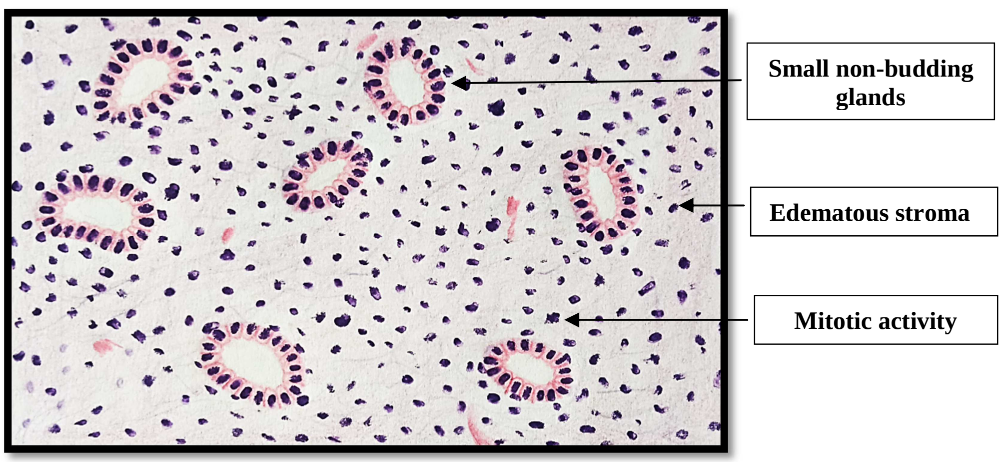


Figure 5: Proliferative phase of endometrium

2. Secretory Phase

It is divided into 3 phases:

1. Early secretory phase,
2. Mid secretory phase, and
3. Late secretory phase

Ovulation occurs at around day 14, characterised by 50% of the cells showing subnuclear vacuolations in around 50% of the glands[17].

Early secretory phase (Days 15 to 19)-

It is marked by the formation of subnuclear vacuoles, displacing the nuclei toward the centre and creating a characteristic piano key appearance.

By day 17 to 18- Vacuoles became uniform and migrated toward the lumen,

By day 19- Nuclei start returning to the base.

Mitotic activity is absent in this phase.

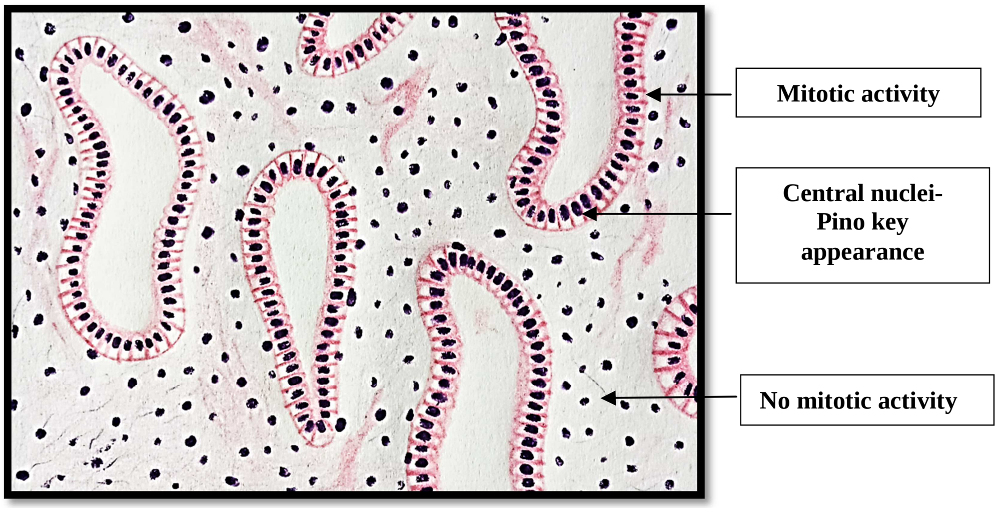


Figure 6: Early secretory phase of endometrium

Mid-secretory phase (days 19–25)-

It is characterized by secretory exhaustion.

Day 20- Peak in intraluminal secretions,

Day 21, 22- Increased stromal edema and prominent spiral arterioles,

Day 23- Stromal predecidualization begins, and

By day 24- Predecidual cells extend to bridge vascular elements.[17]

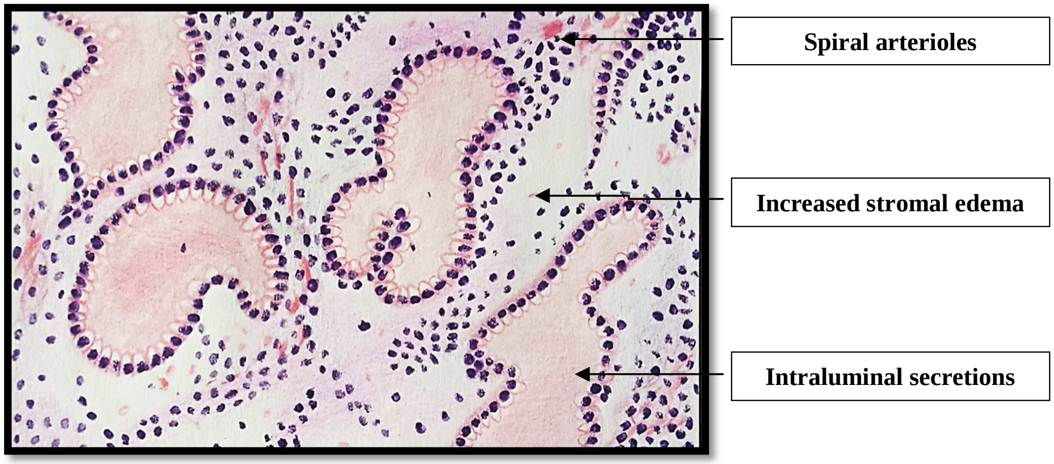


Figure 7: Mid- secretory phase of endometrium

<u>Late secretory phase</u> (days 26–28)-

Extension of predecidual changes deep into the stratum compactum.

By day 27- Granulated lymphocytes appear. The glands in the central spongy part of the endometrium acquire a characteristic sawtooth appearance [15,18]

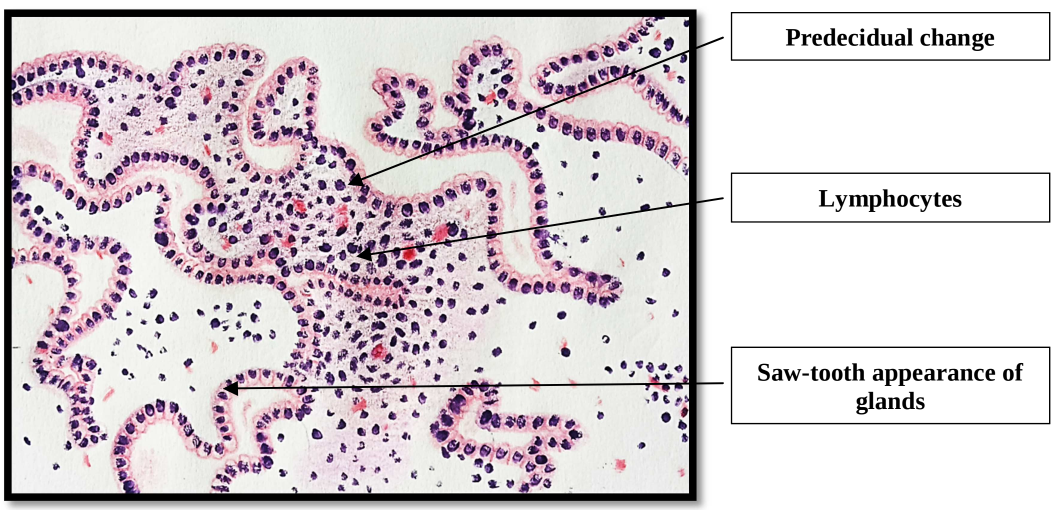


Figure 8: Late secretory phase of endometrium

3. Menstrual Phase

If one does not conceive, the late secretory phase progresses to the menstrual phase, which begins 14 days after the ovulation.[17]

Histologically, it is marked by the breakdown of stromal tissue, haemorrhage in the superficial layers and glandular degeneration. The stromal cells will aggregate into dense clusters, while glands exhibit a deep blue colour, and the epithelial cells show signs of degeneration.[18, 19]

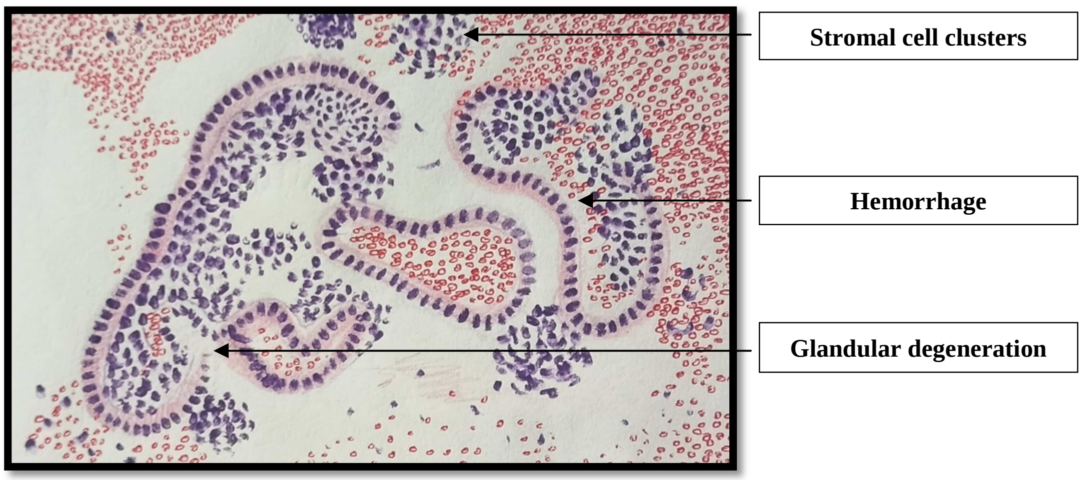


Figure 9: Menstrual phase of endometrium

<u>Endometrial Hyperplasia (EH)</u>

The proliferation of endometrial glands of irregular size and shape with a raise in the ratio of gland-to-stroma is known as endometrial hyperplasia.

This is compared to a normal proliferative endometrium. Multiple studies favor the association of unopposed estrogenic stimulation in the formation of endometrial hyperplasia and endometrioid-type endometrial carcinoma, which is the commonest type of endometrial carcinoma. Increase in both endogenous and exogenous estrogen can lead to endometrial hyperplasia, such as obesity, anovulatory cycles, and exogenous hormones, increasing the body mass index (BMI)[17]. Endometrial polyp, Arias-stella reaction and cystic atrophy can mimic endometrial hyperplasia.[20]

Classification:

World Health Organization (WHO) and International Society of Gynaecologic Pathologists (ISGYP) gave a “4-tier classification” in 1994[17], which gave 4 categories of endometrial hyperplasia-

1	Simple hyperplasia
2	Complex hyperplasia
3	Simple atypical hyperplasia
4	Complex atypical hyperplasia (AH)

Table 2: Classification of endometrial hyperplasia, WHO 1994

In 2014 WHO[21] simplified it into “2 tier classification”-

1	Non-atypical endometrial hyperplasia (Benign hyperplasia) (Hyperplasia without atypia)
2	Atypical endometrial hyperplasia, or Endometrial Intraepithelial Neoplasia.

Table 3: Classification of endometrial hyperplasia, WHO 2014.

Hyperplasia Without Atypia

Increased gland-to-stromal ratio. Glands exhibiting variable degrees of architectural patterns, including different sizes and shapes. They can be simple cystically dilated glands with outpouching to complex branched glands with irregular outlines and infolding into glandular lumens, giving rise to a papillary appearance. The epithelial lining of these glands is composed of stratified columnar cells with oval, basally located, bland nuclei with smooth contours, and an amphophilic cytoplasm.

The stroma surrounding the glands can be abundant to compressed depending on the crowding of the glands. The stromal cells are densely packed, plump, and spindle-shaped with enlarged nuclei. Mitotic activity generally does not exceed beyond 5 mitotic figures for each 10 high-power fields (HPF). Epithelial stratification is usually 2 to 4 layers thick.[17] Due to anovulatory cycles in perimenopausal age disordered proliferative endometrium can sometimes mimic simple hyperplasia[20]

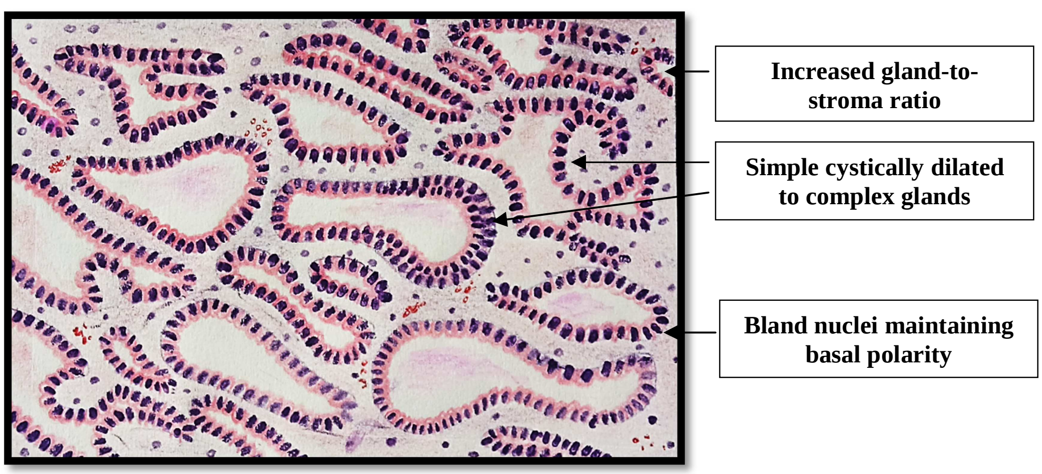


Figure10: Endometrial hyperplasia without atypia

Hyperplasia with atypia (Atypical Hyperplasia)

Architectural patterns can differentiate between simple and complex hyperplasia, supporting the old classification; however, to determine the presence or absence of atypia, the most useful feature remains nuclear atypia. The nuclei of these cells are enlarged, variable in shape and size with an increase in nucleus-to-cytoplasmic ratio, irregular membrane, loss of basal polarity, presence of nucleoli which is prominent, and more rounded shape compared to that of hyperplasia without atypia. Nuclear chromatin show coarse clumping and condensation around the nuclear membrane, giving a vesicular/clear appearance. The glandular architecture, cellular stratification, nuclear atypia, and mitotic activity can be variable[17.]

There is 25 to 40% chances of atypical hyperplasia to transform into endometroid type of endometrial carcinoma.[20]

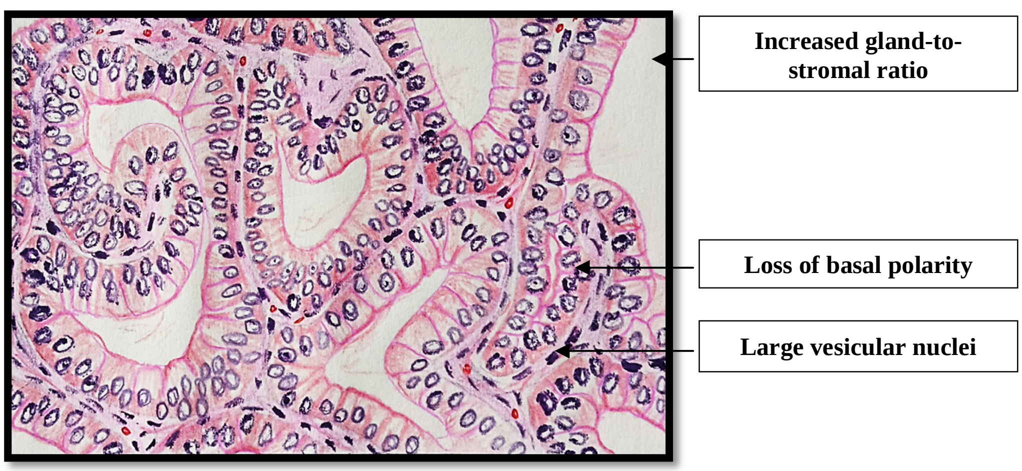


Figure 11: Endometrial hyperplasia with atypia

ENDOMETROID INTRAEPITHELIAL NEOPLASIA (EIN):

Depending on histological, molecular variations and morphometric observation, a new term for EIN was given by the WHO in 2014. Monoclonal proliferations are less associated with estrogenic stimulation and are considered more carcinogenic than polyclonal proliferations, and are thus designated as EIN. In contrast, polyclonal proliferation is observed as a result of excess estrogenic stimulation and is associated with endometrial hyperplasia. This clonal category can help us to apply different treatment approaches for hyperplasia and EIN, Polyclonal lesions can be treated based on symptoms or cause, whereas one should remove or ablate monoclonal lesions.

As performing clonality cannot be possible for most specimens, EIN can be diagnosed when the volume percentage of stroma (VPS) falls below 55%. It is better to use morphometric analysis for the diagnosis of EIN, which uses a D score that determines the gland-to-stroma relationship. When the D score is greater than 1, it does not indicate cancer progression, whereas a D score of 1 or less predicts increasing rates of carcinoma progression.[17]

Criteria for Diagnosis of EIN[20]-

1	Volume percentage of stroma < 55%
2	Cytology of crowded glands differing from background (Cytological demarcation)
3	Linear dimension of the lesion not less than 1mm
4	Excluding benign mimics and carcinoma

Table 4: EIN diagnosing criteria

The recent WHO classification considers both atypical hyperplasia and EIN as equivalent lesions, and the risk of progression to carcinoma is also considered similar.

The European Working Group (EWG) classified proliferative lesions of endometrium which is more reproducible. It decided term “hyperplasia” for hyperplasia without atypia (both simple and complex) and another term “endometrial neoplasia” which compiles both well differentiated carcinomas and hyperplasia with atypia into single entity.[17]

WHO 2020- Classification of endometrial tumors[24]-

Endometrial epithelial tumors and precursors
Endometrial hyperplasia without atypia
Atypical endometrial hyperplasia
Endometrial adenocarcinoma NOS • POLE-Ultramutated endometrial carcinoma • Mismatch repair- deficient endometrial carcinoma • P53- mutant endometroid carcinoma • No specific molecular profile (NSMP) endometroid carcinoma
Serous carcinoma NOS
Clear cell adenocarcinoma NOS
Carcinoma, undifferentiated, NOS
Mixed cell adenocarcinoma
Mesonephric adenocarcinoma
Squamous cell carcinoma, NOS
Mucinous carcinoma, intestinal type
Mesonephric-like adenocarcinoma
Carcinosarcoma NOS

Table 5: Classification of endometrial carcinoma, WHO 2020

TNM classification[24]-

T- Primary tumor

TNM categories	FIGO Stages	Definition
TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
T1	I[a]	Tumor confined to corpus uteri
T1a	IA[a]	Tumor limited to endometrium or invading less than half of myometrium
T1b	IB	Tumor invades one half or more myometrium
T2	II	Tumor invades cervical stroma, but does not extend beyond the uterus
T3	III	Local and/or regional spread as specified here:
T3a	IIIA	Tumor invades the serosa of the corpus uteri or adnexa (direct extension or metastasis)
T3b	IIIB	Vaginal or parametrial involvement (direct extension or metastasis)
N1, N2	IIIC	Metastasis to pelvic or para-aortic lymph nodes
N1	IIIC1	Metastasis to pelvic lymph nodes
N2	IIIC2	Metastasis to para-aortic lymph nodes with or without metastasis to pelvic lymph nodes
T4	IV	Tumor invades bladder/ bowel mucosa

N- Regional lymph nodes

NX	Regional lymph nodes cannot be assessed
NO	No regional lymph node metastasis
N1	Regional lymph node metastasis to pelvic lymph nodes
N2	Regional lymph node metastasis to para-aortic lymph nodes with or without metastasis to pelvic lymph nodes

M- Distant Metastasis

M0	No distant metastasis
M1	Distant metastasis (excluding metastasis to vagina, pelvic serosa, or adnexa, including metastasis to inguinal lymph nodes, intra-abdominal lymph nodes other than para-aortic or pelvic nodes)

Stage

Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
Stage IB	T1b	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3a	N0	M0
Stage IIIB	T3b	M0	M0
Stage IIIC	T1, T2, T3	N1, N2	M0
Stage IIIC 1	T1, T2, T3	N1	M0
Stage IIIC 2	T1, T2, T3	N2	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

Table 6-9: TNM classification and staging of endometrial carcinoma

Endometrial carcinoma-

Histologically, the two major types of endometrial carcinoma are-

1. Endometroid (type 1)
2. Non-endometroid (Type2)

This histological subdivision helps in prediction of outcome and planning of suitable treatment approach as both exhibit different molecular alterations and tumorigenesis.[22]

Type 1 category endometrial carcinoma are considered low to intermediate grade, these are less aggressive, show better prognosis and are more frequently observed in postmenopausal females accompanying a history of endometrial hyperplasia, whereas

Type 2 category is considered high grade, more aggressive, show poorer prognosis and found in atrophied endometrium of postmenopausal women.

Recent molecular study of The Cancer Genome Atlas (TCGA) subdivided endometrial carcinoma into 4 major categories which differs in prognosis, suggesting limitation of above type1/ type 2 classification.[23,24]

RISK FACTORS FOR ENDOMETRIAL CARCINOMA-

ESTROGENS: Excess of endogenous or endogenous estrogen can contribute in development of endometrial hyperplasia and carcinoma. Women who take unopposed estrogens have 3 to 6 times increased risk of developing endometrial carcinoma. This risk can increase up to 9.5 times when unopposed estrogen is consumed for 10 or more years.

Oestrogen dependent tumors are usually well differentiated. Even if estrogen is discontinued, the risk persists for several years of discontinuation. There is not much difference of risk between estrogen taken cyclically or in continuity.[22] Estrogen replacement therapy is a proved risk factor for development of endometrial carcinoma.[17]

Tamoxifen: For prophylaxis and treatment of breast carcinoma tamoxifen is used. It is used for its anti-estrogenic effect but along with that it also shows paradoxical estrogenic stimulation on endometrium when there is absence of ovarian estrogen secretion. It has an increased tendency of developing proliferative lesions like hyperplasia, polyp and sometimes malignant tumors.[25] Its usage is associated with 2-to-3-fold increased risk of developing adenocarcinoma.

Most associated carcinoma are of low- grade but surprisingly a small group of aggressive carcinosarcomas-e.g. malignant mixed müllerian tumors (MMMT)- shows increased risk up to 4 time of expected. Risk increases in proportion to the duration of its use.[22]

Obesity and other constitutional factors: Androgen gets converted into estrogen via aromatization in adipose tissue. Obese women have less concentration of sex hormone binding globulins. Both these factors increase overall estrogen levels in obesity. There is 1.2 to 2.1 increased risk of developing endometrial carcinoma in obesity.

