"TO STUDY THE UTILITY OF PTEN MARKER IN ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA- ONE YEAR PROSPECTIVE AND RETROSPECTIVE OBSERVATIONAL STUDY."

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ABSTRACT

"TO STUDY THE UTILITY OF PTEN MARKER IN ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA -ONE YEAR PROSPECTIVE AND RETROSPECTIVE OBSERVATIONAL STUDY."

BACKGROUND:

Endometrial hyperplasia (EH) is an irregular proliferation of endometrial glands. EH is involved in the development of endometrial cancer (EC) of endometrioid type, which is the most common gynecologic malignancy in the Western World.

The tumor suppressor protein *phosphatase and tensin homolog* (PTEN) has probably been the most studied marker, as it is most commonly mutated in endometrial carcinogenesis.The tumor suppressor gene PTEN was identified on chromosome 10p23.31. It encodes a 403- amino acid PTEN protein (47kDa) with the activity of phosphatase that can act on both polypeptide and phosphoinositide substrates[9-12]. Loss of PTEN is demonstrated by immunohistochemical staining . This research was undertaken to study expression of PTEN in Endometrial hyperplasia without atypia cases.

OBJECTIVE: To study prognostic value of immunohistochemical assessment of PTEN expression in Endometrial hyperplasia without atypia.

METHODOLOGY: Sixty paraffin embedded blocks of histopathologically diagnosed Endometrial hyperplasia without atypia cases were obtained from the Department of Pathology KLE'S DR. PRABHAKAR KORE HOSPITAL, Belagavi, Karnataka and were stained with

Haematoxylin and Eosin (H&E) and immunohistochemical staining with PTEN antibody in KAHER's research laboratory and were evaluated for staining intensity and percentage of tumour cells showing PTEN positivity.

RESULTS:Out of total 60 cases, 2 cases of Complex hyperplasia only showed negative staining depicting loss of PTEN, rest all showed positive staining .The correlation between Endometrial hyperplasia and colour intensity was highly significant with p value of 0.001. Also, correlation between Endometrial hyperplasia and proportion score was statistically significant with p value of 0.001.

Therefore , loss of PTEN expression is rarely seen in cases of hyperplasia without atypia , and mostly seen in cases of Hyperplasia with atypia and Endometrial carcinoma cases.

CONCLUSION: PTEN immunostaining may be an effective tool for screening of malignant and pre malignant endometrial lesions and inactivation of PTEN helps in predicting the response to targeted therapies against carcinogenic pathways .

KEYWORDS: Endometrial Hyperplasia, PTEN, IHC.

LIST OF ABBREVIATIONS

EH	-	Endometrial Hyperplasia
EM	-	Endometrial
EC	-	Endometrial Carcinoma
SH	-	Simple Hyperplasia
CH	-	Complex Hyperplasia
SAH	-	Simple Atypical Hyperplasia
CAH	-	Complex Atypical Hyperplasia
WHO	-	World Health Organisation
EIN	-	Endometrial Intraepithelial Neoplasia
SEIC	-	Serous Endometrial Intraepithelial Carcinoma
EECA	-	Endometrioid Endometrial Carcinoma
IHC	-	Immunohistochemistry
PTEN	-	Phosphatase and Tensin Homolog
PI3K	-	Phosphoinositide-3-kinase
PIP3	-	Phosphatidylinositol 3,4,5 triphosphate
PIK3CA	-	Phosphatidylinositol-4,5-biphosphate 3- kinase catalytic subunit alpha
HRT	-	Hormone Replacement Therapy
MSI	-	Microsatellite Instability
CGH	-	Comparative Genomic Hybridisation
FIGO	-	International Federation Of Gynaecology and Obstetric

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INTRODUCTION

Endometrial cancer is the most common type of uterine cancer and occupies the fourth place among cancers occurring in females among developed countries[1,2]. Non cancerous changes of endometrium are commonly known as hyperplasia. Endometrial hyperplasia essentially implies overgrowth of endometrium. It is almost exclusively associated with a relative excess of endogenous or exogenous oestrogen.

Simple hyperplasia (SH) resembles the normal endometrial tissue growth pattern, while Complex hyperplasia(CH) has a more complex and thus more abnormal architectural growth.Both simple and complex hyperplasia can be associated with cellular atypia (SAH,CAH), which seems to be the most important predictor of malignant potential.

There are two basic types of endometrial carcinoma (ECa): Endometrioid (estrogen related,indolent behaviour) and Nonendometrioid (unrelated to oestrogen, aggressive). Endometrial cancer cells are described as well differentiated , Grade 1 (ECG1), moderately differentiated , Grade 2 (ECG2), or poorly differentiated , Grade 3 (ECG3). Serous carcinoma (SC) represents an example of nonendometrioid carcinoma and it is automatically classified as Grade 3 due to its high aggressiveness[3].

According to World Health Organisation (WHO) classification Endometrial Hyperplasia is of 2 types:

1. Endometrial hyperplasia(EH) with atypia
2. Endometrial hyperplasia(EH) without atypia

EH with atypia is involved in development of endometrial cancer (EC) of endometrioid type, which is most common malignancy in Western world. Since EH can become precancerous due to unopposed action of oestrogens, its malignant potential is highly

variable, with rates of progression of Endometrial carcinoma ranging from less than 1% to over 40%[4,5].

Several biomarkers, like Phosphatase and Tensin Homolog (PTEN), BCL-2, BAX etc. associated with endometrial hyperplasia have been investigated to find out if they can predict prognosis.

The tumor suppressor protein *Phosphatase and Tensin homolog* (PTEN) has probably been the most studied marker, since PTEN gene is the most commonly mutated in endometrial carcinogenesis.

Various studies have been done, and one of the study conducted by **Antonio Raffone et.al** was conducted to know association of PTEN marker which conducted that loss of PTEN immunohistochemical expression in EH is significantly associated with increased overall risk of Endometrial carcinoma[6].

Other study, done by **Zen Zhong Feng et. al.** concluded that PTEN protein was present predominantly in the nuclei of glands in normal endometrium. In carcinoma specimens, the glands showed PTEN negativity while the PTEN staining in nuclei of glands was used as the positive control in each case[7] .

Another study done by **M.Rahul Quddus et al.** reported that PTEN to be altered in endometrial carcinomas and endometrial precancers when no morphologic changes are appreciated[8].

After few studies it is seen that Endometrial carcinoma till shows the highest percentage of PTEN (the phosphatase and tensin homolog, also called MMAC1 and TEP1, MIM 601728) mutations of all tumor types. The tumor suppressor gene PTEN was indentified

on chromosome 10p23.31. It encodes a 403- amino acid PTEN protein (47kDa) with the activity of phosphatase that can act on both polypeptide and phosphoinositide

substrates [9-12].

Sanger sequence has been considered to be the gold standard for detection of PTEN mutations and subsequent loss of PTEN protein function. Alternative methods , such as immunohistochemistry , may be preferred method of assessing functional PTEN loss in patient tumors.

Treatment for Endometrial hyperplasia with atypia and Endometrial carcinoma is hysterectomy or progestins for conservative approach in selected cases and for EH without atypia is only observation or progestins when symptomatic.

Thus, aim of this study is to analyse the prognostic value of immunohistochemical assessment of PTEN expression in EH without atypia.

AIMS AND OBJECTIVES

To study prognostic value of Immunohistochemical assessment of PTEN expression in Endometrial hyperplasia without atypia.

REVIEW OF LITERATURE

EMBRYOLOGY

1) HUMAN EMBRYOLOGY AND DEVELOPMENTAL BIOLOGY, BRUCE M. CARLSON FOURTH EDITION

2) LANGMAN'S MEDICAL EMBRYOLOGY 13 th EDITION

Urogenital system can be divided into two components on the basis of their function:

a) Urinary system

b) Genital system

Two systems are interlinked both anatomically and embryologically as both of them develop from a common mesodermal ridge which is known as 'intermediate mesoderm'' along the posterior wall of the abdominal cavity [6,7].

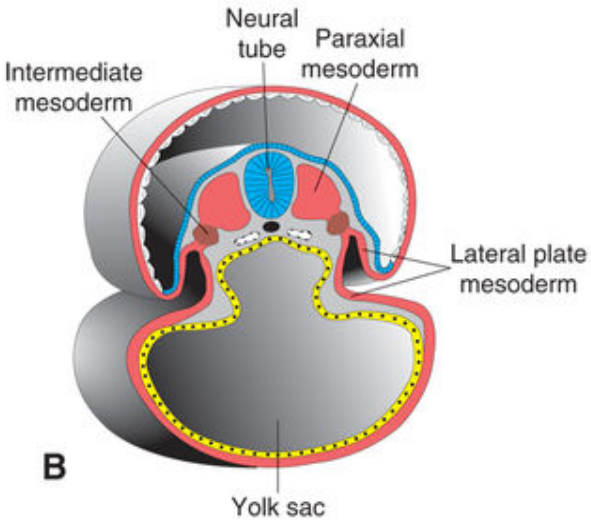


Fig.1. Development of Urogenital system

Gonads do not acquire male or female morphological characteristics until the seventh week of development.