Other factors like early menarche, late menopause, nulliparity (due to long term anovulation) also contributes in increasing the risk of developing endometrial carcinomas. More than 50% cases of endometrial cancer show an association with obesity.[17]

OVARIAN LESIONS: Most commonly occurring lesions are Polycystic ovarian disease, granulosa cell tumor, hyperthecosis and thecoma. These lesions are associated with long term excess estrogen production which can be the cause of endometrial hyperplasia, EIN and endometroid adenocarcinoma (Usually early stage and low grade). 9–13% cases of granulosa cell tumors develop endometrial carcinoma. Approximately 20% thecomas can exhibit endometrial carcinoma.[22]

Hereditary Syndromes:

1. Lynch Syndrome- It is also known by the name of hereditary non-polyposis colorectal cancer (HNPCC). There is alteration in DNA MMR protein which increases risk of various cancers in affected individuals including endometrial cancer, colorectal cancer, and, ovarian cancer, and also cancers of the ureter, renal pelvis, pancreas, stomach, intestine, and brain.[26]

It is the commonest reason for familial endometrial carcinoma.[22] Immunohistochemistry for DNA mismatch repair proteins is a reliable tool for identifying microsatellite instability.[26]

2. Cowden Syndrome- There is PTEN tumor suppressor gene mutation.[22] The only gynaecologic cancer notably associated with Cowden syndrome is endometrial cancer, with lifetime risk estimates ranging from 5% to 28%.[27]

Multiple benign conditions and other malignancies are also associated including thyroid and breast.[22]

Polycystic ovarian syndrome (PCOS): It is linked to a higher risk of developing endometrial carcinoma because of prolonged unopposed estrogen exposure. When ovulation does not occur, estrogen levels remain high without the balancing effect of progesterone, resulting in endometrial hyperplasia, which can progress to endometrial carcinoma.[28, 29]

Types of endometrial carcinoma-

1. ENDOMETRIOID ADENOCARCINOMA-

They are oestrogen dependent tumors which develops slowly from a setting of endometrial hyperplasia or EIN. Few develops in atrophic endometrium as well. Most common age is 6-7th decades. Chances are higher in postmenopausal women.[22]

Gross features:

Grossly, carcinoma of the endometrium can have a variable presentation. The size of uterus can be enlarged, normal or even atrophic/small.[22] The tumor may be seen as a single polypoidal mass or it may infiltrate into the myometrium diffusely.[25] The surface of tumor can present as elevated rough, papillary and shaggy ulcerated lesion or it may also have smooth and haemorrhagic surface. Invasion into myometrium with infiltrative borders can be appreciated by naked eyes but grossly commenting on degree of myometrial invasion becomes difficult. Majority of tumors are seen in corpus but some arise in the lower uterine segment.[22] Tumors in women of younger age have more tendency to involve lower uterine segment.[25] Carcinomas are seen more routinely in posterior wall than compared to anterior wall. When the diameter of tumor exceeds 2cm, it is generally associated with poorer prognosis.[22]

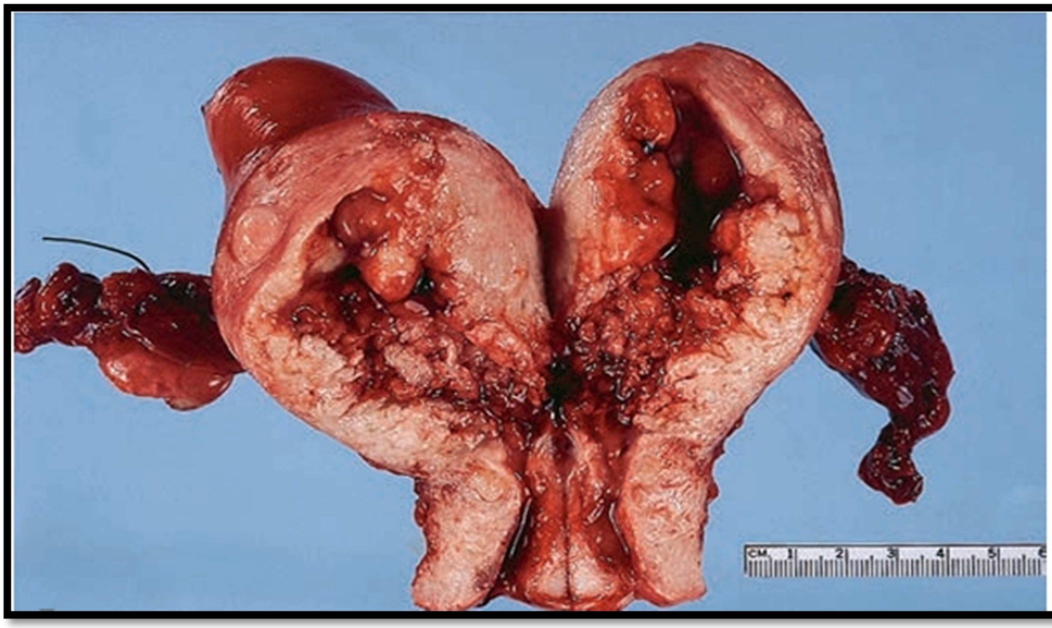


Figure12: Infiltrating endometroid carcinoma.[16]

Microscopic features:

80% malignant epithelial tumors are adenocarcinomas which are divided into 3 categories[23]-

1	Well differentiated	Grade 1
2	Moderately differentiated	Grade 2
3	Poorly differentiated	Grade 3

Table 10: Grading of endometrial carcinoma

The architecture can be complex but it somewhat resembles proliferative phase in terms of cellular and glandular features. Other architectural features are solid, villo-glandular and cribriform. Majority of times epithelial cells show multilayering.[22]

Grade of tumor is determined by nuclear features, architecture and mitotic figures. Extent of solid masses compared to glands decide the architectural grade. Pleomorphism, distribution of chromatin and nucleoli size determines the nuclear grade. Nuclear atypia can upgrade the grade but tumor architecture is key factor in determining grade.[17]

20% endometrial carcinoma show squamous components but it should not be counted in solid areas.[16,17] Grade 1 and 2 are considered low grade.[23]

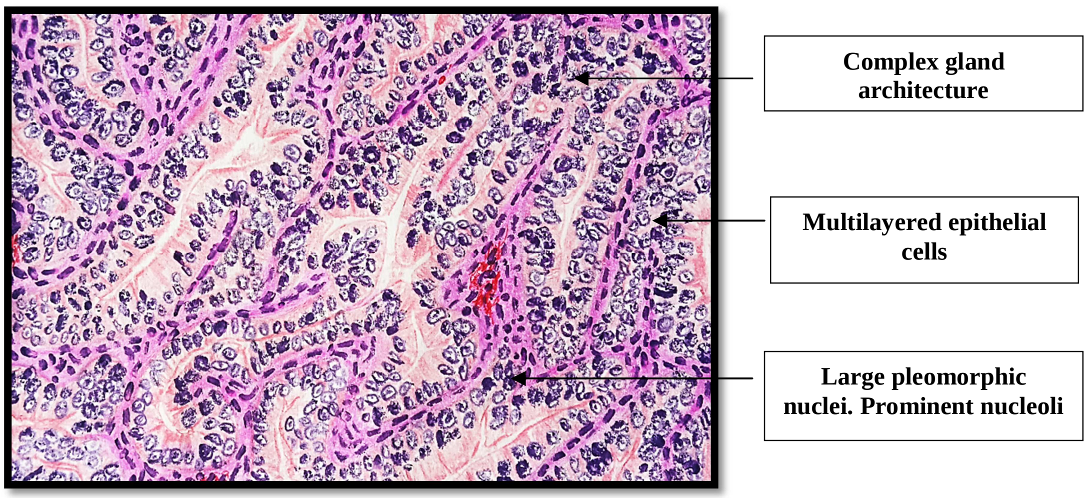


Figure 13: Endometroid Endometrial Carcinoma

The 2023 revision classifies non-aggressive histologic tumors as low-grade endometrioid carcinoma (ECC), whereas more aggressive types include serous carcinoma (SC), clear cell carcinoma (CCC), mixed carcinoma (MC), undifferentiated carcinoma (UC), carcinosarcoma (CS), mesonephric-like carcinoma, and gastrointestinal mucinous-type carcinoma.[30]

2. Endometrial carcinoma with Squamous Differentiation:

Adenocarcinoma should contain at least 10% squamous element to qualify for squamous differentiation. Clinical outcome do not show any difference in endometroid carcinoma with or without squamous differentiation. Grading is still based on glandular architecture.

Low grade tumors usually have nest of squamous epithelium inside the glandular lumen whereas it extends out of glands in high grade tumors and it may appear more atypical.[17] Keratin pearls and intercellular bridging are commonly seen.[22] Glassy cell carcinoma is a type of endometroid carcinoma with squamous differentiation.[25]

3. Mucinous Adenocarcinoma:

Mucinous endometrial carcinoma is a rare subtype, comprising 1–9% of endometrioid carcinoma.[31] The tumor structure typically displays a glandular or villoglandular pattern, comprising at least 50% columnar or pseudostratified epithelial cells with intracytoplasmic mucin. To differentiate a mucinous endometrial tumor from morphologically similar mucinous endocervical adenocarcinomas, an endocervical sampling is essential.

The tumor cells test positive for carcinoembryonic antigen, mucicarmine, and periodic acid-Schiff stain, and they are diastase resistant.[32]

4. Serous Endometrial Intraepithelial Carcinoma:

Diagnosing pure serous EIC can be difficult.[33] It is classified as potentially metastatic in the fifth edition of the WHO tumor classification.[34] It is histologically characterized by markedly atypical nuclei, sometimes with prominent appearing nucleoli, good number of mitotic figures, and also apoptotic bodies.

The cells may display a 'hobnail' appearance, and the overall architecture may resemble a papillary pattern. Multiple studies have described it as being tumor restricted to an endometrial polyp.[33]

5. Microglandular Variant:

It is consisting of tightly packed small to medium-sized glands lined by one or more layers of cuboidal, columnar, or flattened cells. These cells exhibit amphophilic, eosinophilic, or mucin-rich cytoplasm and are often accompanied by intraluminal mucin, neutrophils, and variable mitotic activity. Occasionally, focal areas of solid or squamous differentiation may also be present.[35]

6. Serous Adenocarcinoma:

The serous subtype constitutes roughly 10% of all endometrial cancers. They are not estrogen dependent unlike endometroid carcinoma and they often present at an advanced stage, typically in older women.[36] Tumors with mixed histology, including less than 10% ESC, tend to have a worse prognosis than those with purely grade 3 endometrioid histology.[37] The precursor lesions for endometrial serous carcinoma are endometrial glandular dysplasia and endometrial intraepithelial carcinoma (EIC), which develop in atrophic endometrium.

Histologically it is consist of fibrous stroma and show a papillary pattern of proliferation. Tumor cells are pleomorphic, have prominent nuclear atypia, high N/C ratios, distinctive nucleoli, and frequent nuclear fission. Sixty percent of ESCs contain psammoma bodies. Other pathognomonic cellular features include a ballooned or hobnail-like nucleus and stromal hyalinization.[36]

ESCs are not influenced by hormones and thus do not express ER or PR.[38] Genetic abnormalities in the p53, cyclin E–FBXW7, and PI3K pathways are major factors in the development of uterine serous carcinoma. Out of these p53 mutation occurred in 81.6% of cases.[39] Some studies have indicated that overexpression of HER2/neu is often linked to advanced stages and a poor prognosis in ESC.[40,41]

7. Clear Cell Adenocarcinoma:

Clear cell carcinoma is a rare but aggressive subtype, making up less than 5% of all uterine carcinomas.[42] Patients are typically older, more likely to present with advanced-stage disease, and have a worse prognosis compared to those with endometrioid carcinoma.[43] Endometrial CCC can be seen in four distinct morphological patterns, with the papillary structure being the most frequent, followed by tubular, cystic, and solid forms. The key diagnostic features of CCC are the clarity of the cytoplasm, along with eosinophilic cells and hobnail cells.[44] Evidence indicates that over 80% of patients with clear cell endometrial carcinoma exhibit p53 abnormalities or belong to the NSMP category.[45]

8. Mixed Adenocarcinoma:

Mixed adenocarcinomas of the endometrium are characterized by the presence of both endometrioid and non-endometrioid elements, including serous, clear cell, or mucinous carcinoma. These tumors often exhibit diverse histological patterns, with the endometrioid part generally being less aggressive. In contrast, the non-endometrioid components, like serous carcinoma, are more aggressive and linked to a worse prognosis.[46]

9. Malignant Mesodermal (Mullerian) Mixed Tumour (MMMT) (Carcinosarcoma):

Aggressive and rare, comprising for < 5% of all tumors of uterus.[47] MMMT is the most frequently occurring subtype of uterine sarcomas.[48] The risk factors for developing carcinosarcoma are comparable to those of endometrial carcinoma and include nulliparity, older age, obesity, exogenous estrogen exposure, and prolonged tamoxifen use.[49]

MMMT is a dual-component tumor of the female genital tract, made up of epithelial and mesenchymal tissues. It is also known in the literature by names such as "malignant mesodermal mixed tumor," "metaplastic carcinoma," and "carcinosarcoma.[50]

Carcinosarcomas are classified into two histological subtypes based on the features of the sarcomatous component. The heterologous subtype includes rhabdomyosarcoma, osteosarcoma, chondrosarcoma, or liposarcoma, whereas the homologous subtype typically consists of fibrosarcoma, leiomyosarcoma or endometrial stromal sarcoma. In both cases, the carcinomatous component can exhibit endometrioid, serous, or clear cell characteristics.[51]

10. Undifferentiated Carcinoma:

Both dedifferentiated and undifferentiated endometrial carcinomas are rare, very aggressive forms of cancer, accounting for approximately 10% of all high-grade carcinomas of endometrial and 2% of all endometrial carcinomas.[52] They have a poor prognosis and biological heterogenicity.[52,53]

UDC/DCC, alongside carcinosarcoma, is only subtype of endometrial carcinoma that invariably needs chemotherapy and/or radiation therapy, even in cases of Ia FIGO stage with no residual tumor detected in the specimen of hysterectomy.[54]

DDC describes a tumor featuring a UDC part intermingled with an endometrioid component of low grade, with the UDC part may result from the dedifferentiation of well-differentiated endometrioid element. As a result, the WHO considers UDC and DDC to be a single entity.[54]

11. Mesonephric-like Carcinoma:

Endometrial mesonephric-like carcinomas (MLCa) are uncommon tumors characterized by a combination of architectural and cytologic features. These often display architectural diversity including ductal or tubular pattern, nuclei resembling those seen in papillary thyroid carcinoma- That is overlapping of vesicular nuclei along with grooves, and areas with intraluminal secretions which are eosinophilic. The immunoprofile generally shows less or no expression of hormonal receptors, but focal GATA-3 and/or TTF-1 positivity at least.[55]

Mesonephric-like carcinoma is frequently misdiagnosed, making it essential to combine morphological and immunohistochemical features, along with molecular analysis, when necessary for an accurate diagnosis.[56]

12. Gastrointestinal mucinous-type carcinoma: Gastric-type endometrial carcinoma is an uncommon histological subtype, with its clinical and pathological features and treatment strategies yet to be fully characterized.

The morphological diagnostic criteria for gastric-type endometrial carcinoma (GTEC) were recently established by Wong et al[57], and Kojima et al.[58] for cervical gastric-type adenocarcinoma.

These criteria include:

1. Pale to clear eosinophilic cytoplasm
2. Abundant cytoplasm
3. Well-defined cell borders

Additional criteria specific to GTEC are:

1. Excluding the cervical involvement,
2. Absence of other primary tumor sites,
3. At least focal positivity for one or more gastrointestinal markers (CK20, CDX2, MUC6),
1. Reduced or absent expression of estrogen and progesterone receptors, and
2. Absence of an endometrioid component.[57,59]

Coexisting carcinoma of endometrium and ovary-

Simultaneous occurrence of cancers of the endometrium and ovary may indicate:

- Metastasis from the endometrium to the ovary,
- Metastasis from the ovary to the endometrium, or
- Independent primary tumors.

Differentiating between these possibilities is important because prognosis and treatment can vary.

It has been suggested that when the endometrial carcinoma is small and minimally invasive, the two tumors should be considered independent. One study found that if both carcinomas exhibit an endometrioid pattern, the prognosis is favorable, suggesting that the tumors are likely independent.

Metastatic breast cancer is the most common extragenital tumor to spread to the uterus (47%), followed by metastases from the stomach (29%), cutaneous melanoma (5%), lungs (4%), colon (3%), pancreas (3%), and kidneys (3%).[17]

<u>Tumor invasion</u>-

MYOMETRIAL INVASION:

Extent of depth of myometrial invasion is important for subcategorization of FIGO stage I. Present guidance from ISGyP and ICCR suggests that myometrial invasion depth in each and every endometrial carcinomas should be documented as 'none', 'less than half', or 'more than half'[59]

According to the ESGO/ESTRO/ESP guidelines of endometrial cancer, non-endometrioid carcinomas are considered medium risk when there is no myometrial invasion, however they are categorised as high risk if myometrial invasion is there.[60]

Endometrial carcinomas can show a range of myometrial invasion patterns, such as expansile , infiltrative, adenoma malignum, adenomyosis-like, microcystic elongated fragmented patterns.[61] These patterns can pose challenges in accurately diagnosing the presence and extent of myometrial invasion.

In the dataset of ICCR, the pattern of invasion of myometrium is considered a less important element, as at present is not utilized for risk assessment.[60]

VASCULAR AND LYMPHATIC INVASION: Substantial LVSI is an sole predictor of worse prognosis, related with lymph node and distant metastases.[62] In the FIGO 2023 staging system, low-grade endometrioid ECs with no LVSI or focal LVSI are classified as stages IA and IB. However, the presence of substantial LVSI, defined by the WHO as five or more foci, in cases confined to the uterus results in an automatic upgrade to stage IIB disease.

The updated 2023 FIGO staging system discriminates between micro-metastasis (0.2-2 mm) and macro-metastases (>2 mm) in pelvic (IIIC1) and para-aortic (IIIC2) lymph node metastases by adding "i" for micro-metastasis and "ii" for macro-metastases.[63]

Cervical involvement: The involvement of cervical stroma is an adverse factor associated with a less favorable prognosis in endometrial cancer.[64] Studies indicate that it is challenging to differentiate between a tumor confined to lower uterine segment vs a tumor limited to the upper endocervix, as well as to distinguish amongst cervical stromal and glandular involvement, as they lack clear boundaries.[65]

Peritoneal involvement: In the 2023 FIGO staging system, peritoneal carcinomatosis is separated from distant metastases. When peritoneal carcinomatosis is confined to the pelvis, it is now categorized as stage IIIB2 (a downstage from the previous 2009 stage IVB).

However, when it extends beyond the pelvis, it remains in stage IV but is designated as a separate substage (2023 stage IVB), distinguishing it from other distant intra- and extra-abdominal metastases (2023 stage IVC).[63]

Involvement of the uterine serosa, adnexa, parametrium, and vagina:

Endometrial cancer is classified as pT3a when there is involvement of the uterine serosa and adnexa.[35] Serosal involvement is defined as the tumor infiltration of the entire thickness of myometrium and encroaching the mesothelial layer or else submesothelial fibroconnective tissue, even if the tumor is not visible on the surface or if there is no desmoplastic reaction.[46]

Fallopian tube involvement should be diagnosed when carcinoma involves tubal mucosa (with or without stromal invasion) or wall/serosa, except for endometrioid or clear cell carcinoma arising in endometriosis.

Vaginal and parametrial invasion results in the upstaging of endometrial cancer to pT3b.[35]

It is important to note that LVSI alone in the uterine serosa, parametrium, or adnexal or periadnexal tissues does not qualify as true involvement and should not be considered in staging.[46]

PROGNOSTIC FACTORS-

Age: Younger patients with uterine endometrial carcinoma generally have a more favourable prognosis compared to older women. Younger patients often have a history of conditions associated with estrogen or hormonal imbalances.[66] A poorer prognosis in older patients has been linked to a greater prevalence of grade 3 carcinomas or less favourable subtypes.

Parity: Endometrial carcinoma is traditionally linked to nulliparity, late menopause and obesity.

Histological type: Endometrial carcinomas which are associated with estrogen exposure tend to have better prognosis compared to non-estrogenic carcinomas which invades the myometrium and vascular spaces comparative early and possess more advance stage of disease, hence high tumour grades.[67]

The grade of the glandular component in adenocarcinoma with squamous differentiation is an indicator of prognosis.[68]

Histological grade: Histological grade was strongly linked to myometrial invasion, Vascular invasion and somewhat also to cervix, adnexa, and serosal surface involvement.[69]

Myometrial and vascular involvement: Tumors with a higher histological grade tend to invade more extensively involving more than half of the myometrium and are also associated with lymphovascular invasion.[70] Lymphovascular space involvement (LVSI) is a key prognostic marker for recurrence of disease and reduced age of survival. Both myometrium and vascular involvement have their own independent prognostic significance.[67]

Cervix and serosal surface involvement: Patients with tumors involving the cervix are regarded as having a poorer prognosis compared to those with tumors limited to the corpus. Cervix and Serosal surface involvement independently upstages the grade of endometrial carcinoma, hence are poor prognostic indicators.[46]

IMMUNOHISTOCHEMISTRY

P16 Introduction:

The INK4 family consists of four members:

p16 INK4A, p15 INK4B, p18 INK4C, and p19 INK4D

All of these share similar biological properties related to the cell growth inhibition and tumor suppression.[71] The INK4A locus is located on the short arm of chromosome 9 at position 21.3 (9p21.3).[72] P16INK4A serves as a tumor suppressor and cell cycle regulator and is regarded as a specific biomarker for detecting aging and senescent cells.[73]

Mechanism of action of P16:

P16 is also referred to as inhibitor of cyclin-dependent kinase 4a (INK4A) multiple tumor suppressor-1 (MTS-1), or cyclin-dependent kinase inhibitor 2a (CDKN2A). Cellular senescence is a permanent halt in cell proliferation triggered via 2 primary intrinsic and extrinsic factors in reply to potential oncogenic stress.[73] P16, alongside other tumor suppressors, plays an important role in intrinsic pathway of cellular senescence, serving as an upstream regulator of pRB.[74]

The primary regulatory mechanism of its pathway inhibits the cyclin-dependent kinases CDK4/6 and CDK2, which subsequently regulate the cell growth and cycle.[73] The suppression of p16 restores cell proliferation and enables the bypass of senescence, thereby facilitating tumorigenesis.[75] Recent studies have shown elevated p16 expression following injury.[73] Numerous studies highlight p16 protein detection as an effective indicator of HPV activity, particularly for HPV-16.[76]

Expression of P16 in normal endometrium:

In the normal endometrium, p16 expression is generally low and fluctuates throughout the menstrual cycle. During the proliferative phase, when endometrial cells rapidly divide, p16 expression is carefully regulated to prevent uncontrolled cell growth. In the secretory phase, p16 levels correspond to the necessary balance of cellular activity needed to prepare the endometrium for potential implantation. Recent research has provided further understanding of p16 expression in the endometrium. Studies have demonstrated that the normal cyclical endometrium shows patchy glandular staining and localized stromal expression of p16 in the functional layer during the proliferative phase. This indicates that p16 might play a role in the cyclical regeneration and maintenance of endometrial tissue.[77]

The precise regulation of p16 expression in the normal endometrium stands in stark contrast to its dysregulation in pathological conditions. Abnormal levels of p16, whether overexpressed or loss, have been implicated in disorders such as endometrial hyperplasia and carcinoma, underscoring its essential role as a tumor suppressor. This highlights the significance of p16 in maintaining cellular stability and proper regulation within the normal endometrium.[78]

Role of P16 in endometrial hyperplasia (EH)

In endometrial hyperplasia, P16 overexpression often serves as a biomarker of abnormal cellular proliferation. This dysregulation typically results from mutations in upstream regulatory pathways, such as the PI3K/AKT/mTOR axis, which are frequently altered in hyperplastic and malignant endometrial tissues.[79] Immunohistochemical staining of P16 can help differentiate between benign hyperplasia of endometrium and hyperplasia with atypia or endometrial intraepithelial neoplasia.

Studies indicate that hyperplasia with atypia and EIN frequently exhibit increased P16 expression compared to non-atypical hyperplasia, suggesting its utility in identifying higher-risk lesions.[80] Elevated levels of P16 have been linked to increasing risk of continuation from hyperplasia to endometrial carcinoma. In cases of atypical hyperplasia, P16 overexpression may indicate an underlying predisposition to malignancy, emphasizing its prognostic relevance.[81] Advancing knowledge of P16-associated signaling pathways holds promise for developing targeted treatments aimed at preventing the progression of endometrial hyperplasia to malignancy[82]

Role of P16 in endometrial carcinoma:

Endometrial carcinoma is categorized into Type I and II on the basis of both molecular and clinical characteristics. The expression of p16 varies notably between these two subtypes. Type II tumors typically show pronounced p16 overexpression, which is closely associated to high-grade histologies, for example, serous and clear cell carcinomas, both of which are linked to poorer prognostic outcomes.[83] In Type I tumors, p16 expression is usually less pronounced but may intensify in advanced stages of the disease, especially in higher-grade tumors. This pattern implies that while p16 overexpression in Type I tumors could be associated with tumor progression, it does not have the same poor prognostic significance as seen in Type II tumors.[77]

Stromal p16 expression in non-cancerous lesions like endometrial polyps complicates diagnosis, emphasizing the need for a comprehensive panel of markers to differentiate benign and malignant lesions.[80]

Significance of p16 expression in endometrial carcinoma

P16 overexpression serves as an important indicator of poor prognosis in Type II endometrial carcinoma, as it is linked to increased tumor grade, greater invasiveness, and a higher likelihood of metastasis.[75] The presence of p16 in Type II carcinomas can be used to differentiate them from benign conditions or other malignancies, making it a valuable diagnostic marker. In Type I tumors, p16 overexpression has been suggested as a marker of tumor progression, especially in advanced-stage cases. However, it does not carry the same prognostic weight as in Type II tumors. In summary, p16 immunohistochemistry is an essential tool in the management of endometrial carcinoma, providing key insights for both diagnosis and prognosis.[82,84]

MATERIALS AND METHODS

Study design: Cross sectional study

Study population and data collection: All histologically diagnosed cases of endometrial carcinoma specimens received in the department of pathology at KLE's DR. PRABHAKAR KORE HOSPITAL AND RESEARCH CENTER, a teaching hospital attached to KAHER'S JAWAHARLAL NEHRU MEDICAL COLLEGE, BELAGAVI.

Study period: 3 years

1 year retrospective (January 2022-december 2022)

2 years prospective (From January 2023 – December 2024)

Sample size: Total cases= 80

40 cases of endometrial carcinoma

40 cases of endometrial hyperplasia

Selection criteria:

Inclusion criteria:

1. All histopathologically diagnosed cases of endometrial carcinoma including endometrial curettage, biopsies and resected hysterectomy specimens if available.
2. Histologically diagnosed cases of endometrial hyperplasia (Including both hyperplasia with atypia and without atypia)

Exclusion criteria:

1. Inadequate samples
2. Improperly preserved or improperly fixed specimen

Ethical clearance: The ethical clearance was acquired from Institutional Ethics Committee, JNMC, Belagavi prior to the commencement of study.

Method of data collection:

Procedure: All curettage, biopsy, and hysterectomy specimens of endometrial carcinoma received at the histopathology laboratory were collected, numbered, and fixed in 10% formalin overnight. The following day, the specimens were subjected to gross examination, and representative tissue were labelled placed in separate capsules. These capsules were subsequently processed in a tissue processor (Leica TP1020).

The processed tissues were then removed from the capsules and embedded in molten paraffin wax and blocks were prepared. Sections measuring 4 microns in thickness were cut and stained using hematoxylin and eosin (H&E). Histological examination, grading and histopathological evaluation was done using Nikon Ei microscope.

The slides intended for immunohistochemistry (IHC) were precoated with Poly-L-Lysine and stained for IHC using a specific mouse monoclonal antibody to p16 (biogenex). Normal epithelial and stromal cells of proliferative endometrium were used as a positive control, while negative control IHC staining was performed without the primary antibody. After immersion in xylene, the slides were mounted with a coverslip using Dibutylphthalate Polystyrene Xylene (DPX).