Gonads initially appear as a pair of longitudinal ridges, the genital or gonadal ridges which are formed by proliferation of the epithelium and a condensation of underlying mesenchyme. Germ cells do not appear in the genital ridges till the sixth week of development.

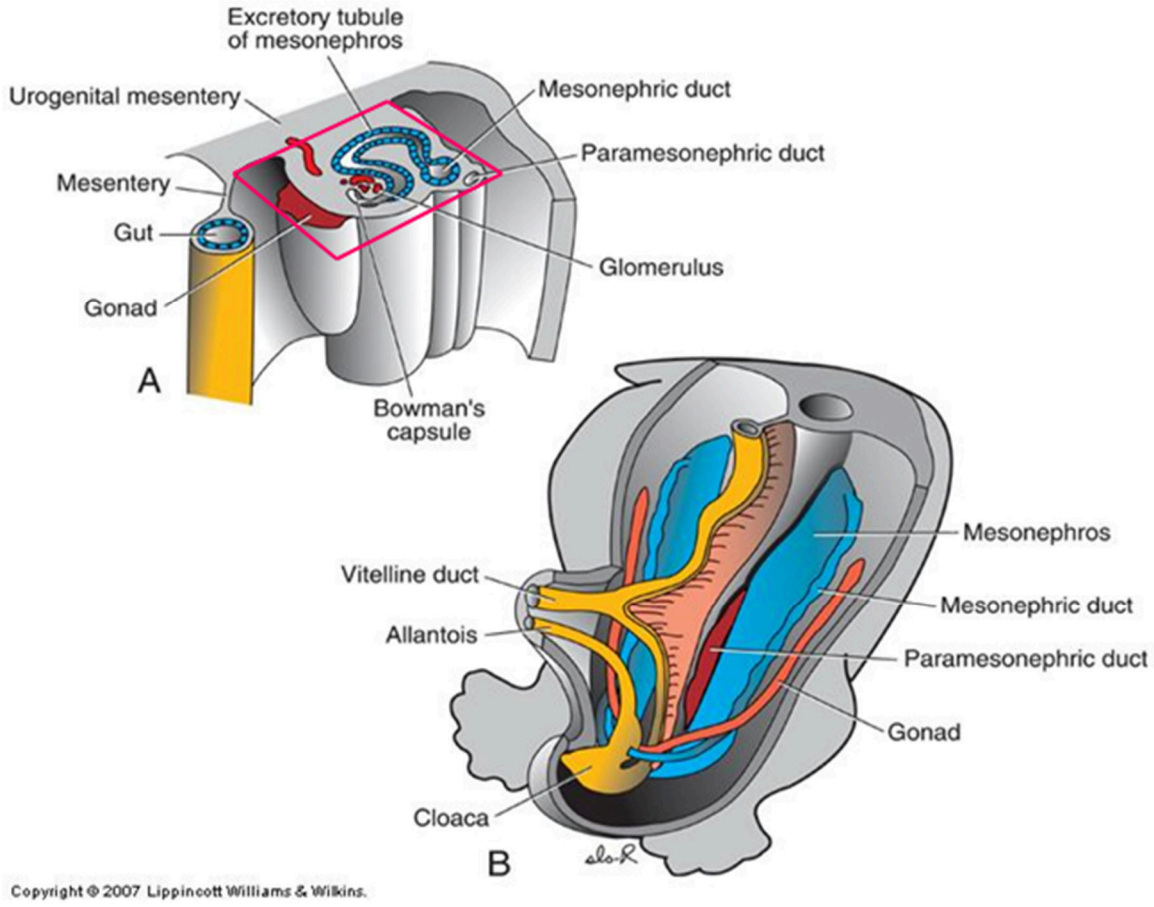


Fig.2. Sixth week of development

Firstly, both male and female embryos have 2 pairs of genital ducts:

1. Mesonephric (Wolffian) duct
2. Paramesonephric (Mullerian) duct

The Paramesonephric duct arise as a longitudinal invagination of the epithelium on the anterolateral surface of the urogenital ridge.

Cranially, this duct opens in the abdominal cavity with funnel-like structure and caudally it runs lateral initially, to the mesonephric duct and then crosses it ventrally to grow caudomedially. In the midline it comes in contact with paramesonephric duct which comes from the opposite side.

Sinus tubercle is a swelling formed when caudal tips of the combined ducts projects in the posterior wall of the urogenital sinus. The mesonephric ducts open on either side of the tubercle into the urogenital sinus.

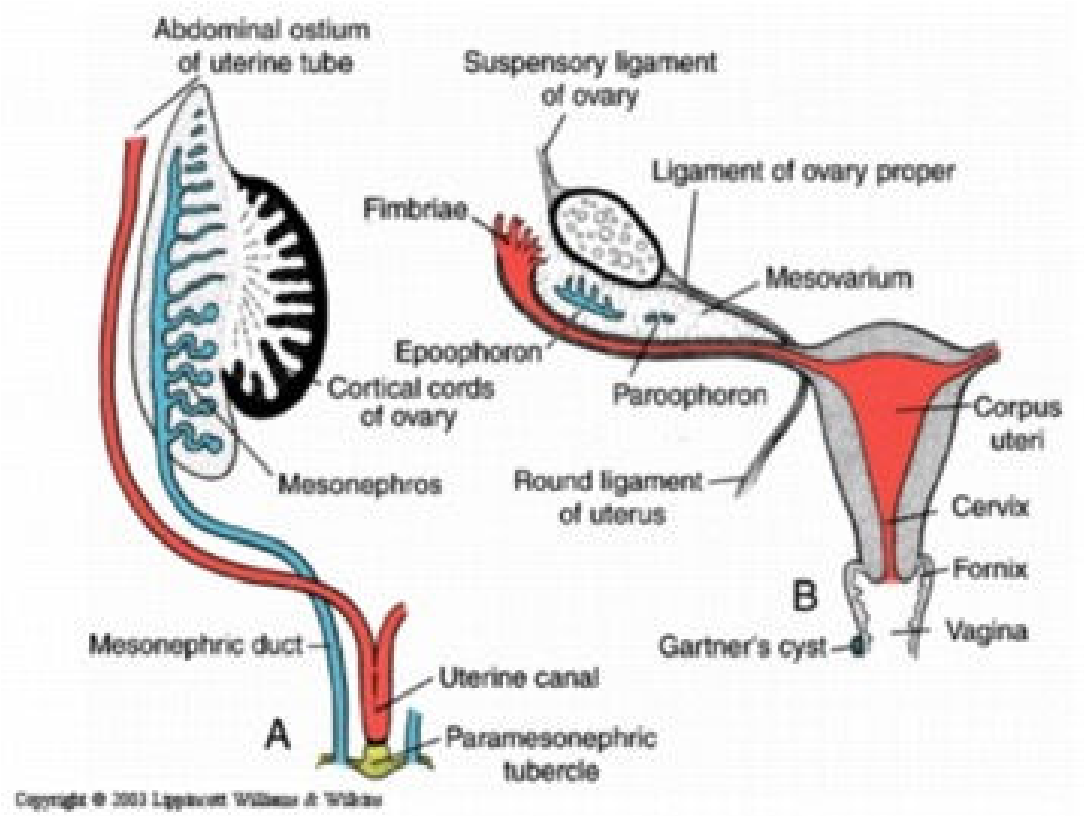


Fig.3. Differentiation into Mesonephric and Para mesonephric duct

Paramesonephric ducts develop into main genital ducts of the female in presence of estrogen and absence of testosterone and AMH (MIS).

Three parts seen in each duct are:

1. Cranial vertical portion which opens in the abdominal cavity.
2. Horizontal part which crosses mesonephric duct.
3. Caudal vertical part which fuses with the other side side duct from opposite side.

Alongwith the descent of ovaries, the first 2 parts develop into uterine tube and the caudal parts fuse to form uterine canal.

The urogenital ridges come to lie in transverse plane as the 2nd part of the paramesonephric ducts moves mediocaudally. A broad pelvic fold is formed when the ducts fuse in midline and this extends from the lateral sides of the fused paramesonephric ducts towards the wall of pelvis and is known as broad ligament of uterus.

Anatomically, ovary lies on its posterior surface and uterine tube lies in the upper border of the ligament. The fused paramesonephric ducts forms corpus and cervix of the uterus and also the upper portion of vagina.