Both H&E and p16 IHC staining procedures were performed and the slides were mounted. The procedure of staining is explained in ANNEXURE.

All the slides of endometrial carcinoma were examined, classified and reported using WHO classification and FIGO grading system by a pathologist on H and E staining as:

The FIGO three-grade system[20] categories of tumors according to the proportion of solid to glandular areas:

SN	Grade	Solid area
1	Grade 1	5% or less solid growth
2	Grade 2	5% to 50% solid growth
3	Grade 3	More than 50% solid growth[23]

Table 11: FIGO architectural grading of endometroid carcinoma

According to nuclear grading-

SN	Grade	Nuclear features
1	Grade 1	Oval, mildly enlarged, and have evenly dispersed chromatin.
2	Grade 2	Features intermediate to grades 1 and 3
3	Grade 3	Markedly enlarged and pleomorphic, with irregular, coarse chromatin and prominent eosinophilic nucleoli.

Table 12: FIGO nuclear grading of endometroid carcinoma

Tumors with an architectural grade of 1 or 2 were considered a higher grade if there was significant nuclear atypia (grade 3 nuclei) present in more than 50% of the tumor.[17]

Major histological grading was given using WHO 2021 classification[85] as:

SN	Grade	Categories
1	Low grade	Endometroid carcinoma FIGO grade 1 and 2
2	High grade	Endometroid carcinoma FIGO grade 3 and all Non-endometroid carcinoma

Table 13: WHO grading of endometrial carcinoma

Equal number (n= 40) cases of endometrial hyperplasia were collected, reported and classified according to WHO 2012 classification as follows:

1. Endometrial hyperplasia without atypia
2. Endometrial hyperplasia with atypia[21]

The p16 slides were assessed under Nikon Ei microscope.

CRITERIA USED TO INTERPRET p16 EXPRESSION INCLUDES-

1 Site of staining

Nuclear	Cytoplasmic	Both nuclear and cytoplasmic

2 Percentage of tumor cells staining and staining intensity[86]

Percentage of positive cells	Score	Staining intensity	Score
Less than 10%	0	Negative	0
10 to 24%	1	Weak	1
25 to 50%	2	Moderate	2
50% or more	3	Strong	3

1. Pattern of tumor cells staining[87]

Pattern of tumor cells staining	Score
Negative	O
Focal/ patchy	1
Strong/ Diffuse	2

Table 14, 15, 16: P16 IHC reporting criteria

For evaluation of p16 immunohistochemical staining, the evaluation was done using the x400 magnification of the microscope. All the cases showing a visible brown staining for nucleus and cytoplasm was taken as positive for p16 marker.

Appropriate scoring was done for the percentage of tumor cells in endometrial carcinoma and epithelial cells in hyperplasia, staining pattern and intensity of tumor cells in carcinoma and epithelial cells in hyperplasia.

Statistical analysis

Data obtained was entered in Microsoft Excel software and analysed and expressed in percentage and proportions.

Cases showing p16 positivity and its association with histopathological grading were studies.

A p-value of less than 0.05 was considered statistically significant.

RESULTS

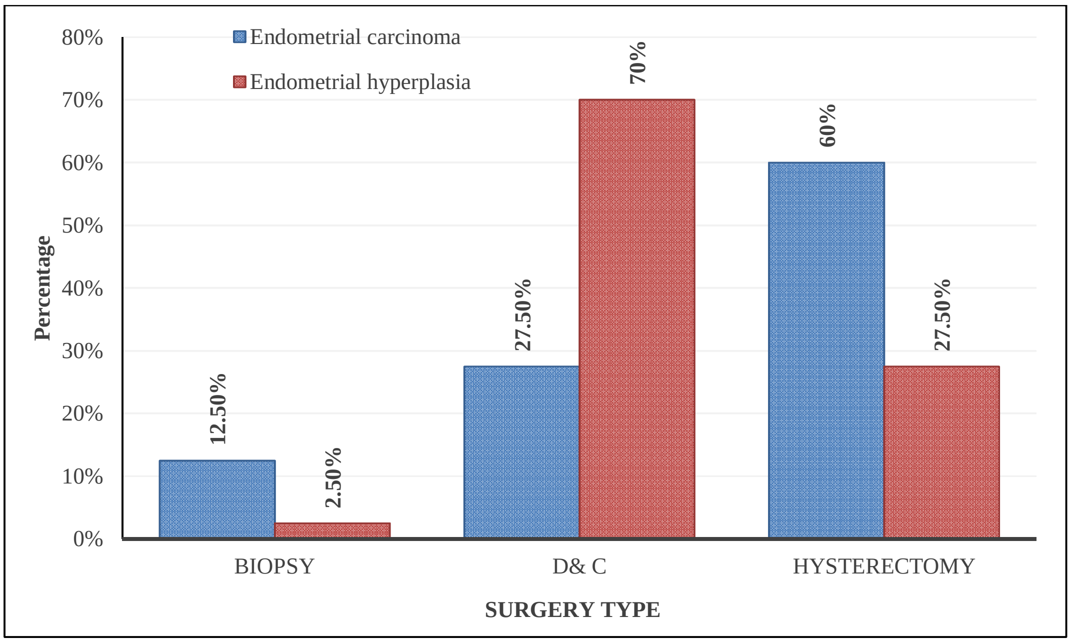
In the present study, 40 cases of endometrial carcinoma and 40 cases of endometrial hyperplasia were studied. All cases were evaluated for H&E stain for histological grading followed by assessment of p16 expression. Case showing p16 positivity and its association with histopathological grading were studied.

The dataset includes total 80 cases, with 40 classified under endometrial carcinoma and 40 under endometrial hyperplasia. In these cases, majority (39) were dilatation and curettage specimens, followed by 35 cases of hysterectomy and 6 cases of endometrial biopsy.

Table 17: Comparison of procedure type over groups.

Surgery Type	Endometrial carcinoma (n 40)	Endometrial hyperplasia (n 40)	Total (n 40)
Biopsy	5 (12.5%)	1 (2.5%)	6 (7.5%)
D& C	11 (27.5%)	28 (70%)	39 (48.75%)
Hysterectomy	24 (60%)	11 (27.5%)	35 (43.75%)

Figure 14: Comparison of procedure type over groups.

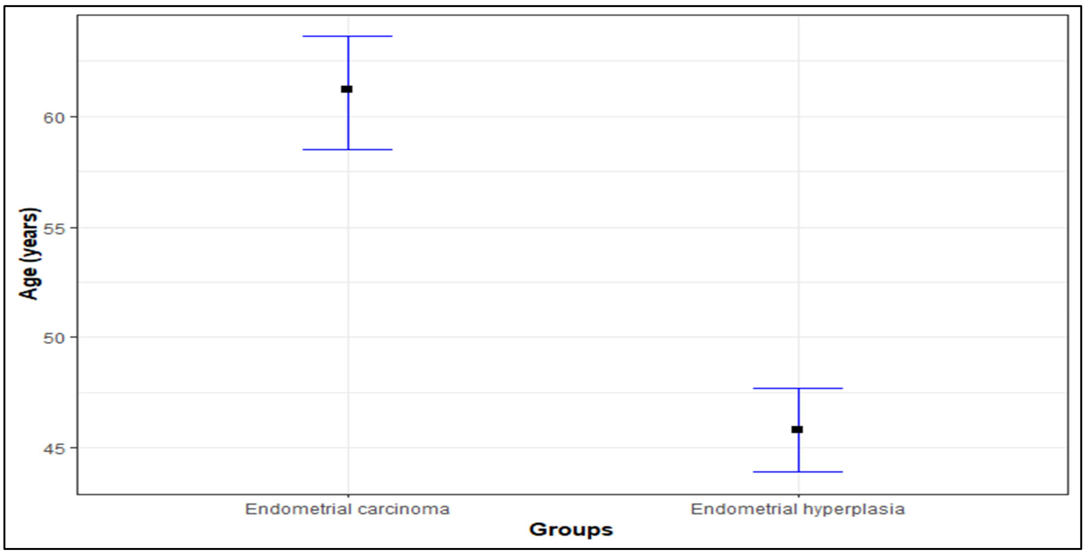


Hysterectomy was the predominant surgical procedure for endometrial carcinoma, whereas D&C was the most common procedure in endometrial hyperplasia.

Table 18: Comparison of age over groups

Age (years)	Endometrial carcinoma (n 40)	Endometrial hyperplasia (n 40)	Total (n 80)	p-value
≤ 40	1 (2.5%)	9 (22.5%)	10 (12.5%)	**< 0.001**^{MC}*****
41-50	3 (7.5%)	24 (60%)	27 (33.75%)	
51-60	16 (40%)	6 (15%)	22 (27.5%)	
61-70	14 (35%)	1 (2.5%)	15 (18.75%)	
>70	6 (15%)	0	6 (7.5%)	
Mean ± SD	61.15 ± 8.27	45.77 ± 6.29	53.46 ± 10.64	**< 0.001**^{MW}*****

Figure 15: Mean plot of age over groups.



In the endometrial carcinoma group, the highest proportion of cases were in the age groups 51-60 years, followed by 61-70 years. In contrast, endometrial hyperplasia was more prevalent in younger age groups, with the majority in the 41-50 years group, followed by ≤40 years.

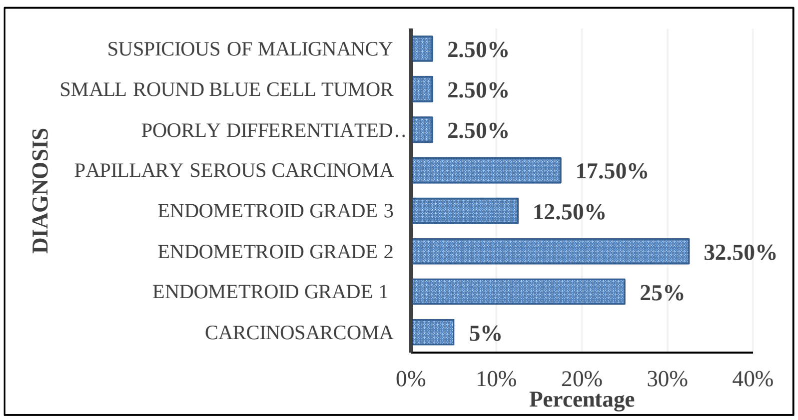
The mean age in the endometrial carcinoma group was 61.15 ± 8.27 years. In the endometrial hyperplasia group, the mean age was 45.77 ± 6.29 years.

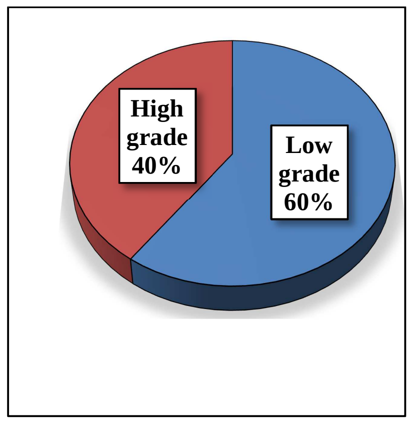
From Chi-square test, it is observed that the age difference between two groups was statistically significant (p-values < 0.001).

Table 19: Distribution of endometrial carcinoma as per WHO classification and FIGO grading system

Endometrial carcinoma types	Number of subjects (n 40) (%)	Grade	
		Low grade 24 (60%)	High grade 16 (40%)
Carcinosarcoma	2 (5%)	0	2
Endometroid FIGO grade 1 ca	10 (25%)	10	0
Endometroid FIGO grade 2 ca	13 (32.5%)	13	0
Endometroid FIGO grade 3 ca	5 (12.5%)	0	5
Papillary serous carcinoma	7 (17.5%)	0	7
Poorly differentiated carcinoma	1 (2.5%)	0	1
Small round blue cell tumor	1 (2.5%)	0	1
Suspicious of malignancy	1 (2.5%)	1	0

Figure 16, 17: Distribution of Endometrial carcinoma cases as per WHO classification and FIGO grading system





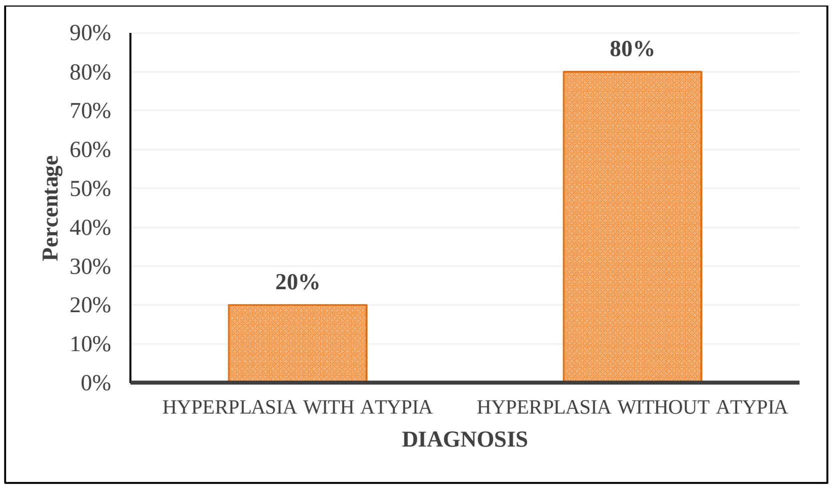
Among the 40 cases of endometrial carcinoma, the most common category was Endometrioid grade 2 carcinoma, followed by Endometrioid grade 1 carcinoma. The least common types included Poorly differentiated carcinoma and small round blue cell tumor.

Low grade endometrial carcinoma is more common (60%) compared to high grade endometrial carcinoma (40%).

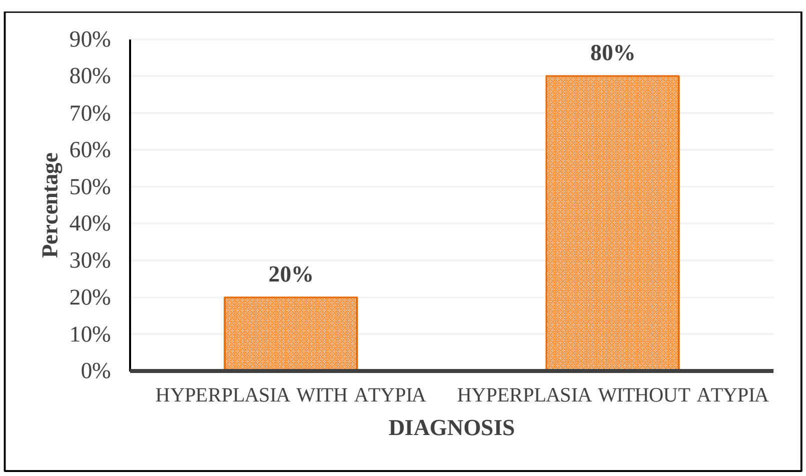
Table 20: Distribution of Endometrial hyperplasia cases as per WHO classification.

Endometrial hyperplasia	Number of subjects (n, %)
Hyperplasia with atypia	8 (20%)
Hyperplasia without atypia	32 (80%)

Figure 18: Distribution of Endometrial hyperplasia cases as per WHO classification



Among 40 subjects with Endometrial hyperplasia, the majority of cases were diagnosed as hyperplasia without atypia (80%), while hyperplasia with atypia was less common (20%).

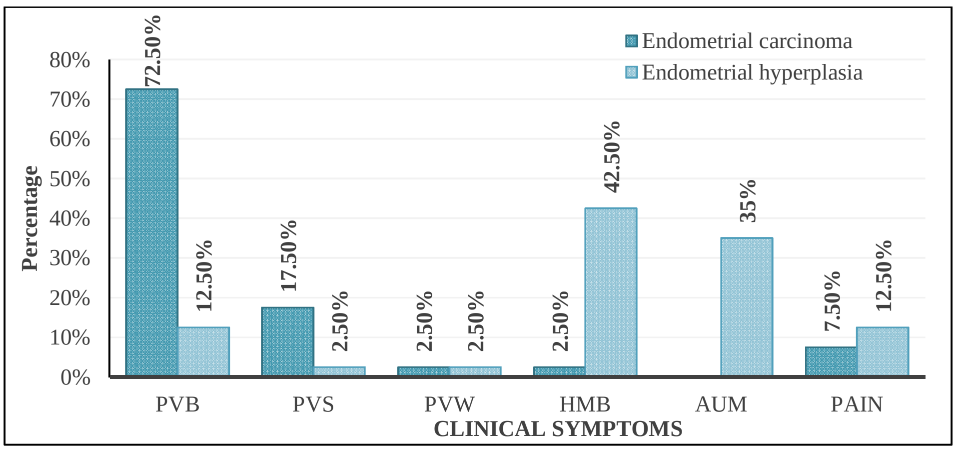


Among 40 subjects with Endometrial hyperplasia, the majority of cases were diagnosed as hyperplasia without atypia (80%), while hyperplasia with atypia was less common (20%).

Table 21: Comparison of clinical symptoms over groups.

Symptoms	Endometrial carcinoma (%)	Endometrial hyperplasia (%)	Total	Total
Per vaginal bleeding (PVB)	29 (72.5%)	5 (12.5%)	34 (42.5%)	**< 0.001^{C}***
Heavy menstrual bleeding (HMB)	1 (2.5%)	17 (42.5%)	18 (22.5%)	**< 0.001^{C}***
Abnormal uterine bleeding (AUB)	0	14 (35%)	14 (17.5%)	**< 0.001^{C}***
Per vaginal spotting (PVS)	7 (17.5%)	1 (2.5%)	8 (10%)	0.0675^{MC}
Per vaginal white discharge (PVW)	1 (2.5%)	1 (2.5%)	2 (2.5%)	1^{MC}
Pain	3 (7.5%)	5 (12.5%)	3 (7.5%)	0.7246^{MC}

Figure 19: Distribution of clinical symptoms over groups.



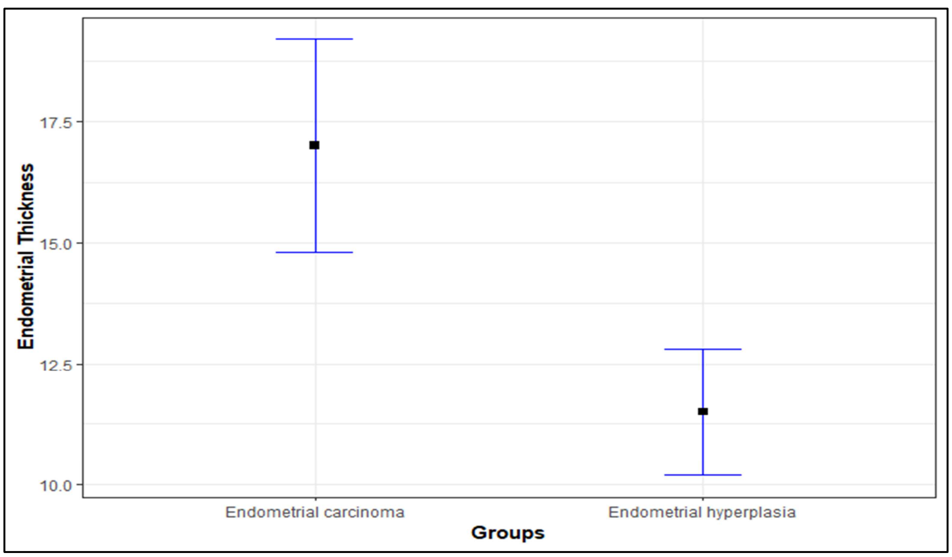
Per vaginal bleeding was significantly more common in endometrial carcinoma, compared to endometrial hyperplasia (p-value < 0.001).

Heavy menstrual bleeding and abnormal uterine bleeding (AUB) was predominantly observed in endometrial hyperplasia, whereas it was rarely present in case of endometrial carcinoma (p-values < 0.001). Per vaginal spotting, white discharge and abdominal pain were less commonly seen and the difference were statistically not significant (p-value = 0.0675).

Table 22: Comparison of endometrial thickness over groups.

Endometrial Thickness	Endometrial carcinoma (n 40)	Endometrial hyperplasia (n 40)	Total (n 80)	p-value
<5	0	1 (2.5%)	1 (1.25%)	**0.0020^{MC}***
5-10	7 (17.5%)	22 (55%)	29 (36.25%)	
>10	33 (82.5%)	17 (42.5%)	50 (62.5%)	
Mean ± SD	16.95 ± 7.18	11.47 ± 4.25	14.21 ± 6.48	**< 0.001^{MW}***

Figure 20: Distribution of endometrial thickness over groups.



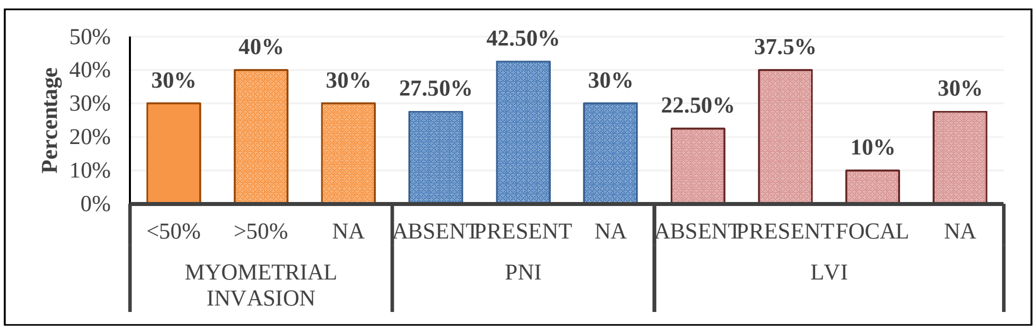
The mean endometrial thickness in endometrial carcinoma was 16.95 ± 7.18 mm. In endometrial hyperplasia, the mean thickness was significantly lower at 11.47 ± 4.25 mm.

There is statistical significant difference in the distribution of endometrial thickness between endometrial carcinoma and endometrial hyperplasia (p-values < 0.05).

Table 23: Distribution of Endometrial carcinoma cases according to Invasion Parameters.

Variables	Sub Category	Number of subjects (%)
Myometrial Invasion	<50%	12 (30%)
	>50%	16 (40%)
	Not applicable (NA)	12 (30%)
PNI	Absent	11 (27.5%)
	Present	17 (42.5%)
	Not applicable (NA)	12 (30%)
LVI	Absent	9 (22.5%)
	Present	19 (37.5%)
	Focal	4 (10%)
	Not applicable (NA)	12 (30%)

Figure 21: Distribution of Endometrial carcinoma cases according to different invasions.



Myometrial invasion, perineural invasion and lymphovascular invasion was reported in 28 (70%) of cases, but it was not applicable in 12 (30%) curettage and biopsy samples.

Out of those 70%, cases showing more than 50% myometrial invasion, perineural and lymphovascular invasion are more compared to those exhibiting less than 50% myometrial invasion, lacking perineural and lymphovascular invasion.

Table 24: Comparison of p16 dominant staining site across types of endometrial carcinoma.

Diagnosis (Endometrial ca)	Dominant Staining site			p-value
	Cytoplasm	Nuclear	Equally stained	
Carcinosarcoma	1 (50%)	0	50%)	0.0020^{MC}*
Endometroid grade 1	5 (50%)	0	5 (50%)	
Endometroid grade 2	4 (30.77%)	1 (7.69%)	8 (61.54%)	
Endometroid grade 3	0	2 (40%)	3 (60%)	
Papillary serous carcinoma	0	5 (71.43%)	2 (28.57%)	
Poorly differentiated carcinoma	0	1 (100%)	0	
Small round blue cell tumor	0	1 (100%)	0	
Suspicious of malignancy	0	1 (100%)	0	
Grade				0.0035^{MC}*
Low grade	9 (37.5%)	2 (8.33%)	13 (54.17%)	
High grade	1 (6.25%)	9 (56.25%)	6 (37.5%)	

Figure 22: p16 staining site distribution across types of endometrial carcinoma.