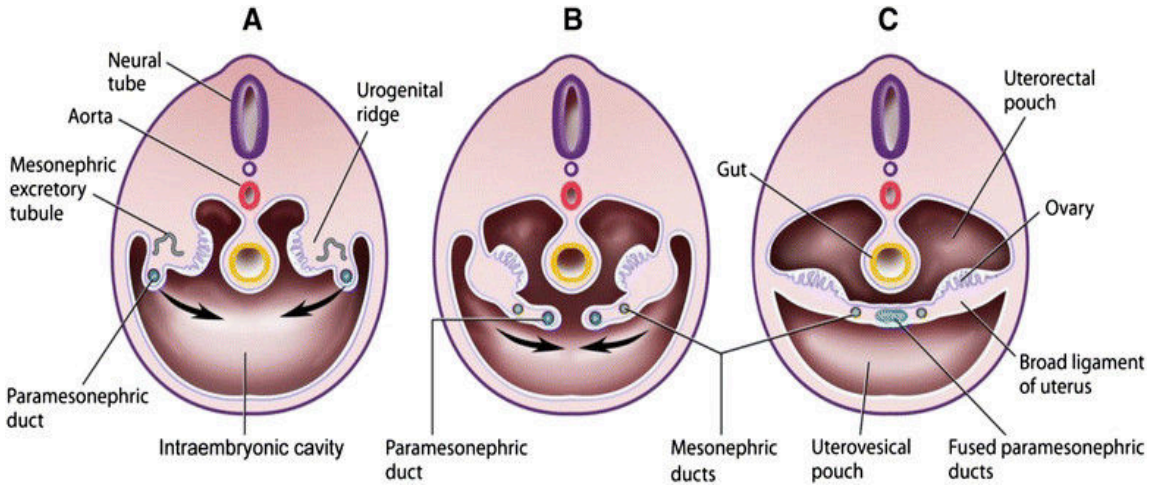


Fig.4. Anatomical Relations with genital ducts

The pelvic cavity is divided into uterorectal and uterovesical pouch by the uterus and broad ligaments. A layer of mesenchyme surrounds uterus which forms muscular coat , myometrium and peritoneal covering , perimetrium.

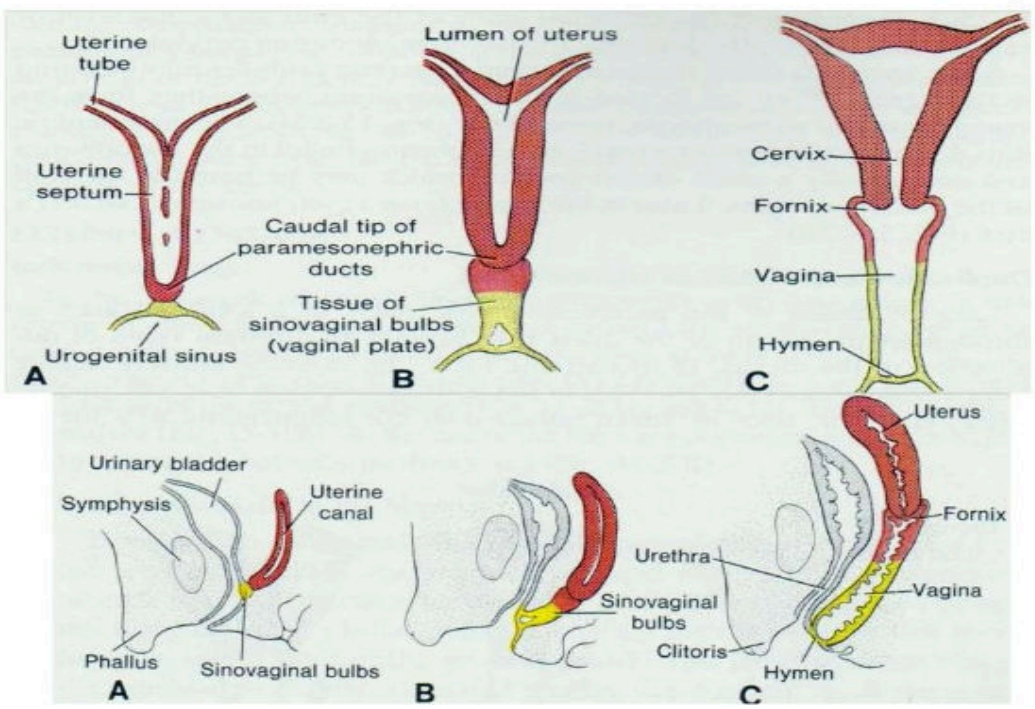


Fig.5. Development of Uterus and Vagina

The tip of paramesonephric ducts fuse with urogenital sinus , and 2 solid evaginations grow out from the pelvic part of the sinus , they are known as sinovaginal bulbs which proliferate and form solid vaginal plate.

At the cranial end of the plate , proliferation continues and leads to increase in distance between the uterus and urogenital sinus.

Vagina has a dual origin , the upper portion is derived from uterine canal and lower portion from urogenital sinus. Thin tissue plate, the hymen separates lumen of vagina from urogenital sinus .

Hymen consists of thin layer of vaginal cells and epithelial lining of sinus. [13,14]

ANATOMY

Rosai and Ackerman's SURGICAL PATHOLOGY Tenth edition

The Nulliparous adult (non gravid) uterus is a pear shaped , hollow organ which weighs around 40-80 gms and measures about 7-8 cm along the longest axis. It is located in between the rectum posteriorly and urinary bladder anteriorly .

Uterus is supported by round ligament and utero-ovarian ligaments. It is also covered with peritoneum. Two divisions of uterus are:

a) Cervix b) Corpus

Fundus - Part of the corpus which is cephalad (lies superior) to the line which connects the insertion of the two fallopian tubes.

Cornua - Lateral region of the fundus which includes the intramural part of the fallopian tubes.

Lower uterine segment or the isthmus -Portion which connects the cervix to the corpus.

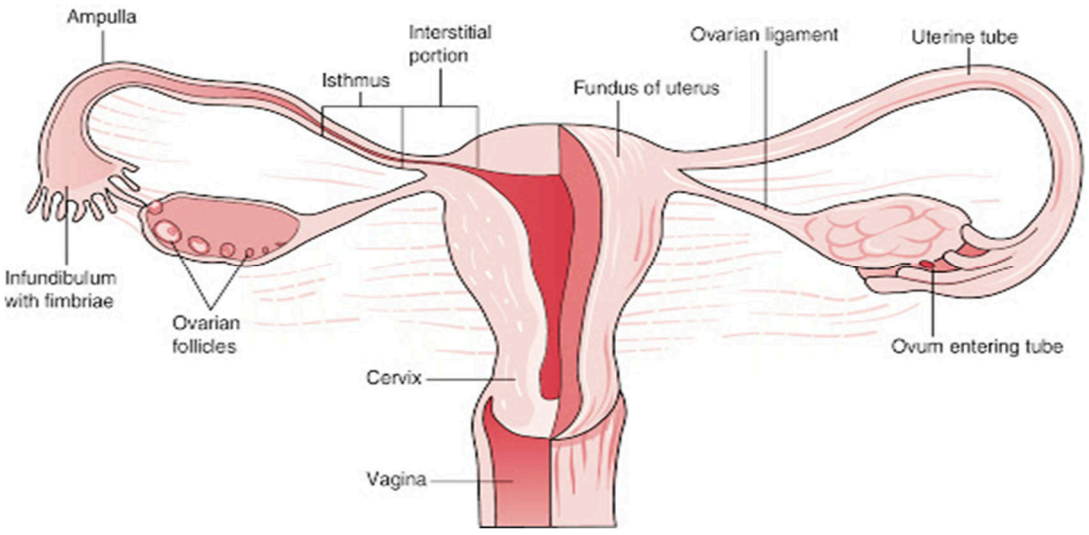


Fig.6. Anatomy of Uterus

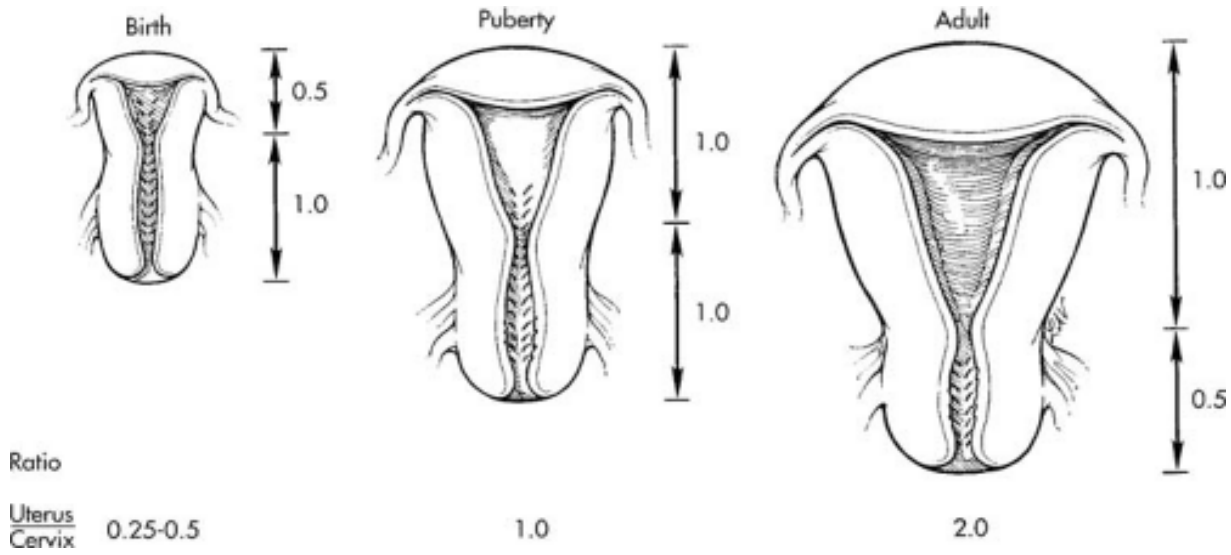
The uterine cavity measures about 6cm in length and is triangular in shape and lined by endometrium. The endometrial mucosa lines inner layer and outer layer is formed of thick muscle layer which is known as myometrium. Outermost layer is called serosa which extends till the point of peritoneal reflection. It helps in identifying the anterior wall and posterior wall in a hysterectomy specimen because they are different from each other. The point of peritoneal reflection in the posterior wall is lower than the anterior wall. Insertion of the fallopian tube into the uterus, which is posterior to the insertion of round ligament is the other point of identification.