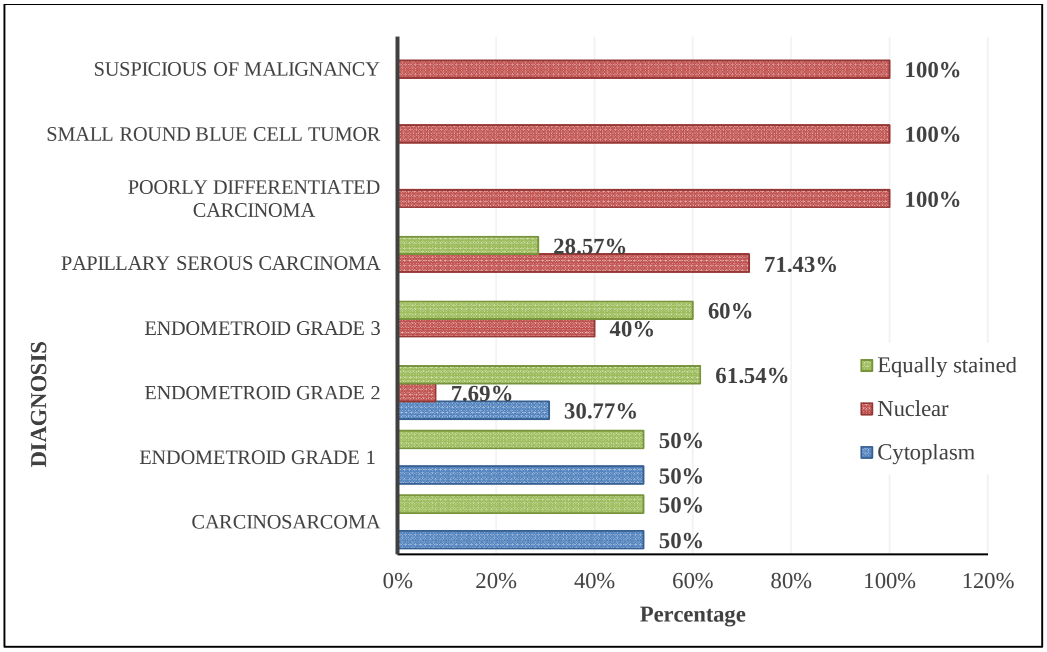
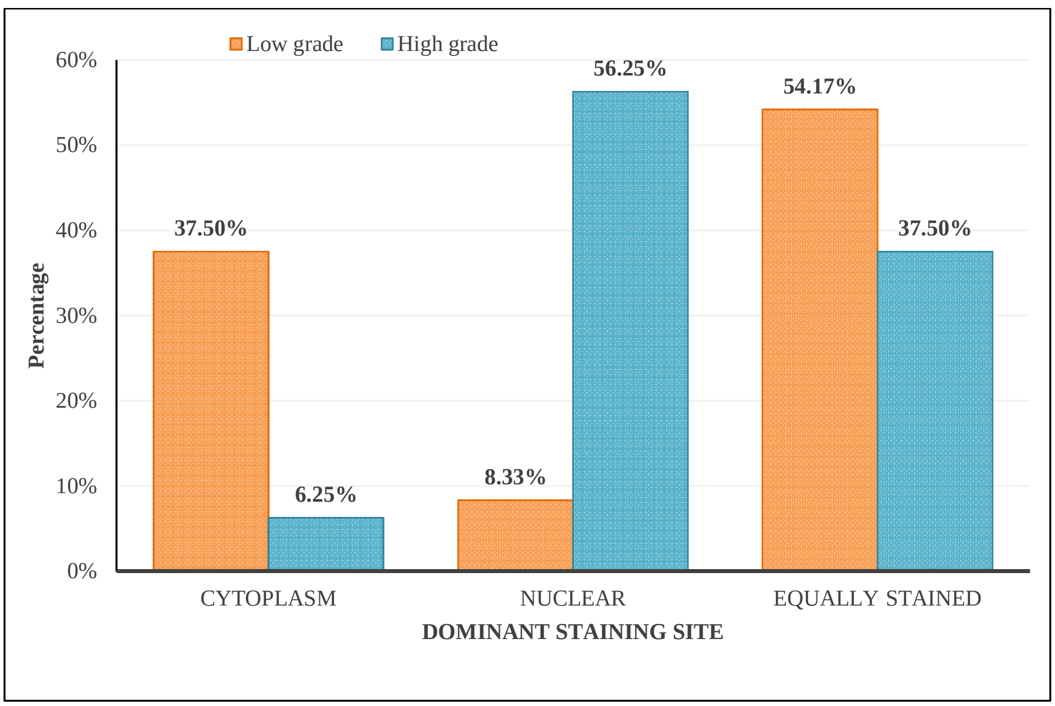


Figure 23: Distribution of dominant staining site across grades of endometrial carcinoma.



More aggressive carcinomas like Endometroid grade 3, serous, poorly differentiated have a nuclear or both nuclear and cytoplasmic staining compared to less aggressive subtypes like endometroid grade 1 and 2 carcinoma which shows a greater number of cytoplasmic or nuclear and cytoplasmic staining with occasional case of nuclear staining.

Similarly high grade endometrial carcinoma shows more cases of nuclear and nuclear plus cytoplasmic staining when compared to low grade carcinoma which show more cases of nuclear plus cytoplasmic or cytoplasmic staining.

This gives a significant difference in the distribution of dominant staining site over types and grades of endometrial carcinoma (p-values = 0.0020, 0.0035).

Table 25: Comparison of percentage of positive tumor cell across types and grades of endometrial carcinoma.

Diagnosis	**Positive Tumor Cells**				**p-value**
	<10%	**10-24%**	**25-50%**	**>50%**	
Carcinosarcoma	0	0	0	2 (100%)	0.2654^{MC}
Endometroid grade 1	2 (20%)	4 (40%)	4 (40%)	0	
Endometroid grade 2	0	3 (23.08%)	3 (23.08%)	7 (53.85%)	
Endometroid grade 3	0	0	0	5 (100%)	
Papillary serous carcinoma	0	0	0	7 (100%)	
Poorly differentiated carcinoma	0	0	0	1 (100%)	
Small round blue cell tumor	0	0	0	1 (100%)	
Suspicious of malignancy	0	0	0	1 (100%)	
Grade					**$< 0.001^{MC}$***
Low grade	2 (8.33%)	7 (29.17%)	7 (29.17%)	8 (33.33%)	
High grade	0	0	0	16 (100%)	

Figure 24: Distribution of percentage of positive tumor cells across types of endometrial carcinoma.

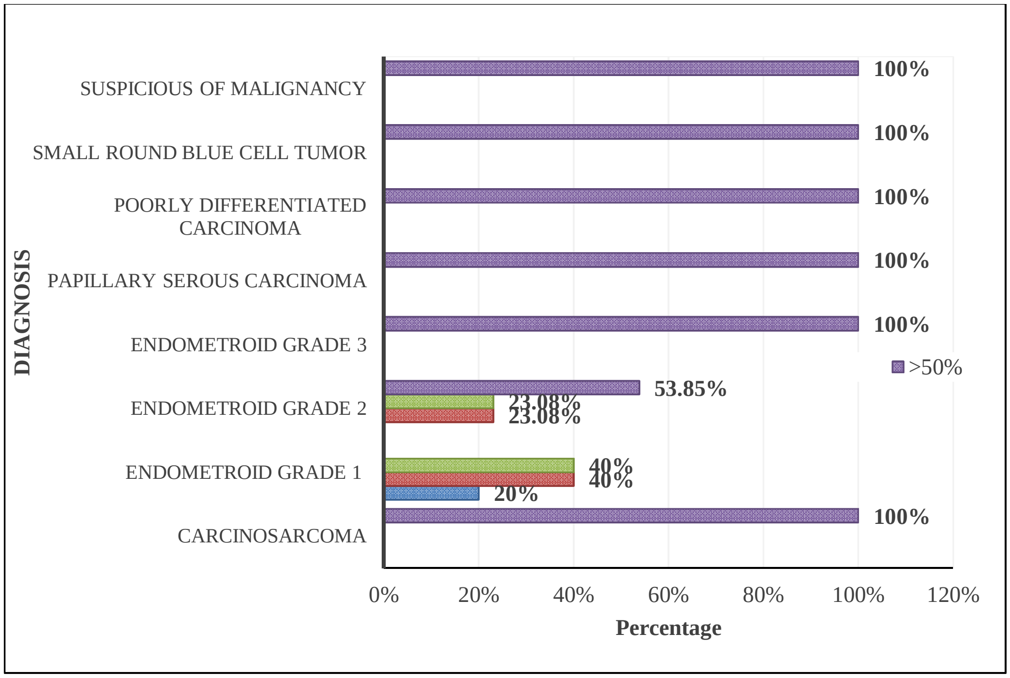
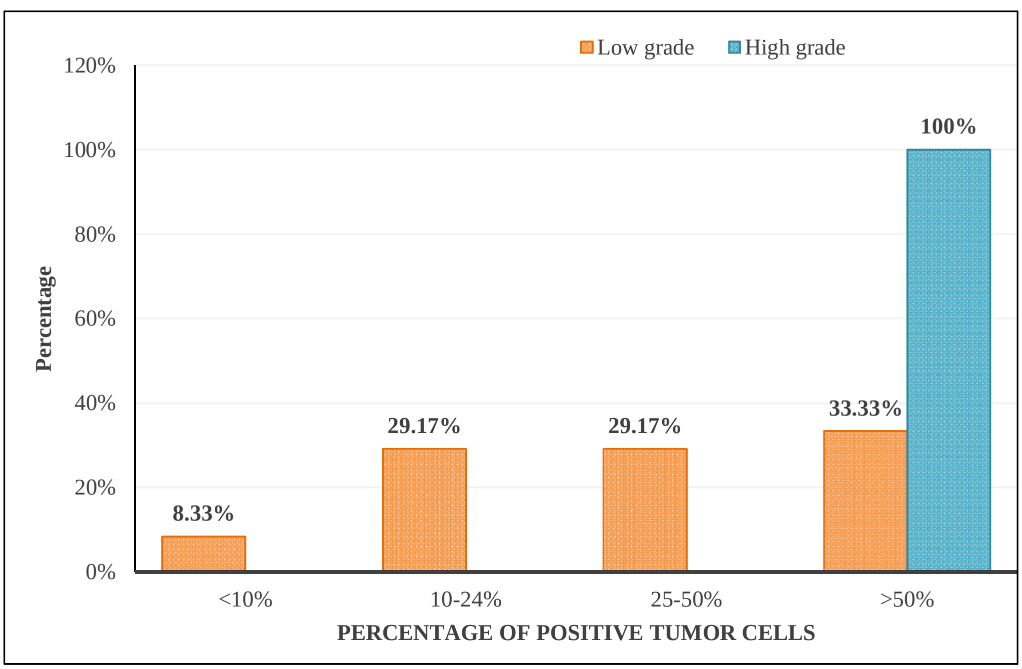


Figure 25: Distribution of percentage of positive tumor cells across grades of endometrial carcinoma.



There is no significant difference in the distribution of percentage of positive tumor cells over different types of endometrial carcinoma (p-value = 0.2654).

However, when expression of p16 is compared between major histological grades it is observed that cases of high grade endometrial carcinoma show >50% tumor cell positivity when compared to low grade carcinoma which shows variable degree of percent positivity of tumor cells. there is significant difference in the distribution of percentage of positive tumor cells over major histological grades of endometrial carcinoma (p-value < 0.001).

Table 26: Comparison of staining intensity across types and grades of endometrial carcinoma.

Diagnosis	Staining Intensity			p-value
	Strong	Moderate	Weak	
Carcinosarcoma	0	1 (50%)	1 (50%)	$< 0.001^{MC}$*
Endometroid grade 1	0	3 (30%)	7 (70%)	
Endometroid grade 2	0	8 (61.54%)	5 (38.46%)	
Endometroid grade 3	4 (80%)	1 (20%)	0	
Papillary serous carcinoma	7 (100%)	0	0	
Poorly differentiated carcinoma	1 (100%)	0	0	
Small round blue cell tumor	1 (100%)	0	0	
Suspicious of malignancy	1 (100%)	0	0	
Grade				$< 0.001^{C}$*
Low grade	1 (4.17%)	11 (45.83%)	12 (50%)	
High grade	13 (81.25%)	2 (12.5%)	1 (6.25%)	

Figure 26: Distribution of staining intensity across types of endometrial carcinoma.

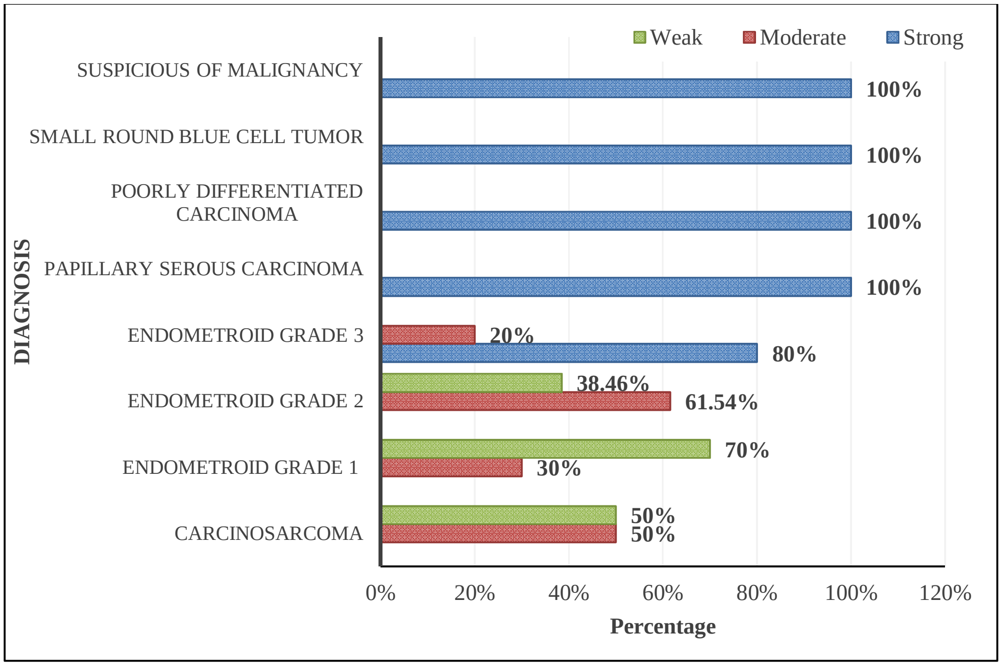
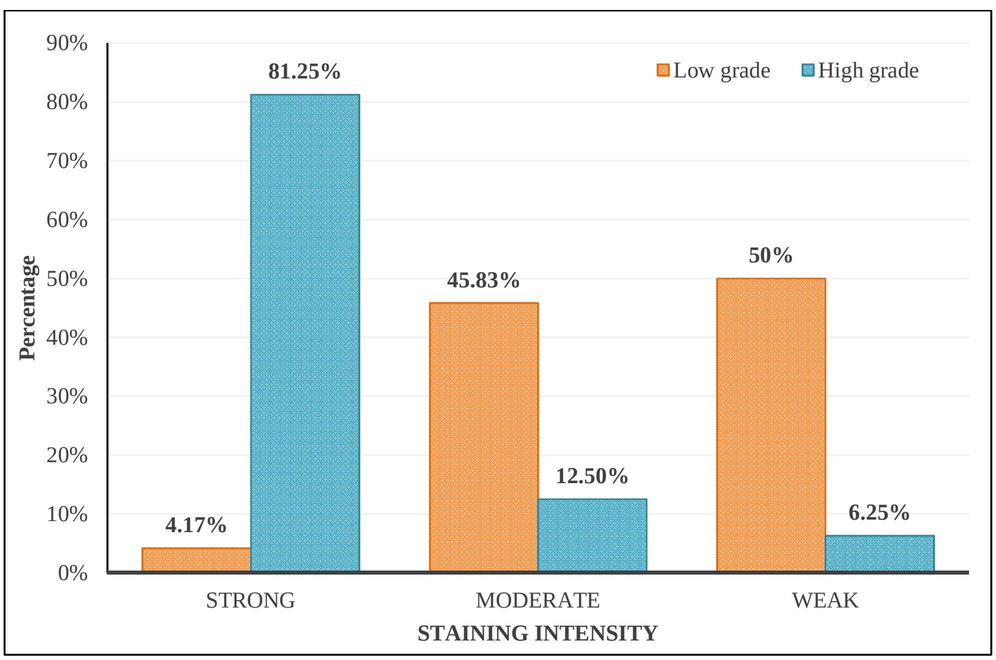


Figure 27: Distribution of staining intensity across grades of endometrial carcinoma.



Papillary serous, poorly differentiated, endometroid grade 3 and rare categories like small round blue cell tumor and suspicious of malignancy show more strong positivity when compared to endometroid grade1, 2 and carcinosarcoma which shows more weak to moderate staining.

High grade carcinoma show more frequency of strong staining intensity than moderate to weak. Low grade carcinoma show more weak and moderate staining intensity than strong.

Hence, that there is significant difference in the distribution of staining intensity over different types and major histological grades of endometrial carcinoma (p-values < 0.001).

Table 27: Comparison of staining distribution across types and grades of endometrial carcinoma.

Diagnosis	Staining Distribution		p-value
	Diffuse	Patchy	
Carcinosarcoma	2 (100%)	0	**< 0.001^{MC}***
Endometroid grade 1	0	10 (100%)	
Endometroid grade 2	0	13 (100%)	
Endometroid grade 3	2 (40%)	3 (60%)	
Papillary serous carcinoma	7 (100%)	0	
Poorly differentiated carcinoma	1 (100%)	0	
Small round blue cell tumor	1 (100%)	0	
Suspicious of malignancy	1 (100%)	0	
Grade			**< 0.001^{C}***
Low grade	1 (4.17%)	23 (95.83%)	
High grade	13 (81.25%)	3 (18.75%)	

Figure 28: Distribution of staining distribution across types of endometrial carcinoma

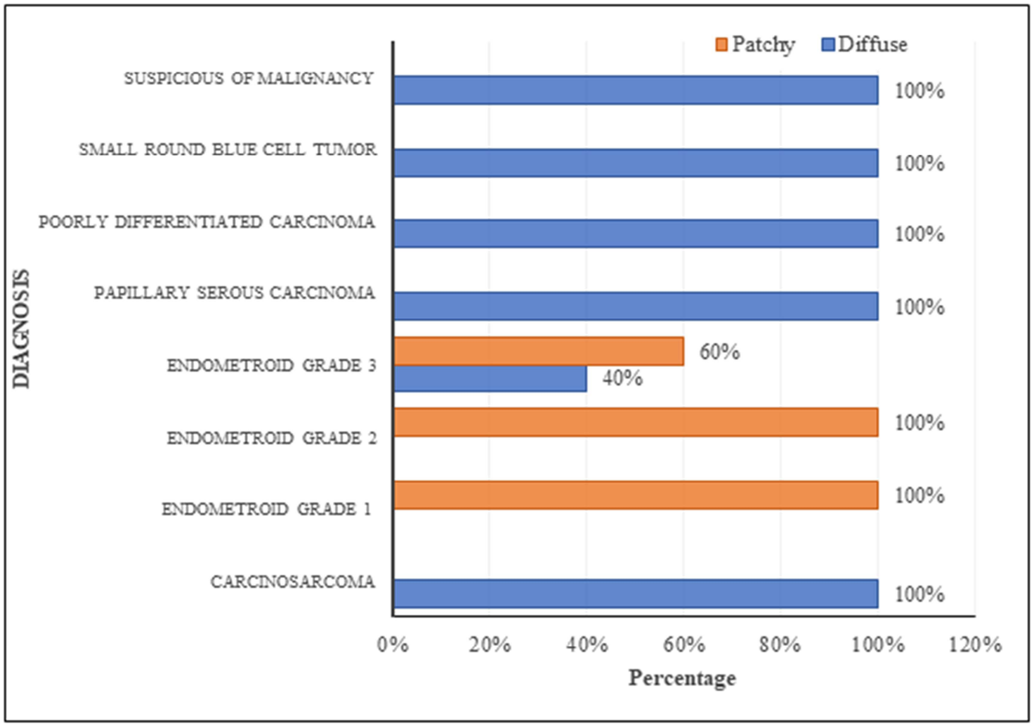
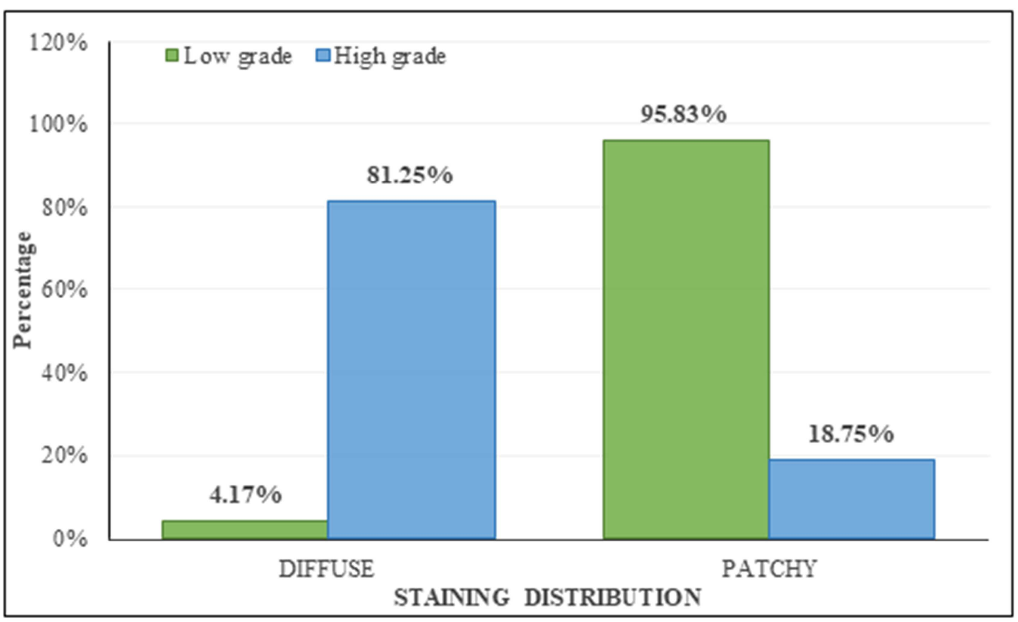


Figure 29: Distribution of staining pattern across grades of endometrial carcinoma



Diffuse staining pattern is seen more commonly in aggressive carcinomas like papillary serous, poorly differentiated, carcinosarcoma and few cases of endometroid grade 3 carcinomas when compared to Patchy staining pattern in less aggressive carcinoma like endometroid grades 1 and 2.

Similarly high grade endometrial carcinoma show more diffuse than patchy staining distribution, this when compared to low grade carcinoma, exhibited mostly patchy distribution and very occasional diffuse staining distribution.

There is significant difference in the distribution of staining distribution over types and major histological grades of endometrial carcinoma (p-value < 0.001).

Table 28: Comparison of IHC characteristics across different FIGO grades of Endometrioid Carcinoma.

IHC characteristics	Sub Category	Endometroid FIGO grade 1	Endometroid FIGO grade 2	Endometroid FIGO grade 3	p-value
Staining distribution	Diffuse	0	0	2 (40%)	**0.0285^{MC}***
	Patchy	10 (100%)	13 (100%)	3 (60%)	
Percentage of positive tumor cells	<10%	2 (20%)	0	0	**0.0080^{MC}***
	10-24%	4 (40%)	3 (23.08%)	0	
	25-50%	4 (40%)	3 (23.08%)	0	
	>50%	0	7 (53.85%)	5 (100%)	
Dominant staining site	Cytoplasm	5 (50%)	4 (30.77%)	0	0.0960^{MC}
	Nuclear	0	1 (7.69%)	2 (40%)	
	Equally stained	5 (50%)	8 (61.54%)	3 (60%)	
Staining intensity	Strong	0	0	4 (80%)	**< 0.001^{MC}***
	Moderate	3 (30%)	8 (61.54%)	1 (20%)	
	Weak	7 (70%)	5 (38.46%)	0	

Figure 30: Distribution of staining pattern across different FIGO grades of Endometrioid Carcinoma

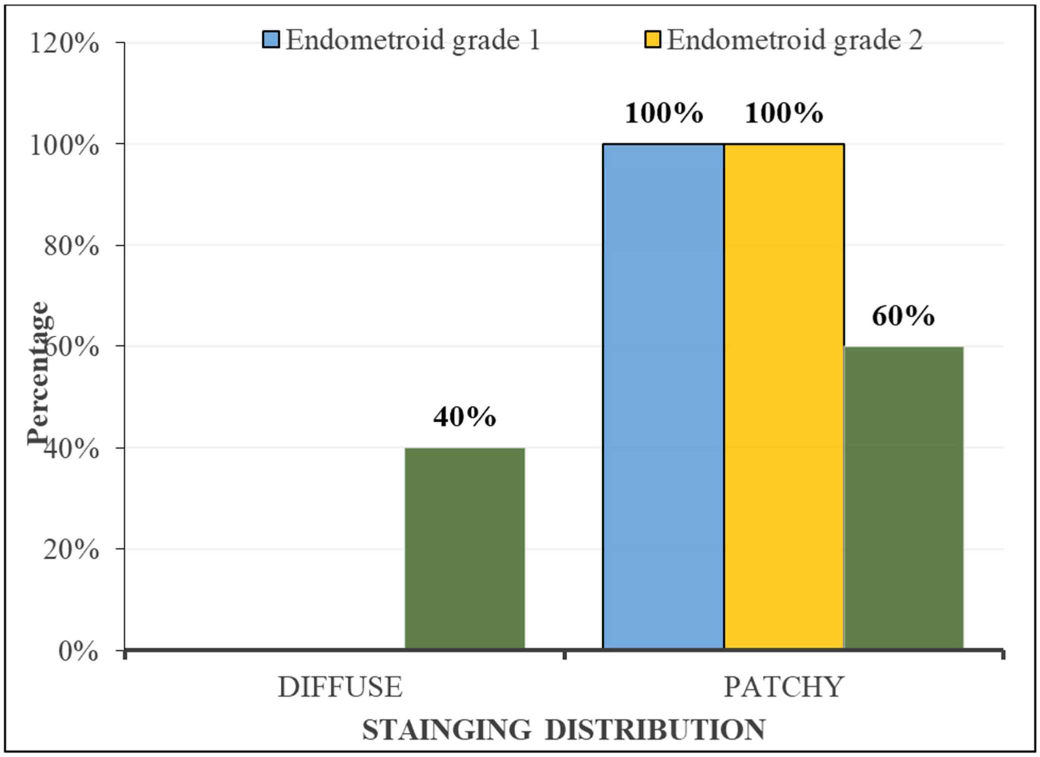


Figure 31: Distribution of Percentage of positivity of tumor cells across different FIGO grades of Endometrioid Carcinoma.