In the pre pubertal period endometrium remains inactive and most of the uterus is formed by the cervix. During the reproductive years, the weight and the size of the uterus depends upon the parity.

Nulliparous uterus weighs about 4-100gms and measures about 8x5x2.5cm whereas multi gravid weigh 250gms and measure 10-12x5-7x2.5cm.

Stage	Uterine Length (cm)	Uterine Body-to-Cervix Ratio*
Neonatal	3.5	2:1
Pediatric	1–3	1:1
Prepubertal	3–4.5	1–1.5:1
Pubertal	5–8	1.5–2:1
Reproductive	8–9	2:1
Postmeno-pausal	3.5–7.5	1–1.5:1

Fig.7. Ratio of Uterine body to Cervix



DIFFERENCE BETWEEN NULLIPAROUS AND PAROUS UTERUS

	NULLIPAROUS	**PAROUS**
Size	8x5x2.5 cm	10-12x5-7x2.5 cm
Weight	4-100 gms	250 gms
Ratio between Body/ Cervix	Equal	2:1
Upper surface of fundus	Less convex	More convex
Uterine cavity	Convex	Concave
Scar for placental attachment	Absent	Present
External os	Round	Transverse
Internal os	Circular, well defined	Ill defined, margin wrinkled

POSITION OF OVARIES

In Nulliparous adult	Below the pelvic brim , in the ovarian fossa
The newborn	Above the pelvic brim
Multiparous female	May prolapsed in the recto-uterine pouch
Ectopic ovary	In the inguinal canal or in the labium major

Anterior uterovesical and posterior rectovaginal folds are formed when parietal peritoneum is reflected over genital tract . Broad ligaments are the lateral folds and extends on both sides of the uterus to the lateral pelvic walls further continuing with the peritoneum.

Ligaments of pelvis are:

a) Round ligament

b) Uterosacral ligament

c) Transverse cervical ligament

d) Pubocervical ligament

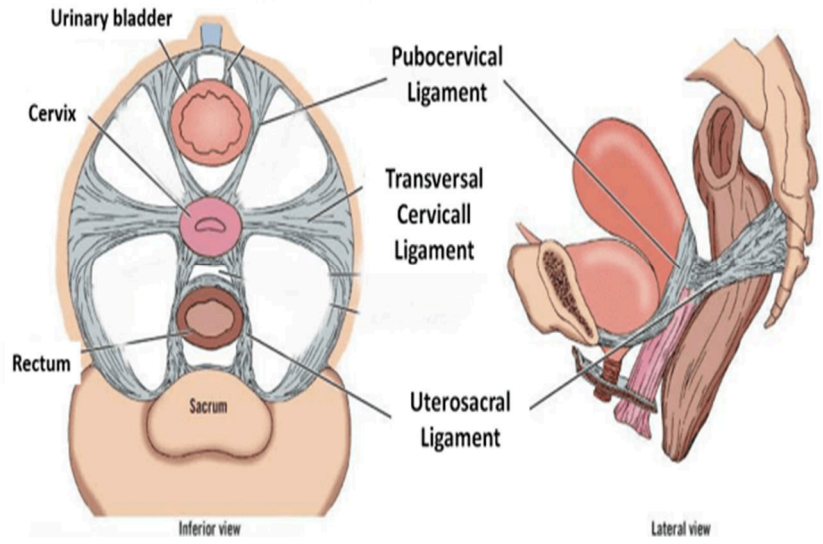
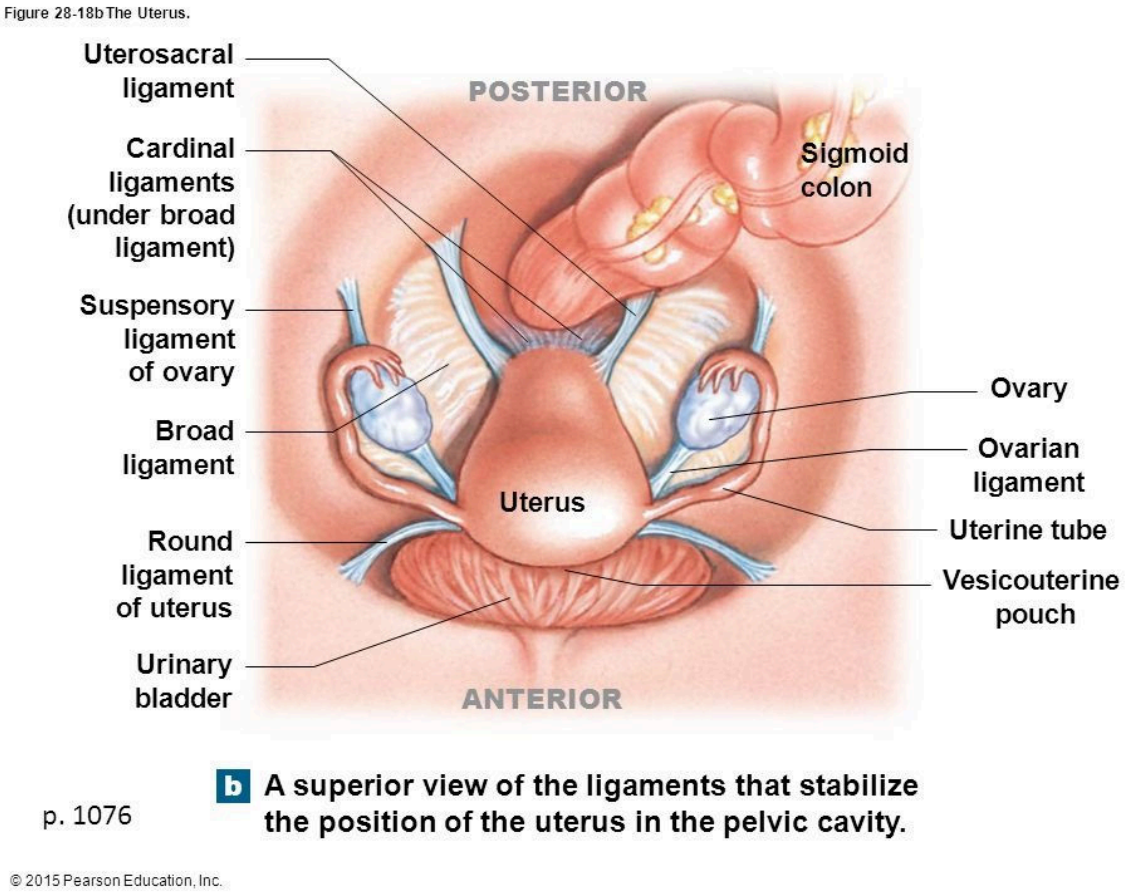


Fig.8. Ligaments of Uterus



Vascular supply and lymphatic drainage:

Uterine artery is the main artery to supply the uterus which arises as a branch of anterior division of Internal Iliac artery. Each uterine artery gives off many branches. The branches enter the wall of the uterus and divide, run circumferentially as anterior and posterior arcuate arteries. While reaching the anterior and posterior midline arcuate arteries become narrow. The terminal branches which are present in the muscular layer becomes tortous and called as helicrine arterioles. In both myometrium and endometrium series of capillaries forming plexus are found .

The radial arteries of the myometrium supplies the overlying endometrium. They penetrate the endometrium forming basal arteries which divide to form horizontal and vertical branches.

Basal layer of endometrium is influenced by the steroid hormones secreted by the ovaries and has the horizontal branch of basal arteries. Vertical branches supply the Functionalis layer , which form spiral arteries in the endometrium. (15)

Histology of the normal endometrium :

The nature and intensity of the ovarian hormone determines the histology of the endometrium.

Regional Variation -The mucosa lining the uterus is divided into two parts, one is mucosa of lower uterine segment and other is mucosa lining the corpus proper. The mucosa of lower uterine segment is thinner compared to the mucosa of the fundal part and has weak response to hormones. Therefore, development of the lower uterine segment lags behind the corpus, which responds very well to the hormonal stimulation. Two layers of mucosa are:

a) Basalis - Lowermost

b) Functionalis- Overlying

Basalis layer is composed of weakly proliferative glands and stroma shows dense spindle shaped cells which are seen adjacent to the myometrium. The endomyometrial junction is irregular. The smooth muscles blends with interdigiting endometrial stroma. This feature has to be distinguished from myometrial invasion which is seen in adenocarcinoma and also in adenomyosis during florid state. This layer is known as reserve cell layer of endometrium. The functionalis, the overlying layer sheds off after procedures like curettings and post menstruation period. The layers which remains are deep functionalis and Basalis, which help in the process of regeneration. The glands in Basalis do not show

secretory change and stroma has spindle cells without feature of decidualisation. Exception is during the latter half of pregnancy.

Functionalis layer has feature characteristic of normal endometrium except for latter half of pregnancy where it has two strata:

Strata compactum and Strata spongiosum.

COMPONENTS OF THE ENDOMETRIUM :

a) Epithelial component which is seen in glands and surface epithelium

b) Mesenchymal component which is seen in the stroma and vessels.

The suface and glandular epithelia have four morphologically different cells:

1. Proliferative type - show pseudo stratification of the nuclei and presence of mitotic figures.

2. Basalis type

3. Secretory cells - have non mucinous secretion

4. Ciliated cells.

Post ovulation secretion which is present in the subnuclear region shifts to supranuclear location and discharges into the lumen. On the surface and entire glands exhausted cells are present.

Cells have elongated nucleus with coarse chromatin, moderate amount of eosinophilic cytoplasm and luminal bleb. Mostly near the isthmus during proliferative phase ciliated

cells are seen. Cellular, reticulin and vascular components comprises the mesenchymal elements.

Cellular components in endometrial stroma differ according to different stages of menstruation.

Early proliferative stage - Cells have small, scant cytoplasm with dense fusiform nucleus and size is compared with the size of neutrophils. As menstrual cycle progresses , stromal cells elongate and have abundant cytoplasm. At the end of secretory phase, stromal cells around the vessels become round with more cytoplasm and vesicular nuclei and nucleoli. Hence, there is transformation of stromal cells to sheets of polygonal cells forming predecidualisation. Decidualisation forms in pregnancy for the purpose of implantation.[16,17]

PHYSIOLOGY OF NORMAL ENDOMETRIUM

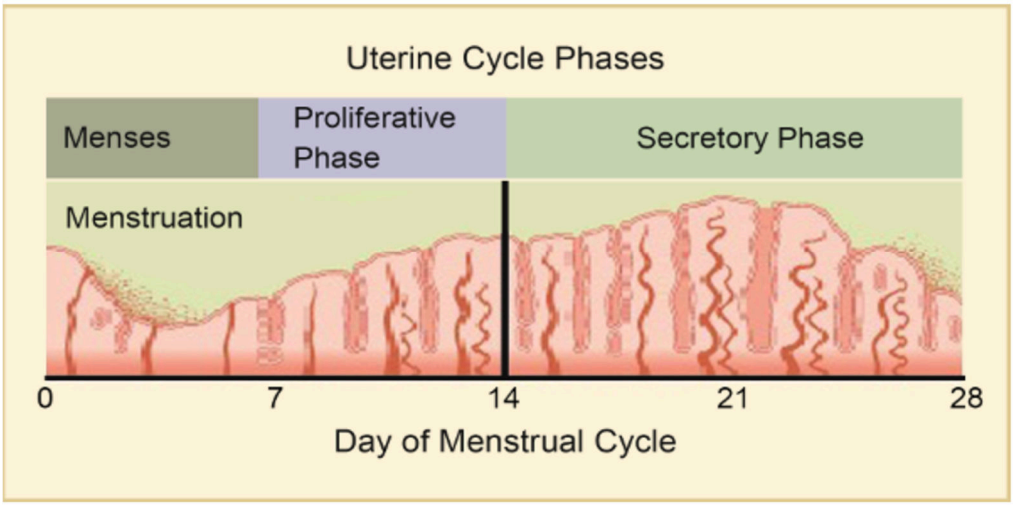


Fig.9. Phases of Menstrual cycle

- The first day of the menstrual cycle when the bleeding starts is taken as day 1 of the cycle.
- Proliferative phase is about 10- 12 days - stimulated by estradiol.

- Then ovulation occurs followed by Secretory phase which lasts for 14+/- 2days - stimulated by both estradiol and progesterone.
- Later, is the period of menstruation that lasts for 4+/- 2days. Then a new cycle starts .[18]

PATTERN OF ENDOMETRIUM

A) **PROLIFERATIVE PHASE**: In this phase the glands of stratum functionalis are non budding and non branching. Glands are uniformly shaped and are distributed throughout the stroma which has cells containing scant cytoplasm and no distinct cell membrane. The gland to stromal ratio is 1:1.The glands are tubular with small lumen which are lined by pseudo stratified elongated tall columnar cells which are mitotically active. Vessels are thin resembling capillaries. The stromal cells show mitosis.

B) **INTERVAL PHASE:** This depicts the late proliferative endometrium where the glands are coiled. There is no pseudo stratification and few of the glands show evidence of ovulation in the form of sub nuclear vacuolation.

C) **EARLY SECRETORY (POD 2-5):** Most glands show cytoplasmic vacuolations and few show features of proliferative stage. There is no sign of predecidualisation. Clinically it may be related to mid cycle spotting which is called as Mittelschmerz sign.

D) **MID SECRETORY PERIOD (POD 6-8):** This is characterised by full coiled glands which is lined by columnar cell with round to vesicular nuclei. There is

presence of luminal secretion . Extensive vacuolation and predecidualisation are absent in mid secretory period. Clinically this period is when implantation takes place.

E) **LATE SECRETORY PERIOD (POD 9-14)**: Prominent spiral arteries which are surrounded by cuff of predeciduated cells are seen in this phase. At POD10 predecidualisation starts around the spiral arteries and later it extends to form islands in the stroma. At the end of this period, these islands become confluent and menstruation is intersected by haemorrhage. Stromal granulocytes are seen and are called as Natural Killer cells.

F) **MENSTRUATION**: This phase has disintegrating fragments of secretory endometrium. Glands in this phase are cystically dilated with flat epithelial lining. Few have frayed or irregular borders, stating secretory exhaustion. Presence of karyorrhectic fragments, predecidualised stroma and fibrin thrombi in the vessels are features of menstruating endometrium [19].

OTHERS PATTERNS: Deviation from normal pattern of secretory endometrium is seen in infertile women. Patients bshowing this change in pattern will present with oligomenorrhea and infertility.

LUTEAL PHASE DEFECT: Occurs due to inadequate amount of progesterone which is secreted by corpus luteum. This defect is mostly diagnosed during evaluation of infertility and abnormal bleeding. The reasons are either the corpus luteum fails to develop or it regresses. Low FSH during follicular phase and LH peak at mid-cycle do not cause luteinisation of granulosa cells. This defect leads to defect in end organ receptors, which results in occurrence of menstruation 6-9 days prior to the LH surge .[20,21]

A) **MIXED SECRETORY PATTERN**: This occurs when there is a difference of 3 days for secretory endometrium to form in different regions of stratum functionalis. This abnormality should be correlated with clinical details.

B) **SECRETORY CHANGE ON ABNORMAL NON SECRETORY PATTERNS**: Pattern in which secretory change is superimposed on proliferative disordered endometrium or endometrial hyperplasia/carcinoma. The glands in this are branching when compared to the coiled structure seen in secretory phase. The atypical extensive secretory change in well differentiated carcinoma is called as secretory carcinoma. It is low grade and has features of well differentiated carcinoma with cribriform pattern, mild nucleomegaly and secretory changes.

PRECURSOR LESIONS OF ENDOMETRIAL CARCINOMA

Endometrial neoplasms show a spectrum of morphological alterations which ranges from endometrial hyperplasia to different types of endometrial carcinomas. Hyperplasia and carcinoma are two extreme points of this spectrum.