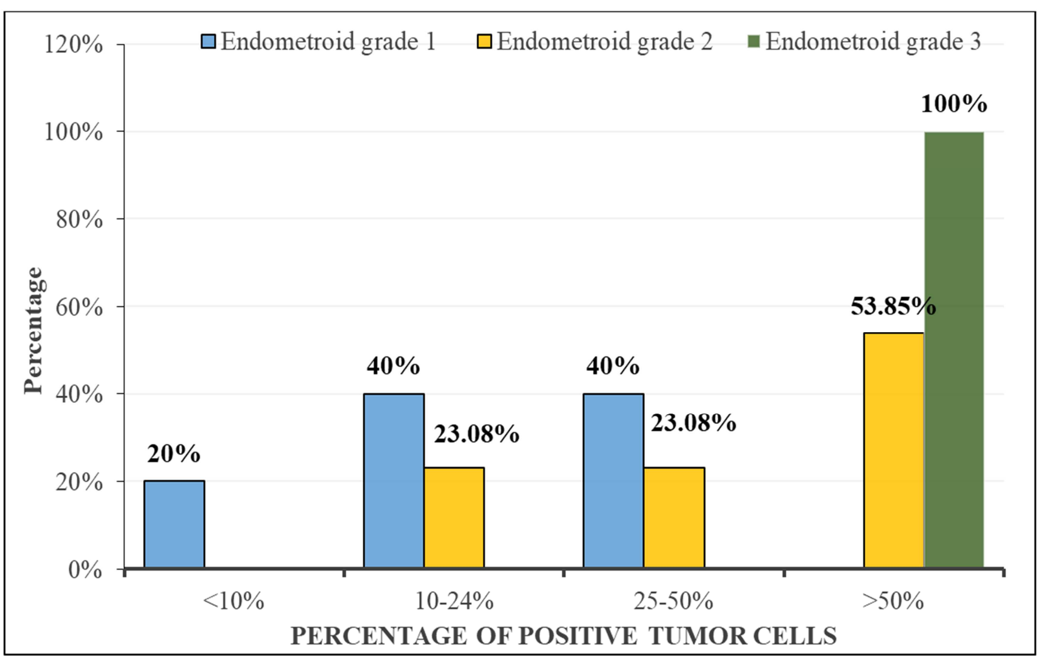
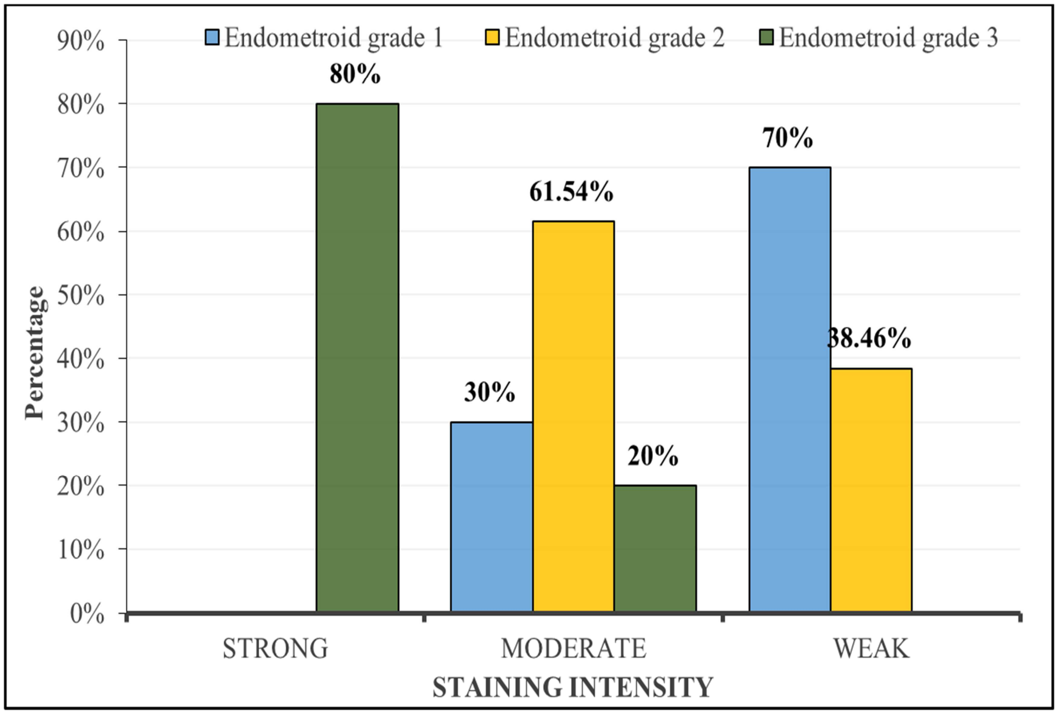


Figure 32: Distribution of staining intensity across different FIGO grades of Endometrioid Carcinoma.



Diffuse staining was observed exclusively in endometrioid grade 3 cases (40%), while patchy staining was present in all grade 1 and grade 2 cases but decreased in grade 3 (60%). This difference was statistically significant (p-value = 0.0285). The proportion of positive tumor cells was significantly higher in grade 3, with all cases showing >50% positivity, whereas lower grades had more cases with lower positivity percentages (p-value = 0.0080). Dominant staining site differences were not statistically significant (p-value = 0.0960), though nuclear staining was observed in 40% of grade 3 cases but was rare in lower grades. Staining intensity was significantly different across grades, with strong staining seen in 80% of grade 3 cases but absent in grades 1 and 2 (p-value < 0.001).

Table 29: Distribution of Endometrial carcinoma subjects according to invasive front.

Invasive Front	**Number of subjects (%)**
Absent	14 (35%)
Present	10 (25%)
NA	16 (40%)

An increasing intensity of p16 expression was noted in the invasive fronts of tumor when compared with centre of tumor. This finding was noted in 10 (25%) and absent in 14 (35%) cases. 16 (40%) cases were of endometrial curettage and biopsy so it was not applicable for categorization.

We further compared expression of p16 between invasive front across other clinical and histological parameters but no significance was observed in any of the variables.

Table 30: Comparison of IHC characteristics across types of endometrial hyperplasia.

Variables	Sub Category	Hyperplasia with atypia	Hyperplasia without atypia	p-value
Staining distribution	Patchy	8 (100%)	32 (100%)	-
Percentage of positive tumor cells	<10%	0	14 (43.75%)	**< 0.001^{MC}***
	10-24%	0	9 (28.13%)	
	25-50%	2 (25%)	9 (28.13%)	
	>50%	6 (75%)	0	
Dominant staining site	Cytoplasm	0	21 (65.63%)	**0.0020^{MC}***
	Nuclear	2 (25%)	4 (12.5%)	
	Equally stained	6 (75%)	7 (21.88%)	
Staining intensity	Strong	6 (75%)	3 (9.38%)	**< 0.001^{MC}***
	Moderate	2 (25%)	11 (34.38%)	
	Weak	0	18 (56.25%)	

Figure 33: Distribution of dominant staining site across different types of endometrial hyperplasia

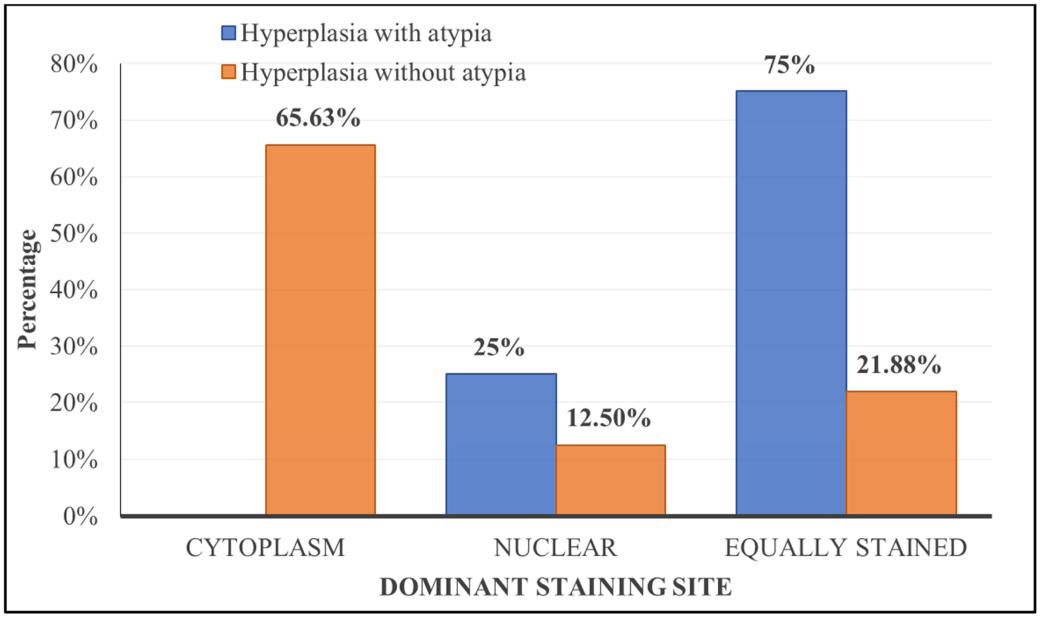


Figure 34: Distribution of percentage of positive tumor cells across different types of endometrial hyperplasia.

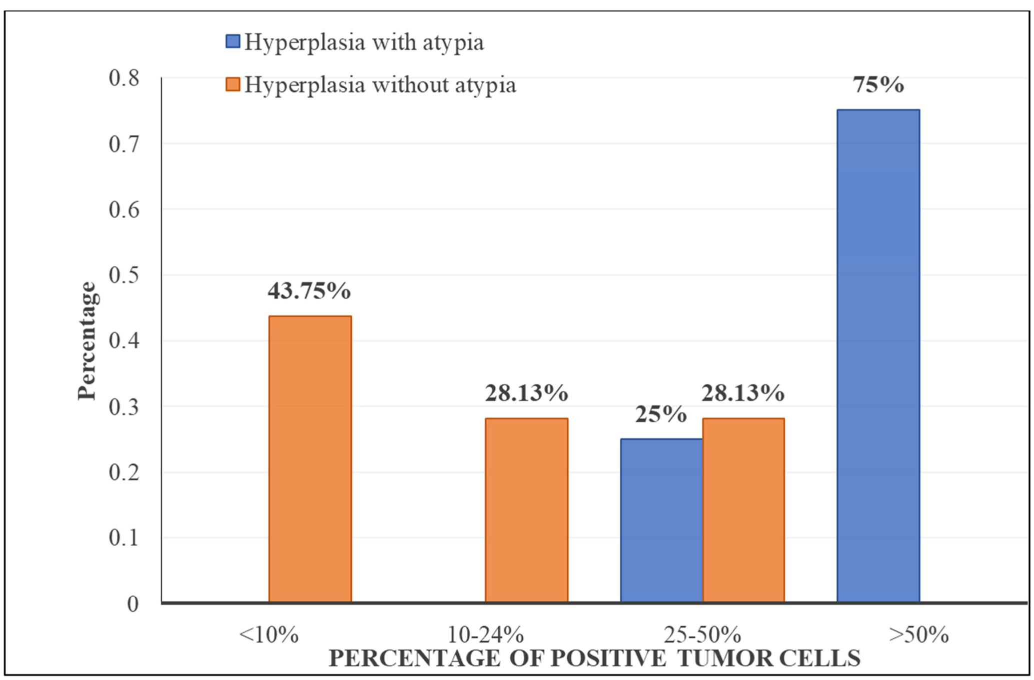
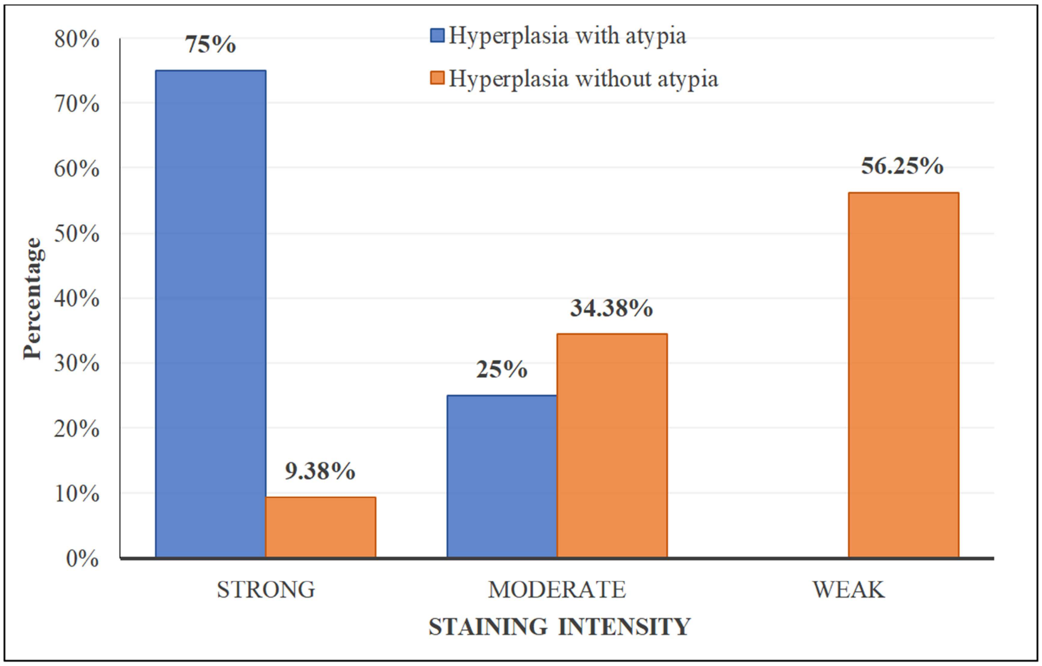


Figure 35: Distribution of staining intensity across different types of endometrial hyperplasia.



All cases of endometrial hyperplasia exhibited patchy distribution. Hyperplasia with atypia show more tumor cell percentage positivity (25-50% and >50%), Nuclear or nuclear plus cytoplasmic staining site and strong to moderate p16 staining intensity.

Whereas, Hyperplasia without atypia had less tumor cell percentage positivity, more cytoplasmic staining compared to nuclear or nuclear plus cytoplasmic and a greater number of weak to moderate p16 staining intensity.

Amongst these 4 parameters, 3 parameters exhibit a significant difference between hyperplasia with atypia and without atypia: percentage of positive tumor cells (p-value < 0.001), dominant staining site (p-value = 0.002) and staining intensity (p-value < 0.001)

Table 31: Comparison of IHC Staining Characteristics over groups.

Variables	Sub Category	Endometrial carcinoma	Endometrial hyperplasia	Total	p-value
Staining Distribution	Diffuse	14 (35%)	0	14 (17.5%)	**$< 0.001^{C}$***
	Patchy	26 (65%)	40 (100%)	66 (82.5%)	
Percentage of Positive Tumor Cells	<10%	2 (5%)	14 (35%)	16 (20%)	**$< 0.001^{C}$***
	10-24%	7 (17.5%)	9 (22.5%)	16 (20%)	
	25-50%	7 (17.5%)	11 (27.5%)	18 (22.5%)	
	>50%	24 (60%)	6 (15%)	30 (37.5%)	
Staining Site	Cytoplasmic	5 (12.5%)	18 (45%)	23 (28.75%)	**0.0030^{MC}***
	Nuclear	2 (5%)	0	2 (2.5%)	
	Both	33 (82.5%)	22 (55%)	55 (68.75%)	
Dominant Staining Site	Cytoplasm	10 (25%)	21 (52.5%)	31 (38.75%)	**0.0388^{C}***
	Nuclear	11 (27.5%)	6 (15%)	17 (21.25%)	
	Equally stained	19 (47.5%)	13 (32.5%)	32 (40%)	
Stain Intensity	Strong	14 (35%)	9 (22.5%)	23 (28.75%)	0.3880^{C}
	Moderate	13 (32.5%)	13 (32.5%)	26 (32.5%)	
	Weak	13 (32.5%)	18 (45%)	31 (38.75%)	

Figure 36: Distribution of Percentage of positive Tumor Cells over groups.

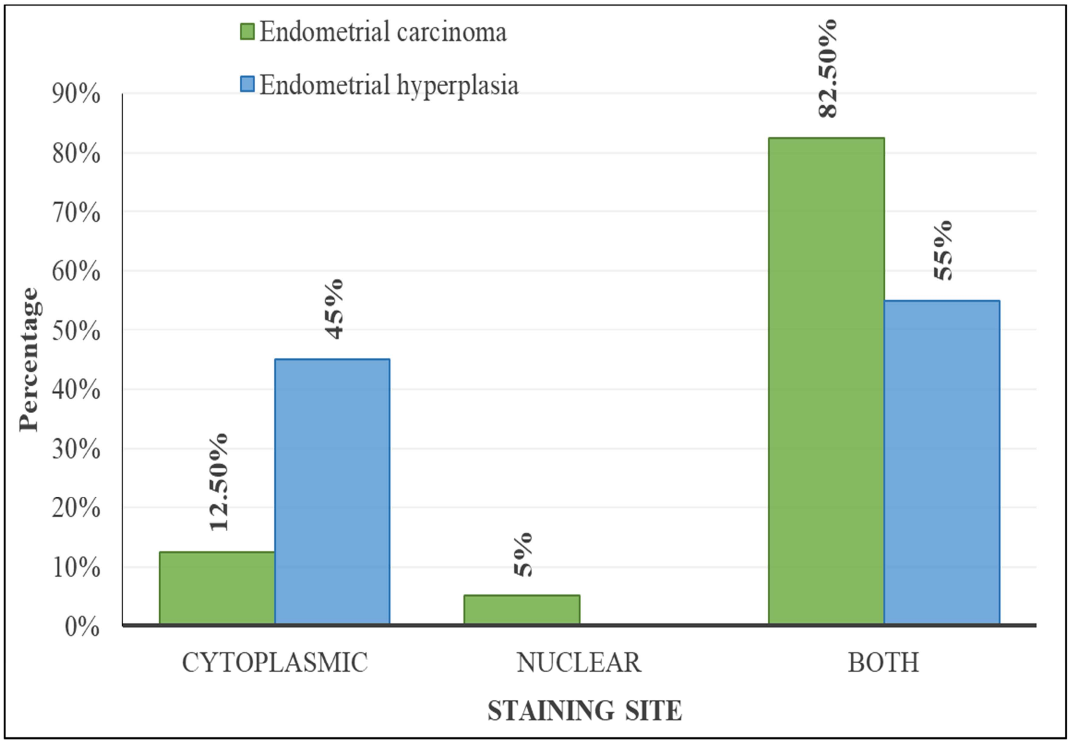


Figure 37: Distribution of Dominant Staining Site over groups

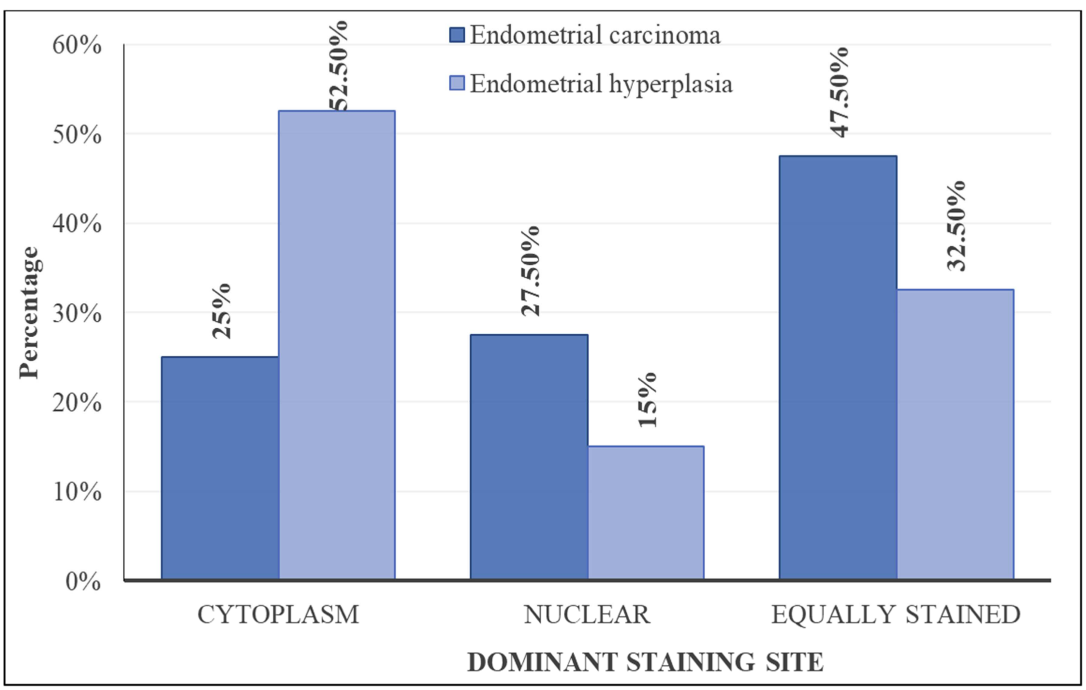


Figure 38: Distribution of Percentage of positive Tumor Cells over groups

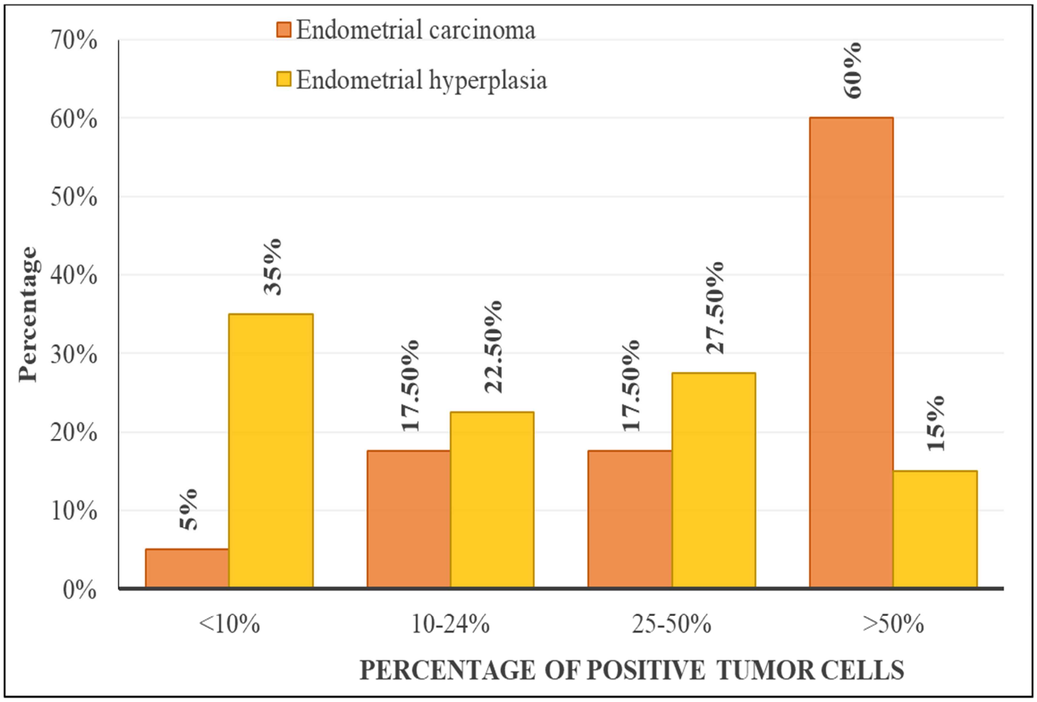


Figure 39: Distribution of Staining intensity over groups.

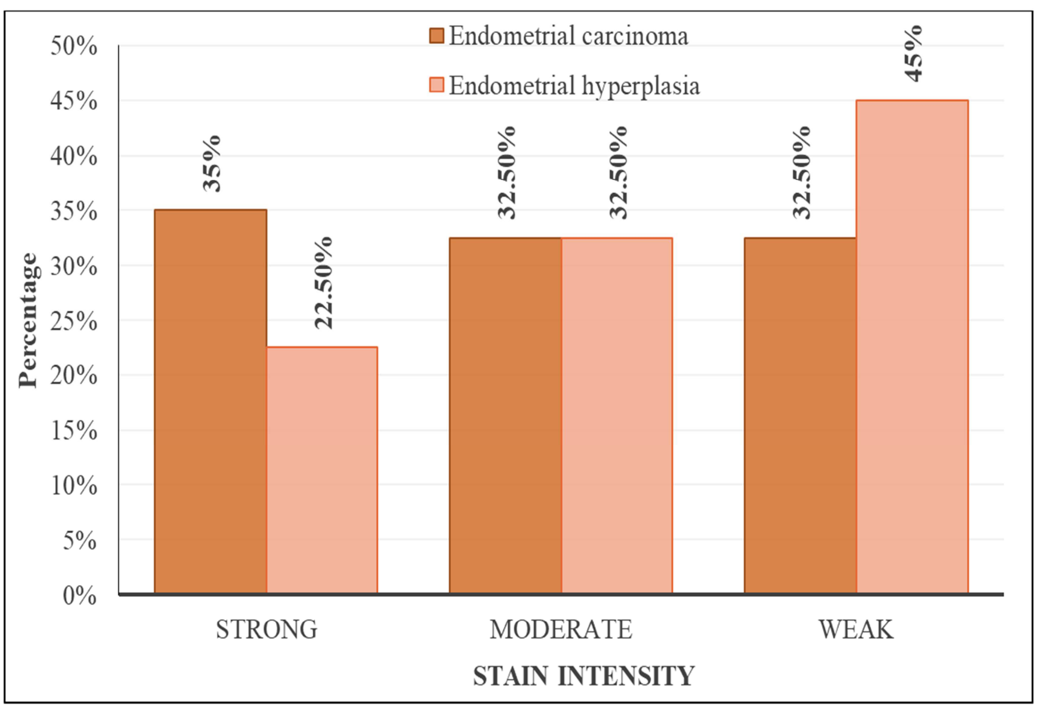
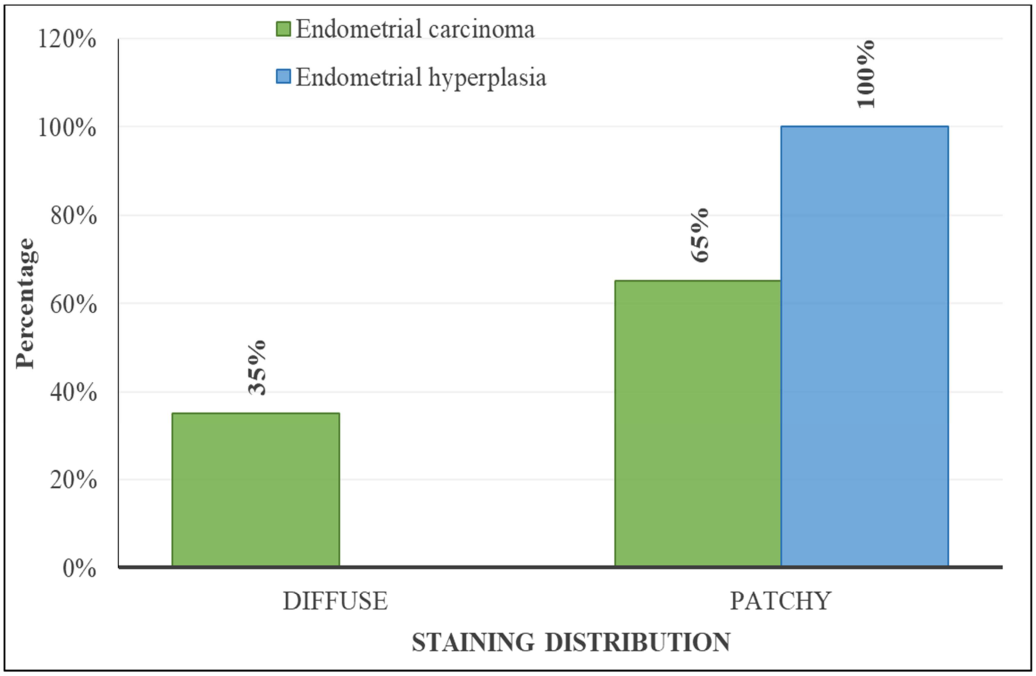


Figure 40: Distribution of staining pattern over groups.