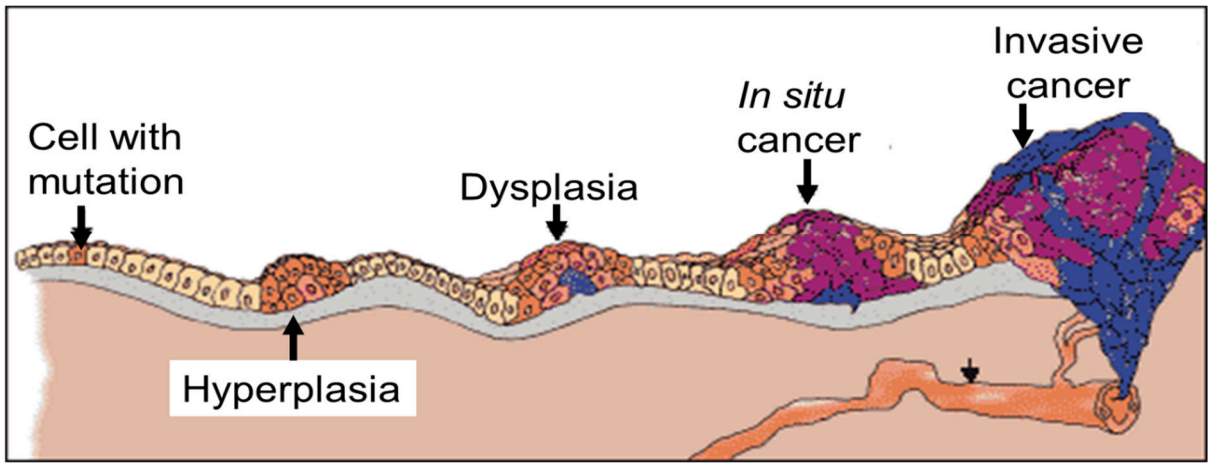


Fig.10. Development of Endometrial Cancer

ENDOMETRIAL HYPERPLASIA - Defined by WHO in 2003 as a spectrum ranging from benign form to premalignant disease in the presence of unfavourable condition of hormonal environment [22]. There is a proliferation of glands exhibiting different sizes and shapes with increase in gland to stromal ratio. Mostly the process is diffuse, but sometimes can be seen focally. [23]

The revised 2014 WHO classification recognizes two types of EH based on the presence of cytologic atypia:

a) EH without atypia (Benign)

b) Atypical EH (Premalignant) [25]

These are further categorized as Simple and Complex depending upon the extent of crowding of the glands.

WHO Classification	Incidence of Endometrial Cancer
Simple Hyperplasia without Atypia	1%
Simple Hyperplasia with Atypia	3%
Complex Hyperplasia without Atypia	8%
Complex Hyperplasia with Atypia	29%

Table 2: Incidence of Endometrial Cancer by WHO Hyperplasia

The 20-year risk of progression of EH without atypia to cancer is less than 5% , thus it may be managed with observation alone and follow-up biopsies. On the other hand, atypical EH requires a total hysterectomy, due to a risk of progression of 29%. [26]

The cytological atypia is more likely to progress into carcinoma than the degree of architectural abnormality in terms of complexity and crowding of glands. For practical use, the hyperplasia with or without atypia is considered as noninvasive proliferative lesion of the endometrium.

The reasons are first of all, simple atypical hyperplasia are rare and most hyperplasias are complex atypical ones. Secondly cytological atypia is much more significant than architectural atypia for its progression to carcinoma.

Patients present with abnormal bleeding [27] . The hallmark of hyperplasia is increase in glandular tissue than stroma with concomitant changes in architecture and cytology. This occurs due to excessive unopposed stimulation by estrogen and it applies mostly for simple hyperplasia. Administration of unopposed estrogen in postmenopausal HRT (hormone replacement therapy) causes development of hyperplasia.

Anovulatory cycles (menopause and post menopause) leads to hyperplasia. But anovulation in menarchal young women and who are bleeding do not develop hyperplasia of endometrium. Polycystic ovarian disease (Stein-Leventhal) like conditions acquire hyperplasia in reproductive period. Development of hyperplasia is dependent upon estrogenic stimulation. A marked withdrawal effect of estrogen causes breakdown of the tissue.

Following menopause estrogen continues to get released from the persistent follicles or by peripheral conversion of andro-steinedione in a fluctuating and inconstant way. The endometrium continues to increase in size. The inconstant secretion of estrogen is not adequate to support such a volume thereby causing breakdown of the tissue. Besides breakdown, the superficial dilated vessels also contribute in the bleeding process.

The changes in the architecture which are in the form of change in shape and size of the glands are diffuse and are present throughout the endometrium. The glands have minimal or focal crowding and are cystically dilated. Some show branching with infoldings and outpouchings. Complex angularity is not seen. Nuclei are oval to elongated with fine chromatin and small inconspicuous nucleoli with pseudostratification . Mitotic activity is also present.

The stroma is abundant and cellular which is composed of small to oval cells with scant cytoplasm and mitotic activity seen. Inconspicuous spiral arterioles are also seen.

Simple Hyperplasia with Atypia	**Complex Hyperplasia with Atypia**
a)Diffuse architectural change.	a) Focal to extensive distribution.
b)Variation in size and shape, glands are small to large and cystically dilated. Minimal crowding and branching with no complex angularity.	b) Marked variation in size and shape, glands are showing marked crowding, branching with papillary infoldings and outpouching. Presence of complex angularity.
c) Increased ratio (Glands: stroma)G:S >1:1	c) Greatly increased G:S ratio >3:1.
d) Size and shape of nuclei are variable. Elliptical to oval, hyperchromatic to vesicular, coarse clumping of chromatin. Prominent enlarged and irregular nucleoli.	d) More aggressive nuclear features.
e) Abundant and cellular stroma with small oval cells with scant cytoplasm	e) Scanty stroma

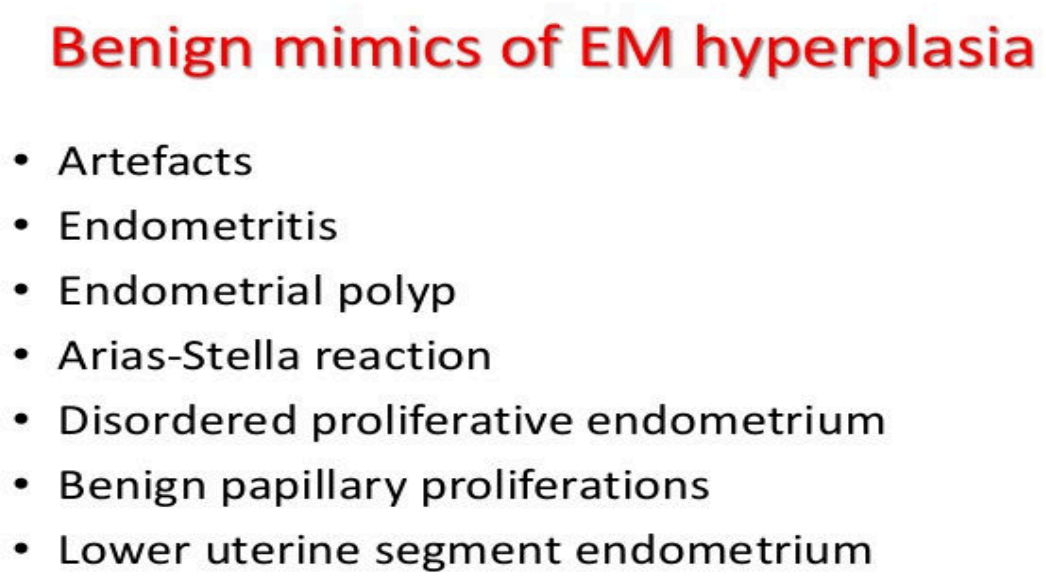


Fig.11. Benign mimics of EM Hyperplasia

EIN SYSTEM [ENDOMETRIAL INTRAEPITHELIAL NEOPLASM SYSTEM] [22] :

Alternative system to WHO classification of endometrial hyperplasia.

Based on:

a) Morphometry

b) Morphology

c) Molecular

d) Prognostic parameters

True precancerous lesions are monoclonal and are derived from polyclonal normal endometrial glands by mutations. They have a benign growth pattern although genetically

these are abnormal. When these genetic damages get accumulated, it leads to malignant transformation.

3 different categories have been recognized.

(1) Endometrial hyperplasia related to benign pattern due to unopposed estrogen.

(2) Endometrial Intraepithelial Neoplasia (EIN)

(3) Adenocarcinoma.

From molecular point EIN is a proliferative lesion which is monoclonal and has molecular alterations similar to endometrial carcinomas. The genetic alterations are PTEN mutations and microsatellite instability .

EIN can be defined as inactivation of the PTEN tumour suppressor gene which is regulated hormonally in the normal endometrium and is mutated in EIN. Alongwith clonal proliferation of abnormal glands they form cohesive clusters of PTEN negative glands. These changes are focal and are identified by their altered architecture and cytology [(28)].

DIAGNOSTIC CRITERIA OF EIN:

A) Maximum dimension on long axis exceeding 1mm which may include 5-10 glands.

B) Volume percentage stroma < 55% (gland area exceeding that of stroma).

C) Cytologically, the lesion should be differentiated from the background endometrial glands.

The mimics are basal endometrium, hypersecretory endometrium, polyp and regenerating endometrium. There is always a risk of confusion with the term neoplasia in a preneoplastic lesion and is a disadvantage of the EIN scheme [22].

ENDOMETRIAL INTRAEPITHELIAL CARCINOMA:

It is a precursor lesion of Type 2/ serous adenocarcinoma of endometrium. Mostly affects postmenopausal women on an atrophic endometrium and restricted to small area or in an endometrial polyp. The lesion is characterized by highly pleomorphic cells which resemble cells of invasive serous carcinoma. They replace the surface cells and do not invade the stroma. The cells show loss of polarity and have large and atypical nuclei with prominent nucleoli. Abnormal mitotic figures are seen. The cells show strong positivity for p53 and ki67. A variety of reactive cases can mimic EIC due to cytological atypia and architecture. Data based study has revealed that these are not p53 positive [22,24].

WELL DIFFERENTIATED ENDOMETRIOID ADENOCARCINOMA VS COMPLEX HYPERPLASIA WITH ATYPIA

It is difficult to distinguish both the entities and histological criterion to differentiate was laid by Kurman and Norris . Main criterion is the invasion into the stroma (present or absent).

The following features define the stromal invasion [23]:

a) Desmoplasia - is the stromal response to the neoplastic glandular proliferation.

b) Pattern of growth ,whether cribriform or confluent.

c) Extensive papillary pattern

d) Squamous epithelium replaces the stroma.

In about 17% cases, stromal invasion was absent in curettings but was seen in hysterectomy specimens in about 17%. The tumour was well differentiated was either confined to the endometrium or invaded myometrium at superficial level. Stroma invasion was present in well differentiated carcinoma and stroma was absent in between the confluent adjacent glands which were arranged in a back to back cribriform glandular growth pattern. Mucinous differentiation was also an indirect indicator for adenocarcinoma with invasion. Malignant potential of all the hyperplasias is difficult to determine because of lack of follow up. It has been noticed with regard to WHO classification that 1- 10% of both the hyperplasia without atypia and >25% of Complex atypical hyperplasia (CAH) progress to carcinoma after 1-20 years. Kurman and his colleagues reported that transformation into carcinoma is very high in premenopausal women with CAH. Hyperplasia without atypia is reversible [22].

Epithelial Intraepithelial Neoplasia

In 1994 WHO had classified hyperplasia into 4 groups. Based on the degree of architectural crowding the system has categorized the entity into :

1.Simple

2.Complex

According to altered nuclear morphology, it is further classified as :

1.Atypical

2. Non- atypical

In recent edition of WHO, the 4 tiered system has been replaced by a two tiered system which comprises of hyperplasia without atypia and atypical hyperplasia [29].

Another alternate system has been postulated based on the studies which determine the clone of the lesions, the morphometry and cancer risk [30]. The excessive proliferating glandular lesions are divided into hyperplasia and endometrial intraepithelial neoplasia.

Further, this new two tier system is currently used for the treatment purpose.

The terms used are hyperplasia without atypia and atypical hyperplasia or Endometrioid intraepithelial neoplasm. Endometrial intraepithelial neoplasia has been now replaced by Endometrioid Intraepithelial Neoplasm. EIN is the precursor of endometrioid endometrial carcinoma.

Fig.12. WHO Classification-2014 of Endometrial Hyperplasia

New WHO classification of endometrial hyperplasias - 2014

New term	Synonym	Genetic changes	Coexistent invasive endometrial carcinoma	Progression to invasive carcinoma
Hyperplasia *without*atypia	Non-atypical EM hyperplasias	Low level of somatic mutations	< 1%	RR: 1.01–1.03
Atypical hyperplasia/endometrioid intraepithelial neoplasia	Atypical EM hyperplasias, EIN	Many of the genetic changes typical for endometrioid endometrial cancer are present	25–33% 2 59% 1	RR: 14–45

1. Antonsen S L, Ulrich L, Hogdall C. Patients with atypical hyperplasia of the endometrium should be treated in oncological centers. Gynecol Oncol. 2012;125:124–128

2. Zaino R, Carinelli S G, Ellenson L H, Lyon: WHO Press; 2014. Tumours of the uterine Corpus: epithelial Tumours and Precursors; pp. 125–126.

ENDOMETRIAL CARCINOMA

It is the most common malignancy of female genital tract in developed countries next to carcinoma of cervix. The increasing incidence of this carcinoma has made it the 4th leading cause of malignancy and 7th leading cause of death [31,32]. The traditional two tiered classification model was postulated by Bokhman in 1980. It was used by both the pathologist and the clinicians. According to the system, endometrial carcinoma was stratified into :

1.Type 1/ Endometrioid type of endometrial carcinoma

2.Type 2/ Serous type endometrial carcinoma .[33]

This carcinoma mostly occurs in post-menopausal women with mean age at presentation around 55 years. There have been many proven studies which demonstrates the association between prolonged, unopposed estrogen exposure and development of endometrial carcinoma .[27]

The factors related to prolonged exposure of estrogen which lead to endometrial carcinoma are [34]:

a) Use of Tamoxifen in breast cancer- increases the risk of developing endometrial cancer by 2-folds as it causes unopposed estrogen stimulation on the endometrium. Postmenopausal women who are on Tamoxifen have to undergo screening test for early detection of endometrial cancer .[34]

b) Unopposed exogenous estrogen treatment: has been the most potential triggering risk factor for endometrial carcinoma. These days combined HRT, includes both estrogen and progesterone and are used to reduce the incidence of this disease. In one

of the study it was noted that postmenopausal women on combined HRT had low risk of developing endometrial carcinoma than the women having only estrogen HRT. [35]

c) Most common endogenous cause of excessive estrogen production- Obesity - Excessive adipose tissue increases peripheral aromatization of androstenedione into estrogen. There is absence of ovulation in post menopausal women which leads to continuous estrogen stimulation. Endometrium proliferates without progesterone effect hence there is no menstrual withdrawal bleeding.

d) Ovarian lesions like thecoma, granulosa cell tumour, polycystic ovarian disease, are all associated with excessive prolonged and unopposed estrogenic effect on the endometrium which leads to endometrial hyperplasia and endometrial carcinoma. [36]

e) Nulliparity or low parity poses a very high risk in development of endometrial carcinoma. [37]

f) Other factors include diabetes, hypertension and are indirect source of estrogen production thereby causing endometrial carcinoma.

Bokhmann in 1980 established characteristics of both the types of endometrial carcinoma and are as follows :[33,38]

	CHARACTERISTICS	**TYPE 1**	**TYPE 2**
1.	Background endometrium	Hyperplstic endometrium	Atrophic endometrium
2.	Obesity, diabetes, hyperlipidemia	Yes	No
3.	Histologic type	Endometrioid	Serous
4.	Histological tumour grading	Low	High
5.	Prognosis	Favourable	Unfavourable
6.	ER/PR status	High	Low

7.	P53	12%	90%
8.	PTEN mutation	80%	10%
9.	PIK3CA	52%	42%
10.	KRAS	43%	8%
11.	ARID1A	48%	11%
12.	CTNNB1	24%	3%
13.	Her2/neu	0%	44%
14.	Microsatellite Instability(MSI)	40%	2%

Pathogenesis:

Endometrial carcinomas have a broad spectrum of phenotypes and exhibits many histological variants. This property of having variants reflects upon the capability of the Mullerian epithelium to differentiate. This carcinoma follows different pathways of tumorigenesis. [25]

It has both sporadic and hereditary population . 90% cases of endometrial carcinoma are sporadic and about 10% belong to hereditary was reported by Norasate et al. It has been seen that women with inherited predisposition for this disease, are also seen having an association with disorders like HNPCC and Cowden syndromes. [39]

Most of the sporadic cases of endometrial carcinoma are Type 1. They usually develop in perimenopausal women. The risk factors for this endometrioid type are hyperestrogenism, anovulation, obesity , late onset of menopause and nulliparity. They seem to develop from a hyperplastic endometrium like complex atypical hyperplasia (EIN).

These are low grade tumours and show minimal myometrial invasion and also have an indolent clinical behaviour. The endometrioid carcinoma and its rare mucinous variant are the prototypes of Type 1.

Most characteristic molecular alterations which are associated with type 1 are mutations of PTEN, K Ras, nuclear accumulation of B catenin and microsatellite instability.

Type 2 carcinomas are less common and are high grade and more aggressive neoplasm. They constitute around 10-20% of the total endometrial carcinoma. These are not related to estrogenic stimulation which is mostly seen in postmenopausal elderly women. They are histologically non endometrioid type and include papillary serous, clear cell, squamous cell and undifferentiated ones. This carcinoma arise in the background of atrophic endometrium.The molecular alterations which are associated with this type are abnormal p53, loss of heterozygosity at several loci, alteration of genes involved in regulating cell division .[40,41]

GENETIC CHANGES IN CARCINOGENESIS OF TYPE 1 ENDOMETRIOID CARCINOMA

Endometrioid endometrial carcinoma generally develops from endometrial hyperplasia which has been proven to be the true precursor for this tumour.

A progression model for Type 1 endometrial carcinoma has been proposed and is very much similar to the Volgelstein progression model for colorectal carcinoma.