For staining distribution, diffuse staining was observed exclusively in endometrial carcinoma 14 (35%), while all cases of endometrial hyperplasia showed patchy staining 40 (100%). In endometrial carcinoma, patchy staining was also predominant 26 (65%). This difference was statistically significant **(p-value < 0.001).**

Regarding percentage of positive tumor cells, the majority of endometrial carcinoma cases had >50% positive tumor cells 24 (60%), whereas most endometrial hyperplasia cases had <10% positive tumor cells 14 (35%). The distribution was significantly different between groups **(p-value < 0.001)**.

The staining site also differed significantly **(p-value = 0.0030)**, with both cytoplasmic and nuclear staining being the most common in endometrial carcinoma 33 (82.5%), whereas endometrial hyperplasia showed predominant cytoplasmic staining 18 (45%). Nuclear staining alone was rare, observed only in endometrial carcinoma 2 (5%).

In terms of the dominant staining site, equally stained cytoplasmic and nuclear staining was the most frequent pattern in endometrial carcinoma 19 (47.5%), followed by nuclear-dominant staining 11 (27.5%) and cytoplasm-dominant staini0 (25%). In endometrial hyperplasia, cytoplasm-dominant staining was the most common 21 (52.5%), with fewer cases showing equally stained 13 (32.5%) and nuclear-dominant staining 6 (15%). This difference was statistically significant **(p-value = 0.0388)**.

Stain intensity did not differ significantly between groups (p-value = 0.3880). In endometrial carcinoma, strong staining was seen in 14 (35%), moderate staining in 13 (32.5%), and weak staining in 13 (32.5%). In endometrial hyperplasia, weak staining was the most frequent 18 (45%), followed by moderate staining 13 (32.5%) and strong staining 9 (22.5%).

Table 32: Comparison of p16 expression over age in endometrial carcinoma

		≤ 40	41-50	51-60	61-70	>70	p-value
Dominant staining site	**Cytoplasm**	0	2 (20%)	6 (60%)	2 (20%)	0	0.2844^{MC}
	Nuclear	0	0	3 (27.27%)	6 (54.55%)	2 (18.18%)	
	Equally stained	1 (5.26%)	1 (5.26%)	7 (36.84%)	6 (31.58%)	4 (21.05%)	
Percentage of positive tumor cells	**<10%**	0	0	2 (100%)	0	0	0.1489^{MC}
	10-24%	0	1 (14.29%)	5 (71.43%)	1 (14.29%)	0	
	25-50%	1 (14.29%)	1 (14.29%)	2 (28.57%)	1 (14.29%)	2 (28.57%)	
	>50%	0	1 (4.17%)	7 (29.17%)	12 (50%)	4 (16.67%)	
Staining intensity	**Strong**	0	0	3 (21.43%)	8 (57.14%)	3 (21.43%)	0.1624^{MC}
	Moderate	0	2 (15.38%)	5 (38.46%)	4 (30.77%)	2 (15.38%)	
	Weak	1 (7.69%)	1 (7.69%)	8 (61.54%)	2 (15.38%)	1 (7.69%)	
Staining distribution	**Diffuse**	0	0	3 (21.43%)	9 (64.29%)	2 (14.29%)	**0.0390^{MC}***
	Patchy	1 (3.85%)	3 (11.54%)	13 (50%)	5 (19.23%)	4 (15.38%)	

Diffuse staining distribution is more observed in advanced age groups, most frequent in age group is 61-70 years, while younger age group did not show diffuse positivity. Patchy distribution is seen in all age groups but more commonly in 51-60 years. Staining distribution is significantly associated with age (p-value = 0.0390).

Staining site, percentage positivity of tumor cells and staining intensity did not show any significance.

Table 33: Comparison of p16 expression over endometrial thickness in endometrial carcinoma

		>10	5-10	p-value
Dominant staining site	**Cytoplasm**	10 (100%)	0	0.2794^{MC}
	Nuclear	9 (81.82%)	2 (18.18%)	
	Equally stained	14 (73.68%)	5 (26.32%)	
Percentage of positive tumor cells	**<10%**	2 (100%)	0	0.8926^{MC}
	10-24%	5 (71.43%)	2 (28.57%)	
	25-50%	6 (85.71%)	1 (14.29%)	
	>50%	20 (83.33%)	4 (16.67%)	
Staining intensity	**Strong**	10 (71.43%)	4 (28.57%)	0.4473^{MC}
	Moderate	11 (84.62%)	2 (15.38%)	
	Weak	12 (92.31%)	1 (7.69%)	
Staining distribution	**Diffuse**	11 (78.57%)	3 (21.43%)	0.6912^{MC}
	Patchy	22 (84.62%)	4 (15.38%)	

None of the staining parameters had significant association with endometrial thickness in endometrial carcinoma cases (p-values >0.05).

Table 34: Comparison of p16 expression over myometrial invasion in endometrial carcinoma

Myometrial Invasion (n = 28)		**<50%**	**>50%**	**p-value**
Dominant staining site	**Cytoplasm**	7 (87.5%)	1 (12.5%)	**0.0030^MC^***
	Nuclear	0	8 (100%)	
	Equally stained	5 (41.67%)	7 (58.33%)	
Percentage of positive tumor cells	**<10%**	1 (50%)	1 (50%)	0.0740^MC^
	10-24%	4 (80%)	1 (20%)	
	25-50%	3 (75%)	1 (25%)	
	>50%	4 (23.53%)	13 (76.47%)	
Staining intensity	**Strong**	0	10 (100%)	**0.0030^MC^***
	Moderate	5 (62.5%)	3 (37.5%)	
	Weak	7 (70%)	3 (30%)	
Staining distribution	**Diffuse**	0	10 (100%)	**0.0020^MC^***
	Patchy	12 (66.67%)	6 (33.33%)	

Endometrial carcinoma cases having >50% myometrial invasion show nuclear or nuclear to cytoplasmic staining site, with strong intensity and diffuse staining pattern. When compared to cases having <50% myometrial invasion which exhibited more cytoplasmic or nuclear and cytoplasmic staining, weak to moderate intensity and patchy distribution.

The dominant staining site, staining intensity and staining distribution in endometrial carcinoma cases showed a significant association with myometrial invasion (p-value < **0.050^MC^**)

Most of the cases with >50% myometrial invasion exhibit >50% tumor cell positivity compared to cases with <50% myometrial invasion shows variable positivity. However, this does not indicate a significant association.

Table 35: Comparison of p16 expression over Lymphovascular invasion in endometrial carcinoma

(n= 29)		LVSI negative	Focal	LVSI positive	p-value
Dominant staining site	**Cytoplasm**	4 (50%)	3 (37.5%)	1 (12.5%)	**0.0090^{MC}***
	Nuclear	0	1 (12.5%)	7 (87.5%)	
	Equally stained	5 (38.46%)	0	8 (61.54%)	
Percentage of positive tumor cells	**<10%**	1 (50%)	1 (50%)	0	**0.0015^{MC}***
	10-24%	3 (60%)	2 (40%)	0	
	25-50%	3 (60%)	1 (20%)	1 (20%)	
	>50%	2 (11.76%)	0	15 (88.24%)	
Staining intensity	**Strong**	0	0	10 (100%)	**0.0030^{MC}***
	Moderate	4 (44.44%)	1 (11.11%)	4 (44.44%)	
	Weak	5 (50%)	3 (30%)	2 (20%)	
Staining distribution	**Diffuse**	0	0	10 (100%)	**0.0015^{MC}***
	Patchy	9 (47.37%)	4 (21.05%)	6 (31.58%)	

Cases with lymphovascular invasion exhibit a greater number of nuclear and cytoplasmic or nuclear staining site. Most cases show >50% tumor cell positivity but no case under <24% cell positivity. More cases of strong intensity and diffuse distribution are observed.

Whereas, cases which did not show lymphovascular invasion exhibited higher number of nuclear and cytoplasmic or cytoplasmic staining. Percentage positivity of tumor cell is variable. All cases exhibit weak to moderate and patchy staining. Focal lymphovascular invasion also follow the similar p16 expressions.

All p16 parameters in endometrial carcinoma cases showed a significant association with lymphovascular invasion (p-value < 0.05)

Table 36: Comparison of p16 expression over perineural invasion in endometrial carcinoma

(n= 28)		PNI absent	PNI present	p-value
Dominant staining site	**Cytoplasm**	4 (50%)	4 (50%)	0.2159^{MC}
	Nuclear	1 (12.5%)	7 (87.5%)	
	Equally stained	6 (50%)	6 (50%)	
Percentage of positive tumor cells	**<10%**	1 (50%)	1 (50%)	**0.0210^{MC}***
	10-24%	1 (50%)	1 (20%)	
	25-50%	3 (75%)	1 (25%)	
	>50%	3 (17.65%)	14 (82.35%)	
Staining intensity	**Strong**	1 (10%)	9 (90%)	0.0790^{MC}
	Moderate	5 (62.5%)	3 (37.5%)	
	Weak	5 (50%)	5 (50%)	
Staining distribution	**Diffuse**	1 (10%)	9 (90%)	**0.0479^{MC}***
	Patchy	10 (55.56%)	8 (44.44%)	

Cases with perineural invasion have higher percentage of tumor cell positivity with both diffuse and patchy staining distribution. On the other hand, cases with no perineural invasion have variable percentage of tumor cell positivity and more patchy distribution giving a significant association with percentage positivity of tumor cells and staining distribution over perineural invasion in endometrial carcinoma **(P values= 0.0210, 0.0479)**

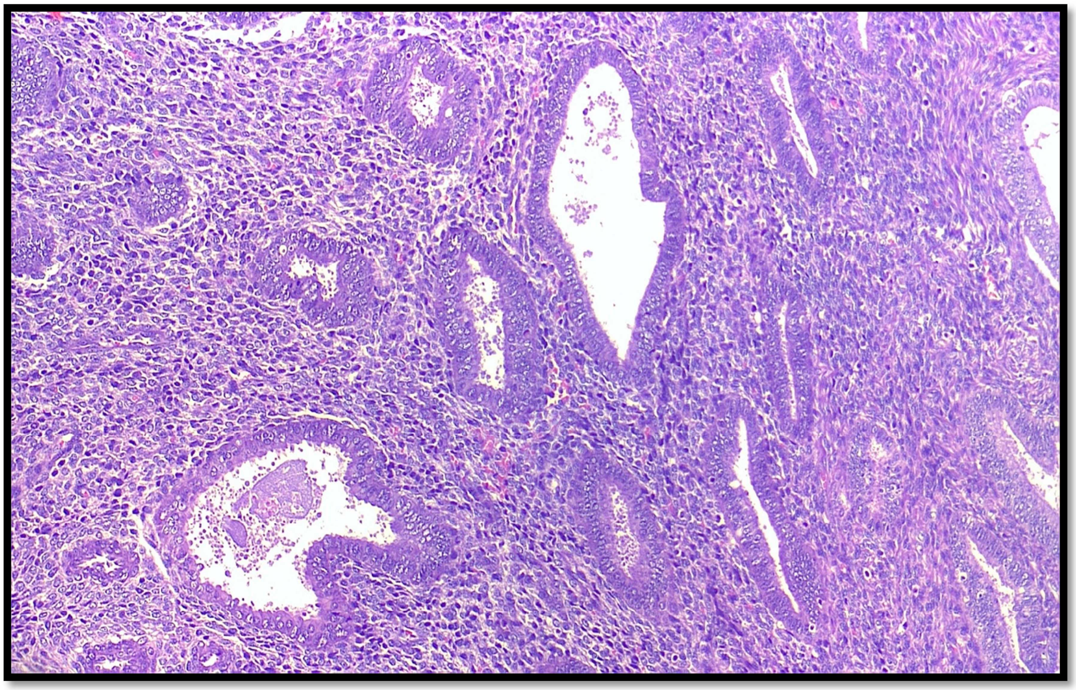
Dominant staining site and staining intensity do not provide any significance with perineural invasion (P values >0.05)

Table 37: Comparison of IHC staining across different clinical parameters

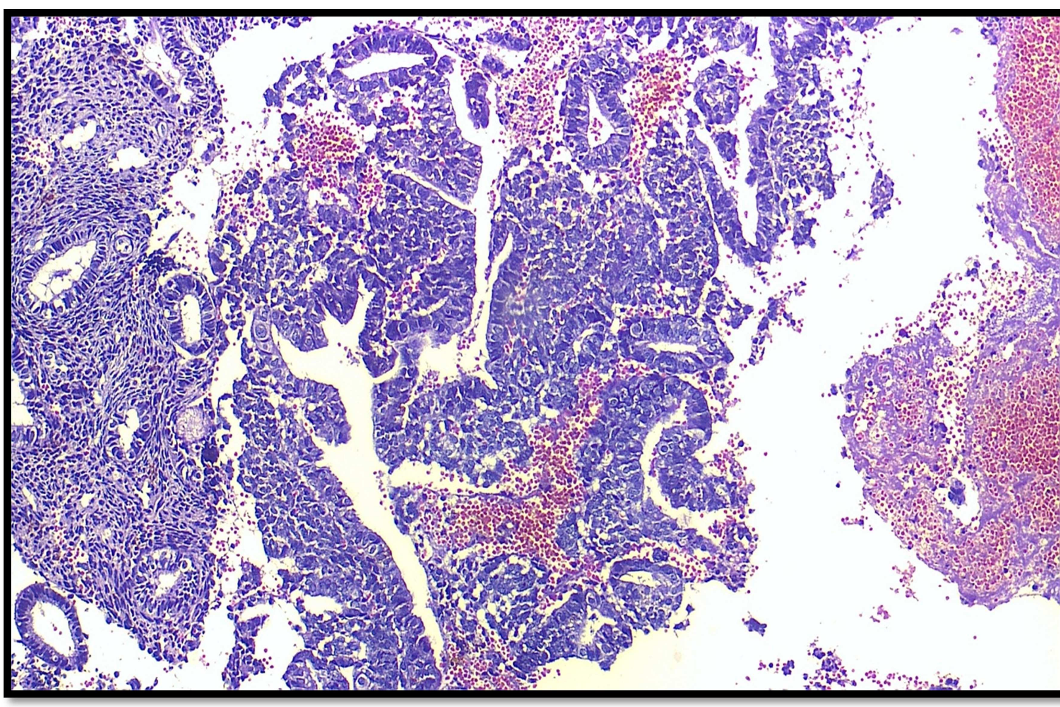
Clinical parameter	Dominant staining site	% positivity of tumor cells	Staining intensity	Staining distribution
Age	0.2844^{MC}	0.1489^{MC}	0.1624^{MC}	**0.0390^{MC}***
Endometrial thickness	0.2794^{MC}	0.8926^{MC}	0.4473^{MC}	0.6912^{MC}
Myometrial invasion	**0.0030^{MC}***	0.0740^{MC}	**0.0030^{MC}***	**0.0020^{MC}***
Lymphovascular invasion	**0.0090^{MC}***	**0.0015^{MC}***	**0.0030^{MC}***	**0.0015^{MC}***
Perineural invasion	0.2159^{MC}	**0.0210^{MC}***	0.0790^{MC}	**0.0479^{MC}***

Presence of more than 50% myometrial invasion, presence of lymphovascular and perineural invasion shows statistically significance difference with expression of p16 IHC when compared to cases with <50% myometrial invasion, no lymphovascular and perineural invasion.

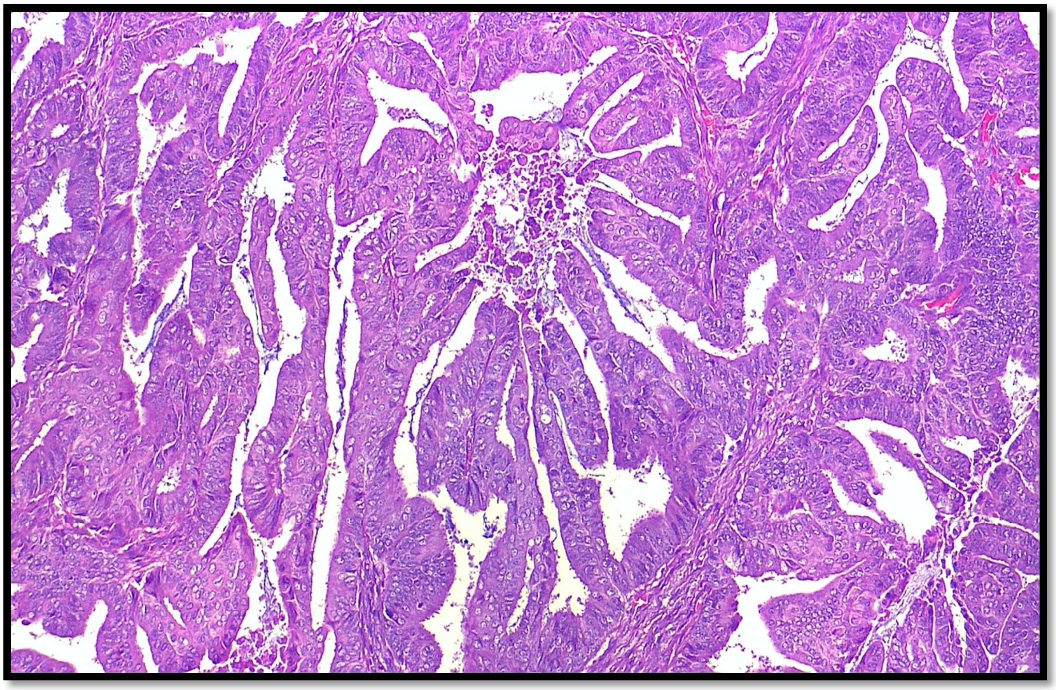
PHOTOMICROGRAPHS



Photomicrograph 1. H&E, 100X- Hyperplasia without atypia

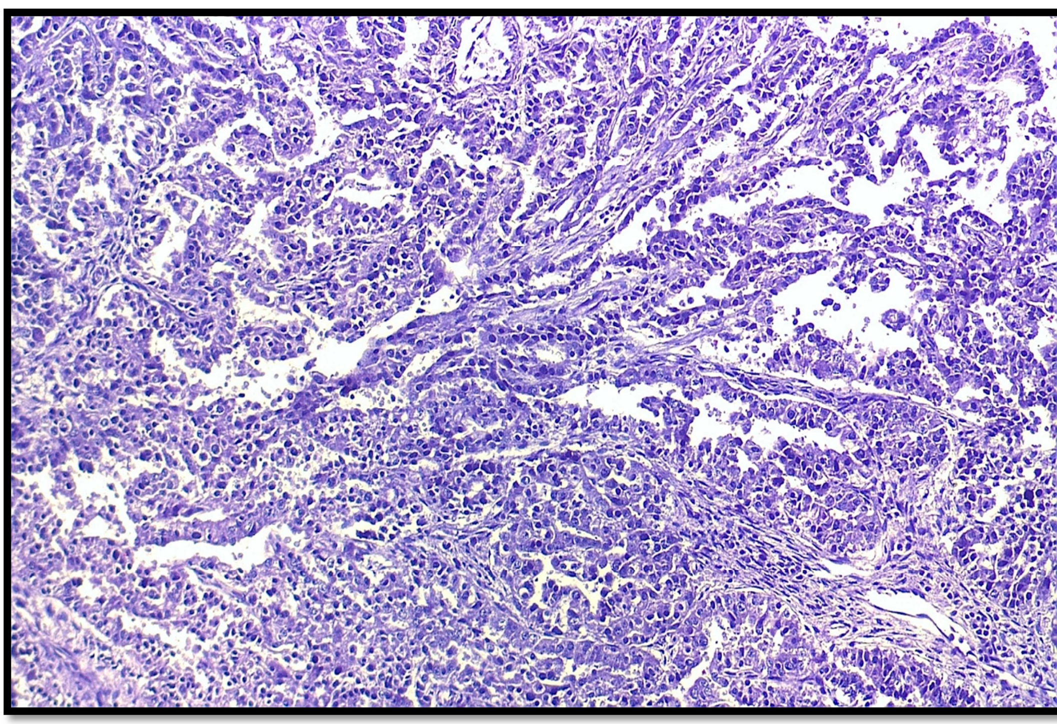


Photomicrograph 2. H&E, 100X-Hyperplasia with atypia

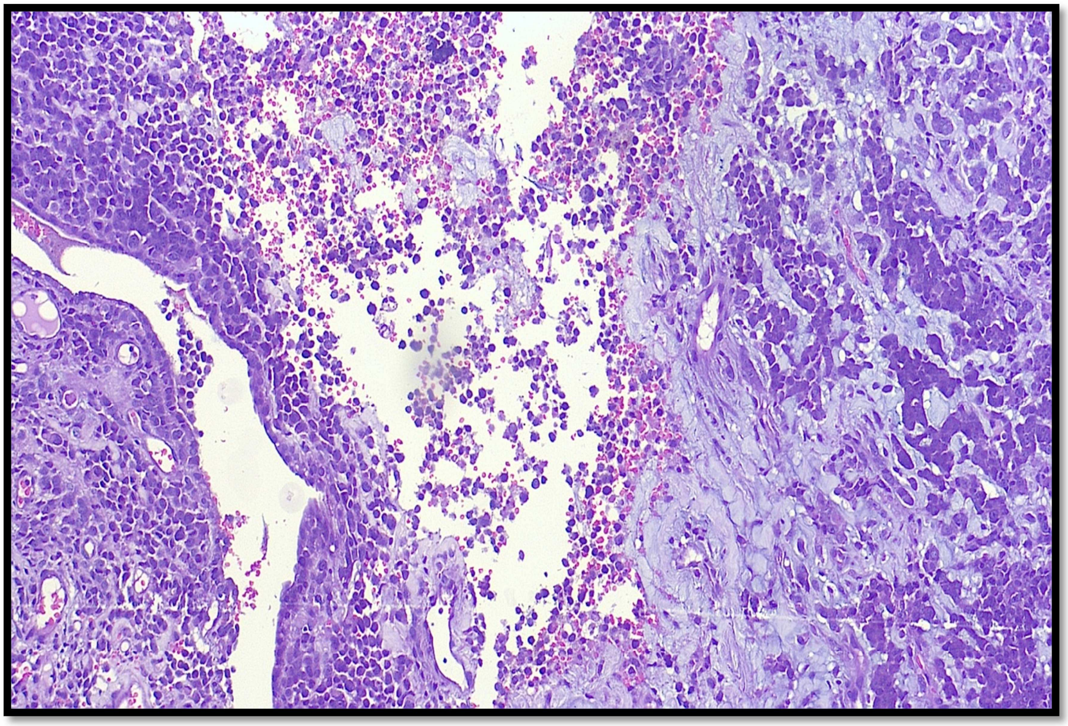


Photomicrograph 3. H&E, 100X- Endometroid endometrial carcinoma

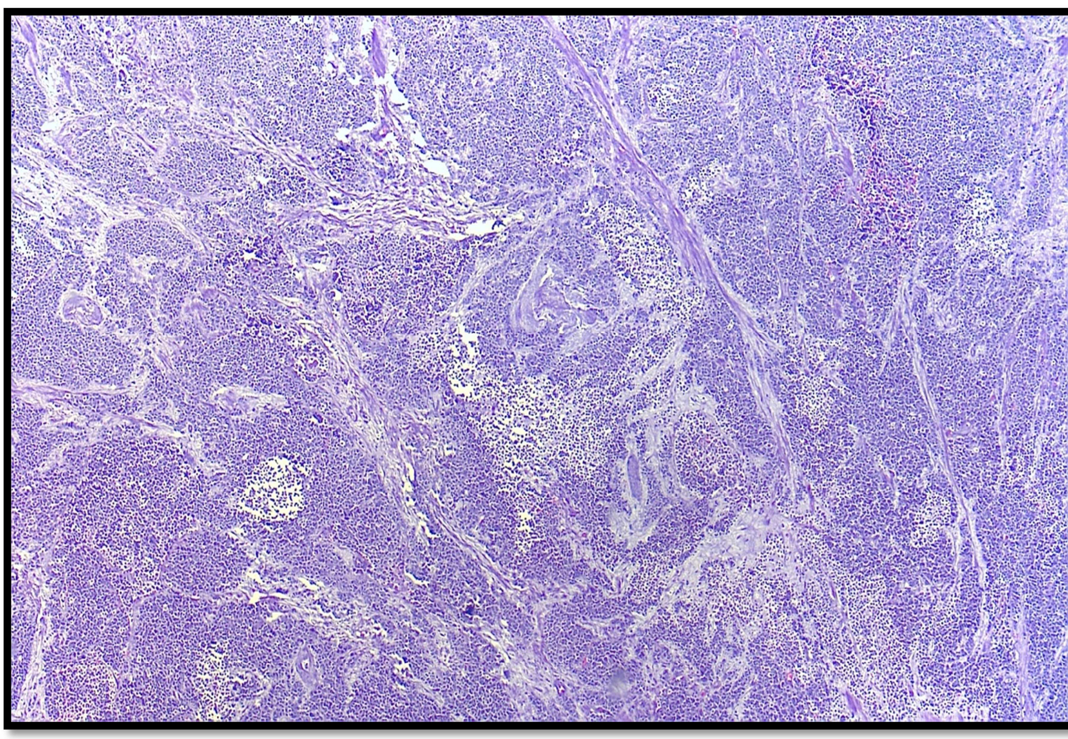
(FIGO grade 2)



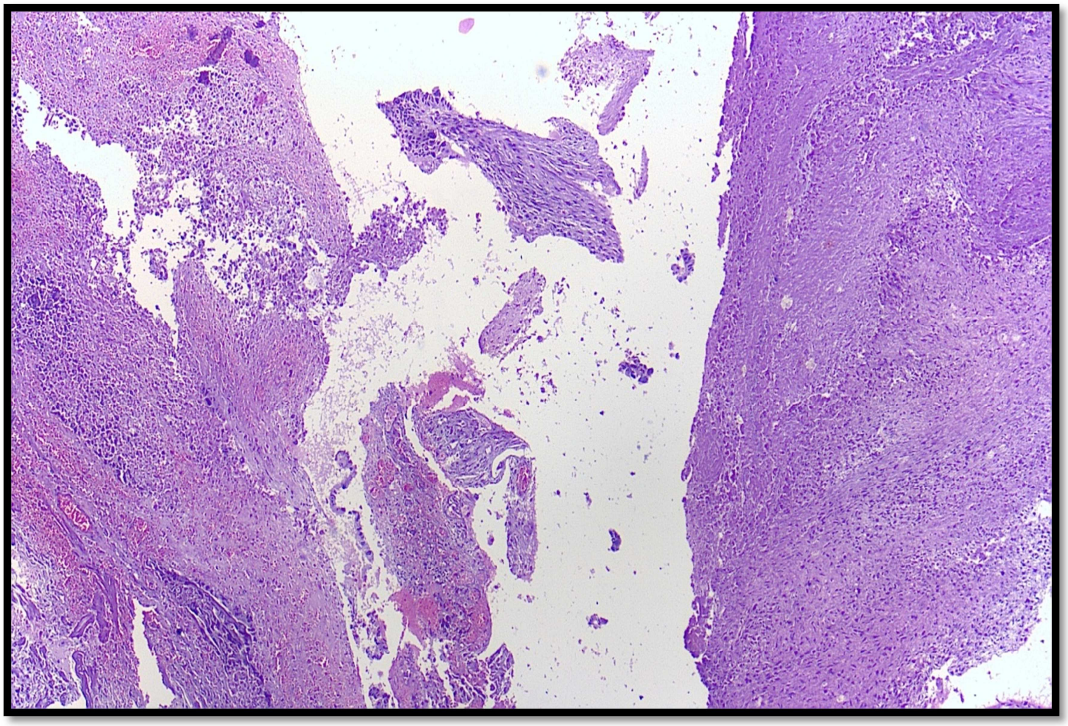
Photomicrograph 4. H&E, 100X- Serous endometrial carcinoma



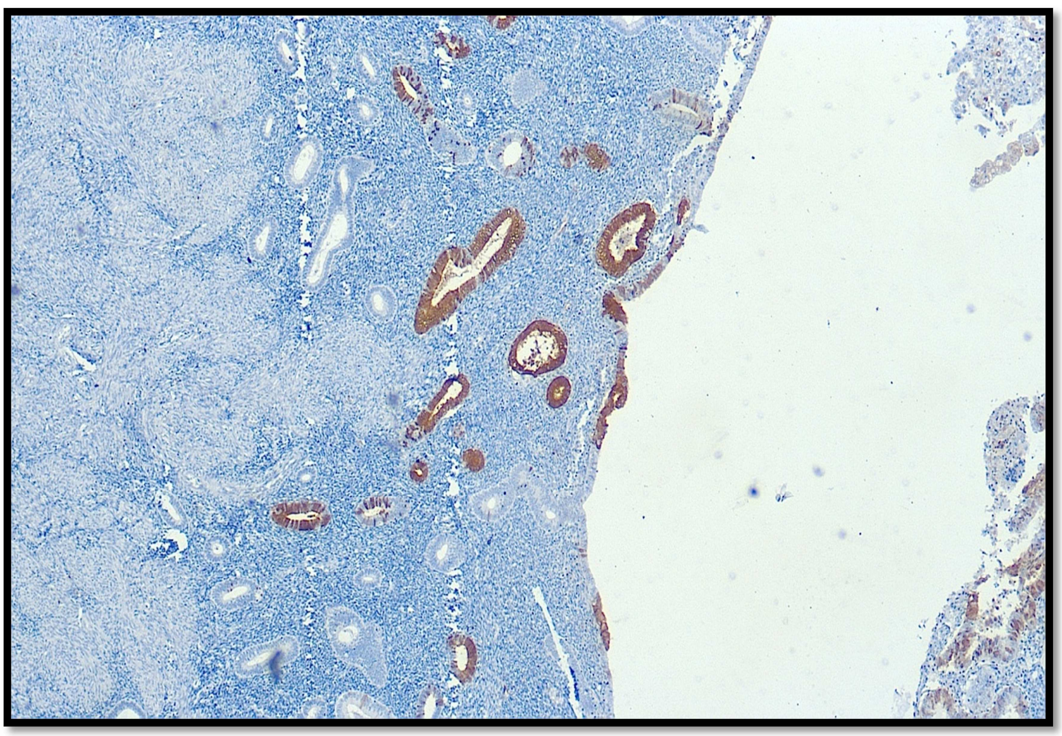
Photomicrograph 5. H&E, 100X- Poorly differentiated carcinoma



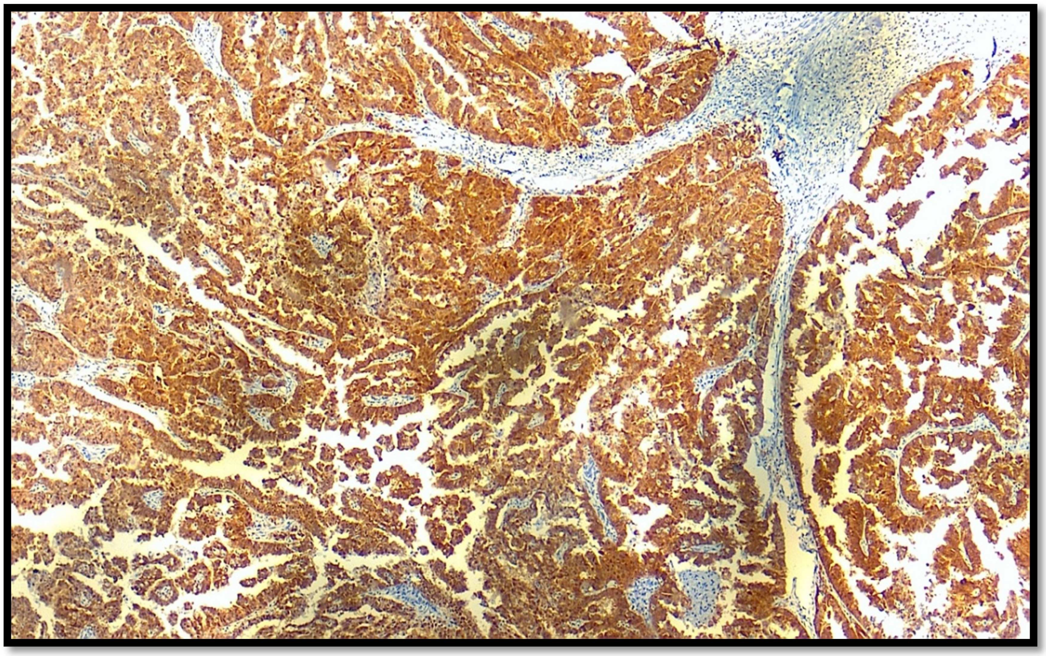
Photomicrograph 6. H&E, 100X-S mall round blue cell tumor



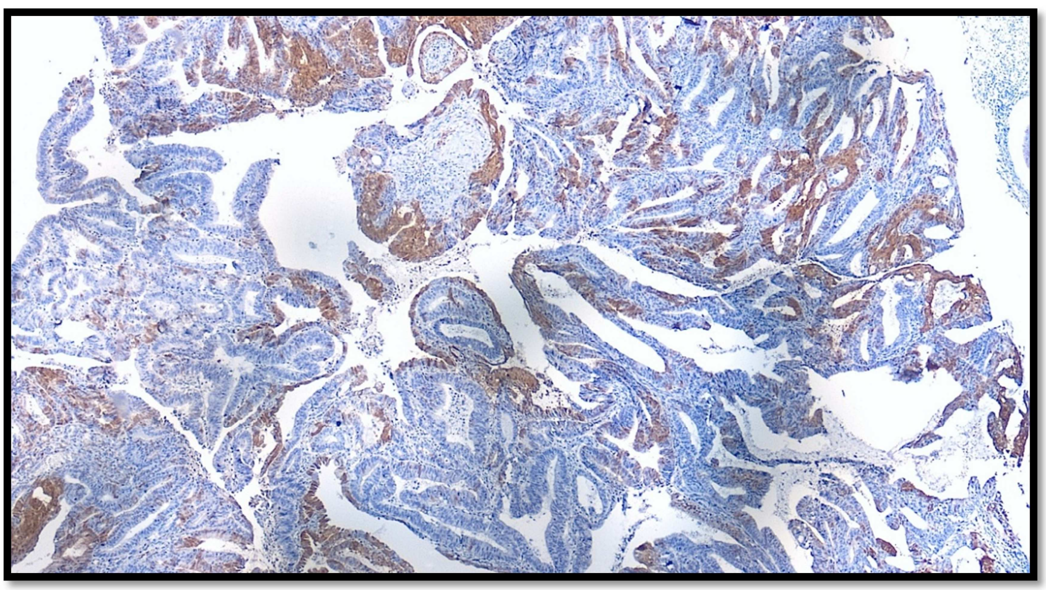
Photomicrograph 7. H&E, 40X-Carcinosarcoma



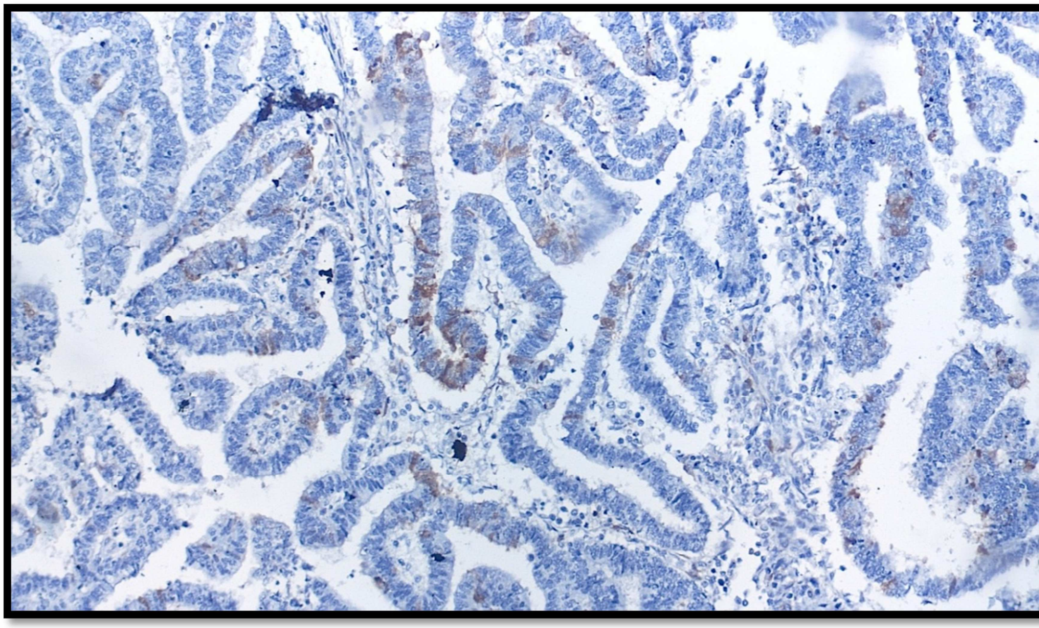
Photomicrograph 8. H&E,100X- Positive control



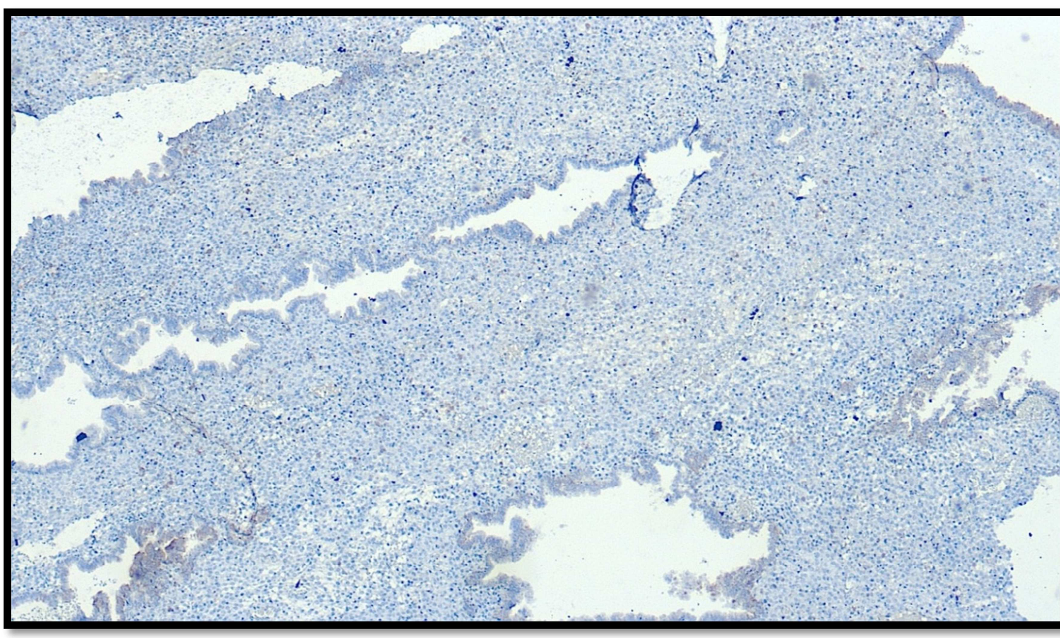
Photomicrograph 9. P16-Diffuse/ Strong/ Nuclear> Cytoplasmic/ >50% of tumor cell positivity seen in a case of serous endometrial carcinoma (IHC, 100X)



Photomicrograph 10. P16- Patchy/ Moderate/ Nuclear= Cytoplasmic/ 25-50% of tumor cell positivity seen in a case of endometroid endometrial carcinoma- FIGO grade 2 (IHC,100X)



Photomicrograph 11- Patchy/ Weak/ Cytoplasmic> Nuclear/ 10-24% of tumor cell positivity in a case of endometroid endometrial carcinoma- FIGO grade 1 (IHC, 200X)



Photomicrograph 12. P16- Patchy/ Weak/ Cytoplasmic/ 0-10% of tumor cell positivity/ Cytoplasmic staining in a case of hyperplasia without atypia (IHC, 100X)

DISCUSSION

Among gynaecological malignancies, endometrial carcinoma constitutes half of all cases. It ranks as the fourth most prevalent cancer affecting women, following those of the lungs, breast, and bowel.[88] Endometrial carcinoma is mostly recognized at an early stage due to its tendency to produce recognizable symptoms.[89] Epidemiological statics indicate a rising incidence of endometrial carcinoma, with 1-3% of women facing the potential risk of developing this condition during their lifetime. Among those diagnosed, approximately 20% succumb to the disease within a five-year period.[90]

Endometrial hyperplasia (EH) occurs approximately three times more frequently than endometrial cancer, with an estimated rate of 133 cases per 100,000 women annually.[91] Approximately 1.5% of women who experience irregular uterine bleeding are diagnosed with endometrial hyperplasia.

Endometrial carcinoma can develop from endometrial hyperplasia, with the risk of cancer significantly elevated in cases of atypical hyperplasia.[90]

Enhancing our knowledge of prognostic indicators could lead to improved survival outcomes through earlier detection of endometrial carcinoma, vigilant monitoring of patients at high risk, and the establishment of a uniform protocol for adjuvant treatment.[92]

In tumor progression, P16 acts as a tumor suppressor gene, demonstrating its capacity to inhibit cell proliferation.[90] Studies indicates that elevated p16 expression is linked to factors such as aging, oxidative stress, and damage to DNA.[93] Studies on endometrial cancer have documented elevated levels of p16 expression.[93,94]

This increase is believed to be a defensive mechanism aimed at halting cell division, which has escalated due to the deactivation of the Rb (retinoblastoma) protein.[95] Additional evidences suggest that malignant transformation may be

linked to reduced expression of p16, which can attribute to homozygous deletion, point mutation, promoter methylation. The prevalence of each varies according to the specific cancer type and may even differ among distinct pathological types of the same malignancy.[92]

According to the meta-analysis conducted by Hu et al., hypermethylation appears to be the primary mechanism responsible for inactivating the p16 gene.[96]

Table 38: Comparing mean age of Endometrial carcinoma and hyperplasia with other studies

Study on endometrial carcinoma	Mean Age (Years)	Study on endometrial hyperplasia (EH)	Mean age (Years)
Gun Yoon[97]	58 (41 to 84)	Kalkan HE[90]	Simple non-atypical EH- 45 Complex atypical EH 50
Kaur S[98]	58.9 (30-88)	Hadi[99]	With atypia- 50-70 Without atypia- 30-50
Kalkan HE[90]	60.5 (39-74)	Gun Yoon[97]	Bening lesion (Include EH without atypia- 46 Precancerous lesion (Including EH with atypia)- 51
Present study	**60.5 (39-74)**	**Present study**	**45 (35-65)**

In the present study, the mean age of endometrial carcinoma is 60. 5 years which is similar to study done by Kalkan HE[90], 90% of cases were more than 50 years old (Postmenopausal) and 10% were below 50 years.

In the present study, mean age of endometrial hyperplasia is 45 years which is similar to most common age range of 40- 50 years of other studies done by Kalkan HE[90], Hadi[99] and Gun Yoon[97]. Some variability in age groups is due to change in classification and limited samples of endometrial hyperplasia with atypia in present study.

In present study most common age range for endometrial carcinoma is 50 to 60 years (Postmenopausal) when compared to age range of endometrial

hyperplasia which is between 40 to 50 (Premenopausal). Similar results are shown in study done by Gun Yoon[97] and Kalkan HE.[90]

In endometrial carcinoma advanced age (61-70 years) show more cases of diffuse p16 staining when compared to younger age group (51-60 years) which show more patchy staining. However, the difference is not statistically significant in endometrial hyperplasia.

Table 39: Comparison of incidence of histological types of endometrial carcinoma with other studies

Study (Total cases)	**Endometroid**	**G1**	**G2**	**G3**	**Serous**	**Others**
Kim JS et al[84] (25)	21	19	3	3	1	4
Salvesen HB[86] (286)	107	64	163	59	29	50 adenosquamous
Kalkan HE[90] (30)	All 30	6	22	2	-	-
Gun Yoon[97] (36)	21	-	-	-	8	7
de Biase D et al[100] (211)	161 (76.3%)	-	-	-	19 (9%)	31 (14.6%)
Kandoth C[101] (373)	307				53	13
Yemelyanova A[102] (201)	101	44	40	17	49	51 adenosquamous
Present study (40)	28 (70%)	10	13	5	7 (17)	5 (12.5)

The incidence of endometroid type of endometrial carcinoma is highest, followed by serous carcinoma which is similar to the various studies done by above mentioned authors. Among endometroid type of endometrial carcinoma FIGO grade 2 is highest in number followed by FIGO grade 1 and 3 in present study, similar to the study done by Kalkan HE[90] and Salvesen HB[86]. Other types include combination of rare variants like carcinosarcoma, poorly/ undifferentiated carcinoma etc. Study done by Salvesen HB[86] and Yemelyanova A[102] are meta-analysis done few years ago before the new WHO classification was launched, thus their other cases include adenosquamous carcinoma which is no longer a terminology in our present study.

Table 40: Comparing incidence of major histological type with other studies

Studies	Total studies	High grade	Low grade
Kim JS et al[84]	25	8 (32%)	17(68%)
de Biase D et al[100]	211	88 (41.7%)	125 (58.3%)
Present study	**40**	**16(40%)**	**24 (60%)**

The incidence of low grade endometrial carcinoma is more in present study compared to high grade carcinoma which is similar to other two studies done by kim JS et al[84] and de Biase D et al[100]. Endometrial carcinomas are detected at an early stage due to obvious clinical symptoms.[89]Thus the incidence of low grade carcinoma is comparatively more.

Table 41: Comparing endometrial thickness with other studies

Study	Endometrial thickness in endometrial hyperplasia	Endometrial thickness in endometrial carcinoma
Kalkan HE[90]	12 (3-29) in simple non- atypical EH 10 (6-20) in complex atypical EH	13.5 (5- 29)
Present study	**11.47+_ 4.25** **10 (4-22)**	**16.95+_ 4.27** **15 (7- 34)**

Endometrial thickness is significantly more in endometrial carcinoma group compared to endometrial hyperplasia in the present study which is similar to the study done by Kalkan HE[90] However, p16 expression did not provide a statistically significant relationship with endometrial thickness in both the studies.

As studies reveal that non- endometroid type (high grade) carcinoma are more diffusely and strongly p16 positive but they mostly arise from a background atrophic endometrium. Thus, this inverse relationship is perhaps the cause of this insignificance.[92,103]

Table 42: Comparing myometrial invasion with other studies

Studies	Total cases	No MI/ Not applicable	<50% MI	>50% MI
Kim JS et al[84]	25	8 (32%)	6 (24%)	11 (44%)
Kalkan HE[90]	30	-	19	11
Kaur S[98]	115	-	53 (46.1%)	62 (53.9 %)
Present study	**40**	**12 (30%)**	**12 (30%)**	**16 (40%)**

The present study found more cases with myometrial invasion similar to study done by Kim JS et al[84]. 12 cases of endometrial curettage were not applicable for these criteria which acts as a limiting factor while comparing with other studies which exclusively used hysterectomy specimens only.

When compared with p16 staining in the present study, cases with more than

50% myometrial invasion had strong/ diffuse, nuclear or nuclear to cytoplasmic p16 staining compared to cases which are under less than 50% myometrial invasion category, where mild to moderate/ patchy, cytoplasmic or nuclear to cytoplasmic patterns are more common. Similarly study done by Kalkan HE[90] found increased expression of p16 in cases with >50% myometrial invasion.

Table 43: Comparing lymphovascular invasion with other studies

Studies	LVI positive	Focal LVI	LVI Negative
Kalkan HE[90]	6		24
Kaur S[98]	19 (16.5%)		93 (83.5)
de Biase D et al[100]	33/211 (16.6%)		
Present study	**15 (37.5%)**	**4 (10%)**	**9 (22.5%)**

In present study, a greater number of lymphovascular positive cases are there compared to other studies. Reason could be the more frequency of high grade cases in present study compared to study done by Kalkan HE[90] which had only endometroid type of carcinoma. Study done by da Biase D et al[100] and Kaur S[98] had a greater number of endometroid and stage 1 endometrial carcinomas when compared with present study.

Presence of lymphovascular invasion was strongly linked to diffuse and strong p16 staining when compared to patchy and weak to moderate staining in absence of focal lymphovascular invasion. Similarly study done by Kalkan HE[90] commented high rate of p16 expression in patients with lymphovascular invasion.

Presence of perineural invasion

In present study more number of cases are there with presence of perineural invasion (17) compared to PNI absent cases (11). PNI positive cases had more diffuse p16 staining where most cases had >50% tumor cell positivity when compared of PNI negative cases which had more cases of patchy staining with variable tumor cell positivity including most cases under <50% tumor cell positivity. However, p16 staining intensity and site was not significant.

Comparing p16 staining in endometrial carcinoma with other studies

Study done by Yemelyanova A[102] and Reid- Nicholson[37] found that all serous carcinoma expressed diffuse p16 staining compared to endometroid type of carcinoma which had patchy distribution. All FIGO grade 1 and 2 (Low grade) had focal expression of p16 as per Reid- Nicholson.[37] Study done by Wei JJ[104] states that FIGO grade 3 endometroid carcinoma cases show diffuse p16 positivity in some cases. Another study by Saad RS[105] on 28 cases of undifferentiated carcinoma found that>50% of the cases of undifferentiated carcinoma express diffuse pattern of p16 staining. The present study demonstrates diffuse expression of p16 in all serous carcinoma, few cases of FIGO grade 3 endometroid carcinoma. Patchy in all FIGO grade 1 and 2 endometroid carcinoma and most cases of FIGO grad 3 endometroid carcinoma. Our findings are similar to the study done by Yemelyanova A[102], Reid- Nicholson[37] and Wei JJ[104]. Poorly differentiated carcinoma in the present study also expressed diffuse p16 expression which is similar to the study done by Saad RS[105]. All of the remaining rare types in our study expressed diffuse expression of p16 which were not included in these studies includes carcinosarcoma, small round blue cell tumor and a case suspicious of malignancy. The expression of p16 distribution amongst different histological types of endometrial carcinoma is statistically significant (p value < 0.001)

Study by Hadi[99] noticed that half of the FIGO grade 3 endometroid carcinoma were positive for p16, while most FIGO grade 1 and 2 were negative. Kalkan HE[90] commented a high rate of p16 expression in all patients of grade 3 and type 2 endometroid carcinoma. The present study found that most all cases of grade 3 and type 2 endometrial carcinoma expressed both nuclear and cytoplasmic positivity, but few cases of FIGO endometroid grade 1 and 2 expressed only cytoplasmic positivity which was considered negative as per other studies.

Our findings are similar to the study done by Kalkan HE[90]. The expression of p16 according to dominant staining site across different types of endometrial carcinoma was statistically significant (p value= 0.0020) study done by Matson[106] found 2 out of 62 cases of serous carcinoma which were completely negative for p16, giving a terminology of “p16 null” to such cases. However, we did not find any such case in our present study.

Study done by Yemelyanova A[102] found that all serous carcinoma expresses 90-100% tumor cell positivity when compared to endometroid carcinoma which expressed variable percentage positivity of tumor cells ranging from 10 to 90%. Similarly, the present study found all cases of serous carcinoma expressing >50% (90-100%) tumor cell positivity in all cases. Endometroid carcinoma expressing variable positivity ranging from <10% and going up to 90%. This finding is similar to the study done by Yemelyanova A[102]. All FIGO grade 3 endometroid carcinoma expressed >50% tumor cell positivity. All rare categories like carcinosarcoma, poorly differentiated, small round blue cell tumor and suspicious of malignancy expressed >50% tumor cell positivity. The difference of percentage positivity of tumor cells amongst histological types of endometrial carcinoma is statistically not significant. (p value= 0.2654)

According to the study done by Yemelyanova A[102] all serous carcinoma express strong 16 positivity while most endometroid carcinoma expressed weak- moderate and occasionally moderate- strong p16 positivity (p value< 0.0001). Similarly, study done by Hadi[99] found strong p16 positivity in all cases of serous carcinoma. Reid-Nicholson[37] demonstrated that FIGO grade 1 and 2 (Low- grade) endometroid carcinoma typically exhibit weak p16 expression but demonstrated stronger p16 expression in FIGO grade 3 endometroid

carcinoma, in epithelial component of carcinosarcoma and clear cell carcinoma. The expression of p16 was strong in serous carcinoma (p value<0.001). Wei JJ[104] also states that strong p16 positivity can be seen in few cases of FIGO grade 3 endometroid carcinoma. In present study all serous carcinoma express strong positivity for p16. All cases of FIGO grade1 and 2 express mild- moderate positivity. Most cases of FIGO grade 3 endometroid carcinoma express strong positivity and occasional case of moderate positivity. This finding is similar to the study done by Yemelyanova A[102], Hadi[99], Reil-Nicholson and Wei JJ[104]. The remaining rare variants in our study like carcinosarcoma, poorly differentiated carcinoma, small round blue cell tumor, one case of suspicious of malignancy were strongly positive. The intensity of p16 expression among different types of endometrial carcinoma is statistically significant (p- value<0.001).

Comparison of p16 expression across FIGO grades of endometroid carcinoma

In study done by Yemelyanova A[102], expression of p16 was not statistically significant among the different FIGO grades of endometroid endometrial carcinoma (p value= 0.5), however, a slight increased expression in FIGO grade 3 was observed compared to other 2 groups. In the present study, all FIGO grade1 and 2 had patchy distribution, most expressed weak-

moderate intensity and had lesser percentage positivity of tumor cells. Whereas, few of the FIGO grade 3 endometroid carcinoma exhibited strong, diffuse, >50% tumor cell positivity. These all parameters (Staining pattern, intensity and percentage positivity of tumor cells) found a statistical significance among different grades of FIGO (P values <0.05). However, staining site across different FIGO grades was statistically not significant (p value= 0.0960).

Expression of p16 was not statistically significant in study done by Yemelyanova A[102] but we found a significance, the reason could be use of more parameters for p16 related to p16 expression in the present study.

Comparison between endometrial carcinoma and hyperplasia

In present study we found that there was statistically significant difference in expression of p16 between endometrial hyperplasia and carcinoma in an increasing manner which can confirm the progression of endometrial carcinoma from hyperplasia

Similarly study done by Kalkan HE[90] on progression of endometrial carcinoma used 4 groups including normal proliferative endometrium, simple no-atypical endometrial hyperplasia, complex atypical endometrial hyperplasia and endometrial carcinoma revealed statistically significant difference of p16 expression amongst the groups ($p<0.001$)

Comparison of invasive front expression of endometrial carcinoma with other studies:

present study found 12 out of 40 samples of endometrial carcinoma which show increased expression of p16 in invasive fronts than in central part of the tumor which is similar to the study done by Horrée N [107] who explored the expression of hypoxia and proliferation related proteins and found 11 of 39 cases for p16.

The explanation provided was increased proliferation and gradual disruption of cell cycle control proteins cyclin E, p16, and cdk2, rather than an enhanced hypoxic response.[107]

CONCLUSION

Our study found a statistically significant correlation between different grade and types of endometrial carcinoma and p16 expression. We observed that tumors with high histological grading exhibited high levels of p16 expressions.

We found a statistically significant correlation between predictive prognostic parameters and p16 expression. Thus, it can help us to assess the aggressiveness of tumor and progression from low grade tumor subtypes to high grade subtypes.

We also found a statistically significant difference in p16 expression between endometrial hyperplasia and carcinoma, its expression increases from hyperplasia without atypia, with atypia to endometrial carcinoma. Thus, it can be used to assess the progression from endometrial hyperplasia to carcinoma

These findings suggest that p16 can serve as valuable diagnostic and predictive prognostic marker along with other IHC markers in endometrial carcinoma and hyperplasia.

Limitations

Sample size was limited in our study due to time constraints. The study on higher number would be better.

Our study was unable to keep up the follow up of the patients due to time constrains. A comparative study between p16 expression and patient's prognosis would be better to understand the prognostic value of the p16 IHC marker.

Future aspects

There are very limited studies on the expression of p16 in the endometrial carcinoma and its predictive value in prognosis. Hence, more studies are needed for a better understanding of its expression in endometrial carcinoma.

SUMMARY

- This cross-sectional study was done at Histopathology laboratory at KLE'S Dr. Prabhakar Kore Charitable Hospital And Medical Research Center, Belagavi by collecting data and blocks of endometrial carcinoma cases between January 2021 to December 2024 and equivalent number of cases of endometrial hyperplasia.

- The objectives of the study were to evaluate expression of p16 in endometrial carcinoma, to compare its expression in endometrial carcinoma and hyperplasia and correlate p16 positivity score with clinicopathological parameters.

- The study took into account 40 samples of endometrial carcinoma and 40 samples of endometrial hyperplasia.

- Of 40 cases of endometrial carcinoma, the patient's ages ranged from 39 to 74 with mean age of 61.15 ± 8.27 years.

- Of 40 cases of endometrial hyperplasia, the patient's age ranged from 35 to 65 years old, with a mean age of 45.77 ± 6.29 years. Most preferred procedure for endometrial carcinoma was hysterectomy (60%), while most preferred procedure for endometrial hyperplasia was dilatation and curettage (70%). Postmenopausal prevaginal bleeding was most common symptom in cases of endometrial carcinoma. Abnormal and heavy menstrual bleeding were most common symptom in case of endometrial hyperplasia.

- 5- 10 mm endometrial thickness range was more common in endometrial hyperplasia with mean thickness of 11.47 ± 4.25. In cases of endometrial carcinoma >10mm endometrial thickness was most prevalent, with mean thickness of 16.95 ± 7.18.

- Of 40 cases of endometrial carcinoma 24 (60%) were low grade and 16 (40%) were of high grade. Out of 40 cases of endometrial hyperplasia 32 (80%) were hyperplasia without atypia and 8 (20%) were hyperplasia with atypia,
- All serous endometrial carcinoma had diffuse
- p16 positivity while all endometroid FIGO grade 1 and 2 exhibited patchy distribution. FIGO grade 3 was having both patchy and diffuse positivity. (p value= 0.001)
- All serous carcinoma had >50% (90- 100%) tumor cell positivity while endometroid carcinoma had variable (<10-80%) tumor cell positivity. The difference was not statistically significant across different types og endometrial carcinoma (p value= 0.2654)
- Among rare variants carcinosarcoma, poorly differentiated carcinoma, small round blue cell tumor and suspicious of malignancy exhibited diffuse p16 distribution with >50% tumor cell positivity.
- All serous, endometroid FIGO grade 3, poorly differentiated carcinomas, small round blue cell tumor and suspicious of malignancy showed nuclear or nuclear and cytoplasmic positivity. Endometroid FIGO grade 1 and carcinosarcoma predominantly exhibited either cytoplasmic or nuclear and cytoplasmic positivity. (p value= 0.0020)
- All serous, 80% endometroid FIGO grade 3 show strong positivity, along with rare cases like poorly differentiated, small round blue cell tumor and

suspicious of malignancy. All endometroid FIGO grades 1, 2 and carcinosarcoma show weak to moderate staining (p value= 0.001). where FIGO grade 1 had more cases (70%) of weak positivity compared to FIGO grade 2 (38%).

- High grade endometrial carcinoma had more cases of diffuse (81%), strong (81%), nuclear (56%) and >50% tumor cell positivity (100%) compared to low grade carcinoma which had more number of Patchy (95%), weak to moderate (50%,45%), nuclear and cytoplasmic (54%) and variable tumor cell positivity (10-90%). (p values= 0.001, 0.0035)

- Among 40 cases of endometrial hyperplasia all cases had diffuse staining distribution of p16. There was statistically significant difference between hyperplasia with atypia and without atypia, based on staining intensity, percentage positivity of tumor cells (p values= 0.001) and dominant staining site (p value= 0.0020).

- While comparing between endometrial carcinoma and hyperplasia, except staining intensity (P value= 0.3880), all the other parameters were statistically significant. (P values< 0.05)

- In endometrial hyperplasia all cases had patchy distribution but in endometrial carcinoma only 65% cases had patchy distribution (p value= 0.001)

- Most endometrial hyperplasia (35%) had <10% tumor cell positivity but most endometrial carcinoma (60%) cases had >50% tumor cell positivity (p value= 0.001)

- More number of cytoplasmic only staining was observed in endometrial hyperplasia (45%) cases compared to endometrial carcinoma (12%). (p value= 0.0030)

- 10 out of 40 cases exhibited increased intensity of p16 in the invasive fronts compared when compared to centre parts of tumor.

- FIGO grade 3 had more cases of diffuse (40%), strong (80%) and >50% tumor cell positivity (100%) compared to FIGO grade 1 and 2 (P values= 0.0285, 0.0080, 0.001)

- There was statistically significant association of p16 expression with myometrial, lymphovascular and perineural invasion in cases of endometrial carcinoma.

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ANNEXURE – I - INFORMED CONSENT FORM

"EXPRESSION OF P16 IN ENDOMETRIAL CRACINOMA- HOSPITAL BASED CROSS-SECTIONAL STUDY"

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Introduction: The purpose of this study is to study the expression of p16 in endometrial carcinoma and hyperplasia and its association with clinicopathological parameters.

Explanation of procedure: During this study, block will be collected from the pathology department and would be used for study purpose for the general benefit.

Possible benefits from participating in the study: This study may give opportunities for novel targeted therapies.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study

Cost of investigations done during the course of study will be paid by the principal investigator**.**

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions with regard to this study, you are free to contact:

Principal Investigator, Department of Pathology, J.N Medical College, Belagavi If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

ANNEXURES II – CONSENT STATEMENT

I am making a voluntary decision to participate in the study "**EXPRESSION OF P16 IN ENDOMETRIAL CRACINOMA- HOSPITAL BASED CROSS-SECTIONAL STUDY**". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURES III - STAINING PROTOCOL H&E

HEMATOXYLIN AND EOSIN STAIN

REAGENTS

1. Erhlich's Haematoxylin solution
2. Eosin Y solution 1%
3. 1% acid alcohol solution

HEMATOXYLIN AND EOSIN STAIN – PROCEDURE

1. Deparaffinise the tissue sections in xylene (Xylene 1 for 5 mins+ Xylene 2 for 5 mins
2. Subject the tissue section to water through reducing grades of alcohol (90% alcohol for 5 mins + 70% alcohol for 5 mins)
3. Keep it in hematoxylin for 8 to 10 minutes
4. Rinse it in tap water for 2 mins
5. Differentiate with 1% acid alcohol for 10 sec
6. For bluing - place in tap water for about 10 minutes
7. Counter stain by eosin 1-2 minutes
8. Rinse in water
9. Dehydration increasing grades of alcohol (70% alcohol for 30 sec + 90% alcohol for 30 sec)
10. Clearing is done by Xylene (Xylene 1 for 5 mins + Xylene 2 for 5 mins
11. Mount it with Dibutylphalate Polystyrene Xylene (DPX).

ANNEXURES IV – STEPS OF P16 IHC PROCEDURE

(TRIS buffer+ EDTA)- Buffer solution required amount of buffer is prepared

Tissue section is cut on a microtome with 3 microns thickness and collected on coated slides

↓

Bake the sections at 37 degree celsius overnight. Before test bake it at 60 degrees Celsius for 1 hour

↓

Deparaffinise steps-

Xylene I-10 minutes

Xylene II- 10 minutes

Absolute alcohol I- 10 minutes

Absolute alcohol II- 5 minutes

Rinse in distilled water- 1 minute

↓

Antigen retrieval (TRIS buffer+ EDTA)- Buffer solution

↓

Required amount of buffer is prepared and cook the slides in pressure cooker for 3 whistles

↓

Allow it to cool to room temperature for 15 minutes

↓

Wash with wash buffer 2 times with gap of 30 seconds each

↓

Wash with water buffer 3 rimes with gap of 30 seconds

↓

Apply 3% hydrogen peroxide- 8 to 10 minutes

↓

Primary monoclonal antibody for p16 protein (biogenex) is incubated for 30 to 60 minutes in closed chamber at room temperature

↓

Wash with wash buffer 3 times with gap of 30 seconds each

↓

Apply polymer Horseradish peroxidase (HRP) for 25 to 30 minutes in closed chamber at room temperature

↓

Wash with wash buffer 3 times with gap of 30 seconds each

↓

Apply Diaminobenzidine (DAB) substrate for 10 minutes

↓

Wash with water for 2 minutes

↓

Wash with distilled water for 1 minute

↓

Counter stain with Haematoxylin- 3 minutes

↓

Counter stain with Haematoxylin- 3 minutes

ANNEXURES - V

MASTER CHART

				IHC STAINING PATTERN													SURGERY TYPE			CLINICAL FEATURES							RADIOLOGICAL FINDING							
BIOPSY NUMBER	AGE	TUMOR TYPE AND FIGO GRADE	MAJOR TYPE	Diffuse	Patchy	<10	10-24%	25-50	>50	Nuclear	Cytoplasmic	More	Strong	Weak	Moderate	Invasive front	SX-hysterectomy	Sx- D& C	Sx- Biopsy	PVB	PVS	PVW	HMB	AUM	Pain	Endometrial Thickness	R-LEMJ	R-lesion	R- EIT	R-collection	R-Fibroid	MI	PNI	LVI
1544	74	Endometroid grade 3	B		+				+	+	+	E	+			-				+						8	+	+				>50	+	+
3128	50	Endometroid grade 2	A		+				+		+	C		+		+	+			+						26	+	+				<50	+	-
961	58	Endometroid grade 1	A		+			+		+	+	E			+	+	+					+				13					+	<50	-	-
593	55	Endometroid grade 1	A		+	+				+	+	C		+		-	+			+						25			+			<50	-	-
1853	69	Papillary serous carcinoma	B	+					+	+	+	N	+			+	+			+						34		+				>50	-	yes
2638	67	Carcinosarcoma+B16	B	+					+	+	+	C			+	NA			+	+						22		+				NA	NA	
4719	73	Endometroid grade 2	A		+			+		+	+	E			+	NA		+			+					8	+					NA	NA	-
3999	72	Endometroid grade 3	B	+					+	+	+	N	+			-	+			+						20	+	+				>50	+	+
5201	71	Endometroid grade 2	A		+				+	+	+	E			+	-	+			+					+	17		+		+		<50	-	+
3169	59	Papillary serous carcinoma	B	+					+	+	+	N	+			NA		+		+						16			+			NA	NA	NA
4414	52	Endometroid grade 2	A		+				+	+	+	E			+	-	+								+	12	+	+		+		<50	+	-
3157	72	Small round blue cell tumor	B	+					+	+		N	+			+	+				+					10		+				>50	+	+
411	71	Endometroid grade 1	A		+			+		+	+	E		+		NA			+	+						12		+				NA	NA	NA
2771	67	Carcinosarcoma	B	+					+	+	+	E		+		-	+			+						13	+	+				>50	+	+
2455	60	Endometroid grade 3	B		+				+	+	+	E			+	NA		+		+						12			+			NA	NA	NA
4363	59	Endometroid grade 2	A		+				+		+	C			+	-	+			+						15		+				<50	+	+
5387	39	Endometroid grade 2	A		+			+		+	+	E		+		NA		+			+					28			+			NA	NA	NA
2074	50	Endometroid grade 1	A		+		+				+	C			+	NA		+					+			26			+			NA	NA	NA
3218	58	Endometroid grade 2	B		+		+			+	+	E			+	-	+			+						10		+				>50	-	-
3079	60	Endometroid grade 2	B		+				+	+	+	E		+		-	+			+						16	+	+				>50	+	+
1690	53	Endometroid grade 1	A		+			+		+	+	C		+		-	+			+						18		+				<50	-	-
3220	70	Papillary serous carcinoma	B	+					+	+	+	N	+			NA		+		+						12	+					NA	NA	NA
723	69	Papillary serous carcinoma	B	+					+	+	+	N	+			+	+			+						13	+	+				>50	+	+
2256	61	Endometroid grade 2	A		+		+			+	+	C		+		+	+				+					28	+					<50	-	Focal
3635	68	Endometroid grade 3	B		+				+	+	+	E	+			NA		+		+						13			+			NA	NA	NA
2712	52	Endometroid grade 1	B		+	+					+	C		+		-	+			+						34	+	+				>50	+	Focal
651	57	Endometroid adenocarcinoma 1	A		+		+			+	+	E		+		NA		+		+						15			+			NA	NA	NA
464	52	Endometroid grade 1	A		+		+			+	+	E		+		+	+			+						10			+			<50	-	-
1059	59	Endometroid grade 1	A		+		+				+	C		+		-	+			+						14	+	+				<50	+	-
4281	68	papillary serous carcinoma	B	+					+	+	+	E	+			+	+			+						7	+	+				>50	+	+
4242	69	Endometroid grade 2	B		+				+	+	+	E			+	-	+				+					12	+	+				>50	-	+
5464	59	Papillary serous carcinoma	B	+					+	+	+	N	+			+	+								+	20				+		>50	+	+
2408	61	Poorly differentiated carcinoma	B	+					+	+	+	N	+			NA			+		+					15	+	+				>50	+	+
2795	62	Endometroid grade 3	B	+					+	+	+	N	+			+	+			+						14	+					>50	+	+

BIOPSY NUMBER	AGE	TUMOR TYPE AND FIGO GRADE	MAJOR TYPE	Diffuse	Patchy	<10	10-24%	25-50	>50	Nuclear	Cytoplasmic	More	Strong	Weak	Moderate	Invasive front	SX- hysterectomy	Sx- D& C	Sx- Biopsy	PVB	PVS	PVW	HMB	AUM	Pain	Endometrial Thickness	R-LEMJ	R-lesion	R- EIT	R-collection	R-Fibroid	MI	PNI	LVI
1157	61	Endometroid garde 2	A		+				+	+	+	E			+	NA		+			+					16	+					NA	NA	NA
3846	57	suspicious of malignancy	A	+					+	+		N	+			NA			+	+						10			+			NA	NA	NA
2893	62	Endometroid garde 2	B		+			+		+	+	N			+	-	+			+						19					+	>50	+	Focal
2491	70	papillary serous carcinoma	B	+					+	+	+	E	+			NA			+	+						11	+	+				>50	+	+
4020	48	Endometroid grade 1	A		+			+		+	+	E			+	NA		+		+						26	+	+				<50	-	-
5247	52	Endometroid grade 2	A		+		+			+	+	C		+		NA		+		+						28	+					<50	-	Focal
769	43	Hyperplasia without atypia			+	+					+	C		+			+						+			12								
2804	46	Hyperplasia without atypia			+	+					+	C		+				+						+		11								
890	47	Hyperplasia without atypia			+		+			+	+	E			+			+					+		+	12								
3833	44	Hyperplasia without atypia			+	+					+	C		+				+						+		8								
3858	50	Hyperplasia with mild atypia			+			+		+	+	E	+				+						+			6								
2028	48	Hyperplasia without atypia			+		+				+	C		+					+					+		9								
3055	46	Hyperplasia without atypia			+	+					+	C		+			+						+			9								
3104	44	Hyperplasia without atypia			+	+					+	C		+			+						+			22								
1327	54	Hyperplasia without atypia			+	+					+	C		+				+					+			11								
3230	48	Hyperplasia without atypia			+			+		+	+	E			+			+						+		8								
2570	43	Hyperplasia with atypia			+				+	+	+	E	+					+						+		10								
473	47	Hyperplasia with atypia			+				+	+	+	E	+					+					+			20								
1540	35	Hyperplasia without atypia			+	+					+	C	+					+					+			12								
-2210	40	Hyperplasia without atypia			+			+		+	+	E			+			+						+		9								
222	46	Hyperplasia without atypia			+		+			+	+	N	+				+						+			18								
274	50	Hyperplasia without atypia			+	+					+	C		+				+						+		10								
957	60	Hyperplasia without atypia			+			+		+	+	C			+		+			+					+	8								
233	38	Hyperplasia without atypia			+	+					+	C		+				+					+			4								
2155	43	Hyperplasia without atypia			+			+		+	+	N	+					+						+		8								
2165	46	Hyperplasia without atypia			+			+		+	+	E			+			+		+						14								
2686	40	Hyperplasia without atypia			+	+					+	C		+				+							+	18								
1221	40	Hyperplasia without atypia			+	+					+	C		+				+					+			8								
1882	58	Hyperplasia without atypia			+		+			+	+	C		+				+					+			9								
1753	44	Hyperplasia without atypia			+			+		+	+	E			+			+						+		9								
1813	38	Hyperplasia without atypia			+		+			+	+	E			+			+					+			12								
1842	65	Hyperplasia without atypia			+	+					+	C		+			+								+	10								
836	39	Hyperplasia without atypia			+		+				+	C		+				+					+			9								
560	45	Hyperplasia without atypia			+			+		+	+	N			+			+					+			18								
507	45	Hyperplasia without atypia			+		+			+	+	C			+		+							+	+	10								
3940	42	Hyperplasia without atypia			+	+					+	C		+				+						+		13								

BIOPSY NUMBER	AGE	TUMOR TYPE AND FIGO GRADE	MAJOR TYPE	Diffuse	Patchy	<10	10-24%	25-50	>50	Nuclear	Cytoplasmic	More	Strong	Weak	Moderate	Invasive front	SX-hysterectomy	Sx- D& C	Sx- Biopsy	PVB	PVS	PVW	HMB	AUM	Pain	Endometrial Thickness	R-LEMJ	R-lesion	R- EIT	R-collection	R-Fibroid	MI	PNI	LVI
2528	46	Hyperplasia without atypia			+			+			+	C			+		+						+			21								
2303	43	Hyperplasia without atypia			+		+				+	C		+			+							+		16								
2648	50	Hyperplasia without atypia			+		+			+	+	E		+			+					+				7								
2674	45	hyperplasia with mild atypia			+			+		+	+	E			+			+						+		9								
3776	36	hyperplasia with atypia			+				+	+	+	N	+					+					+			9								
391	53	Hyperplasia with atypia			+				+	+	+	E	+					+		+						14								
307	51	Hyperplasia with atypia			+				+	+	+	E			+			+			+					10								
201	51	Hyperplasia without atypia			+			+		+	+	N			+			+		+						10								
2441	40	Hyperplasia without atypia			+	+					+	C		+				+						+		17								
2452	42	Hyperplasia with atypia			+				+	+	+	N	+					+		+						9								