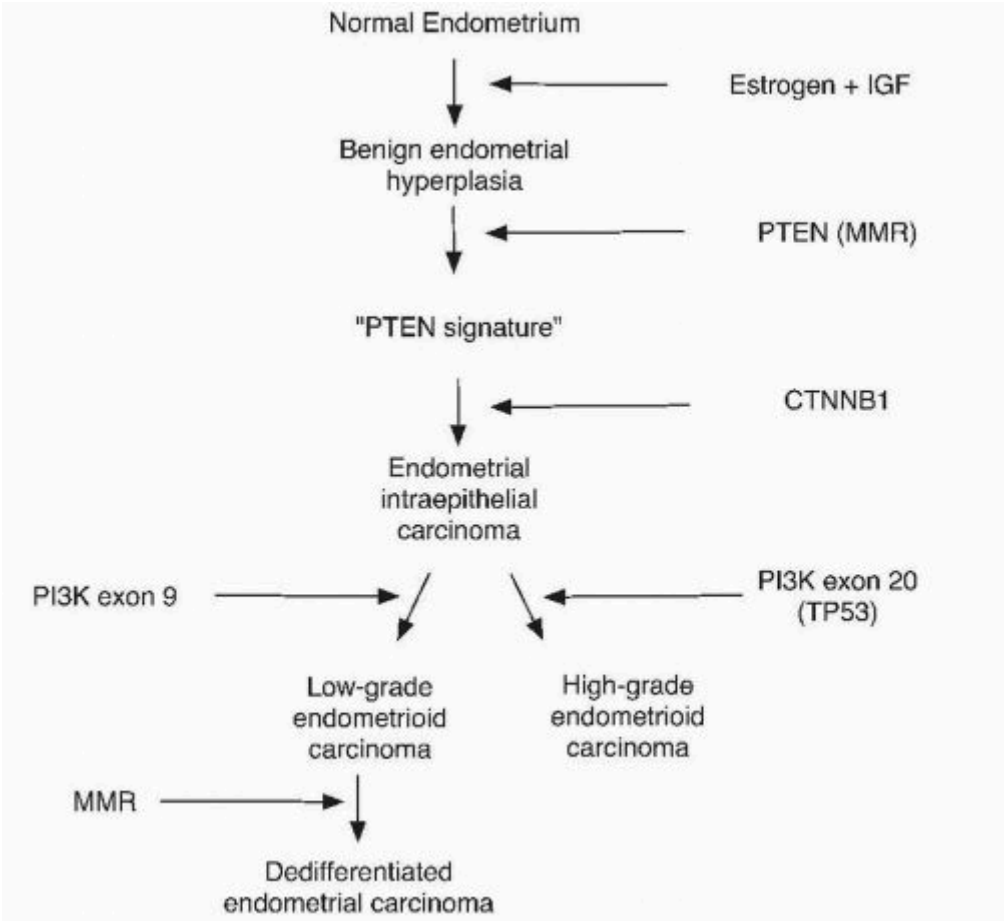
This hypothetical model is based on some facts such as:

(a) Both endometrioid carcinoma and endometrial atypical hyperplasia share some similar type of genetic alterations.

(b) There is an increase in genetic alterations of well differentiated endometrial carcinoma compared to atypical hyperplasia.

(c) The histological grading increases with increase in genetic alterations.

(d)With the help of CGH (Comparative Genomic Hybridization), the detected chromosomal abnormalities were found to be more in endometrial carcinoma than atypical hyperplasia. [25]



Progression model for Type 1

These genetic alterations taking place in endometrial carcinoma are seen to be present at a very early stage but there is no proven study to confirm that which alteration is responsible for the earliest changes of malignant transformation.

Alterations like PTEN inactivation, K-Ras, B catenin and MSI are seen in this type of tumour pathogenesis. These alterations are also seen in Atypical endometrial hyperplasia. PTEN is the most common alteration in Atypical hyperplasia and occurs in 50% of the cases. [25]

PTEN gene is a tumour suppressor gene and is a phosphatase and tensin homologue which is deleted from chromosome 10/MMAC1. PTEN encodes a lipid phosphatase which antagonises PI3K/AKT pathway by dephosphorylating PIP3 (product of PI3K).[42,43]

Cells have complex mechanisms which help in guarding our system against tumour development. These molecular guardians are responsible for the protection and gene which encodes these molecules are known as Tumour suppressor gene.

Tumours arise when the activity of these genes goes away and there are several processes which limit cell division. When there occurs an interaction between the extracellular molecules and their cell membrane receptors, this triggers intracellular signalling via many pathways.

One of this is PI3K pathway and PI3K are a group of several lipid kinases which catalyses the production of second messenger known as PIP3 (Phosphatidylinositol 3,4,5 triphosphate). PIP3 then activates downstream signalling via AKT and m TOR

for regulating diverse cell processes like cell division, survival, invasion and also metabolism.

AKT serine/threonine kinases –PIP3 activation can result from various types of alterations which include:

1. Mutation /Amplification of tyrosine kinase receptor (EGFR and HER)
2. Mutations of Ras
3. Mutation/ Amplification of PIK3CA

4. Inactivation of PTEN (tumour suppressor gene). [44,45]

PTEN desphosphorylates PIP3, therefore, impedes cell activation that is caused by mitogenic extracellular activity.

Endometrial carcinoma develops due to abnormality in PTEN-PI3K pathway. PTEN gene is having both lipid and protein phosphatase activity. When both lipid and protein activity are lost, it leads to loss of apoptosis thereby leading to aberrant cell growth.[46]

50% of Type 1 endometrial carcinomas and 83% of premalignant lesions have shown loss of PTEN. Type 1 carcinomas have the highest frequency of PTEN mutations which is about 34-80% of cases. The normal proliferative endometrium shows PTEN expression which suggests that this genetic alterations appears to be an early change in endometrial carcinogenesis. [26]

PTEN immunohistochemistry has been suggested to be a great tool in knowing the functional status of PTEN [47]. When the nucleus/cytoplasm of the normal endometrial glandular epithelium takes up the brown stain- immunoreactivity is taken as positive and acted as an internal control.

Kapucuoglu et al graded the immunostaining of PTEN-Semi quantitatively by taking percentage % and intensity of the stain in the endometrial glandular epithelial cells. (nucleus /cytoplasm). [48]

According to him:

a) If the number of cells stained were less than 10%- Staining score is negative

b) If 10-50% of the cells take up the stain- Score is 1+

c) If more than 50% of the slide's area was stained positive- Score is 2+.

In terms of intensity- PTEN scoring was done as follows:

a) Absence of staining- Score '0'.

b) When the staining is light brown- '1+'.

c) When the staining is brown to dark brown – '2+'.

In another study which was made by Garg et al, there were two different groups which were performing the IHC for PTEN in endometrial carcinoma. [49]

The aim of their study was to propose a useful scoring system and later the interpretations were cross validated between the two cancer centres, Memorial Sloan Kettering cancer centre and MD Anderson cancer centre.

Immunohistochemistry for PTEN interpreted by both the institutions were as follows:

1) Strong positive staining in the entire tumour / majority of the tumour-Positive (pos)

2) No staining in entire/most of the tumour with strong positivity in the adjacent normal endometrial glands or stromal cells – Negative (neg)

3) Tumour having convincingly positive and negative staining- Heterogenous (het).

H score was calculated for cases with heterogeneous staining and ranged from 'Zero' that signifies 'no staining' to '300' with 'maximum staining'.

Calculation was done based on formula:

H score=1 x % light staining +2 x %moderate staining +3 x strong staining.

A new three tiered scoring system was postulated in which the cases were divided into 3 groups denoting :

1) Positive- all or most cells have taken up the stain.

2) Negative- all or most of the tumour has not taken up the stain.

3) Heterogeneous- there are discrete positive and negative areas.

The three tiered scoring system was demonstrated to yield better and higher reproducibility rates in comparison to previously followed complex semiquantative H Score system.

Inhibitors of PI3K, MTOR and AKT are the chosen drugs for the treatment of endometrial carcinoma which targets the pathway PTEN-PI3K-AKT pathway. [50–52]

Therefore, decrease in PTEN immunoreactivity shows malignant features of endometrium through a premalignant stage.

PIK3CA – is a regulatory subunit in PIK3CA pathway and its activation is reported in 26- 36% in endometrial carcinoma. It coexists with other PI3K activating alterations to cause carcinoma. In endometrium it is present along with K Ras and PTEN mutations to cause malignancy.

K Ras – encodes a member protein of small GTPase family which participates in signal transduction pathway, between cell surface receptors and the nucleus. This mutation is reported in 10-30% of type1 endometrial carcinoma. Some investigators reported complete absence of this mutation seen in serous type. Several studies on Kras mutation reported, equal expression of Kras mutation in both type 1endometrial carcinoma and endometrial hyperplasia and higher frequency of this mutation being present in MSI positive cases. It is found that Kras mutation has no relationship with staging, grading, myometrial invasion, age or clinical outcome . [25]

B catenin (CTNNB1) is a cell adhesion molecule. It is also an important element in Wnt signal transduction pathway which is implicated in embryogenesis and carcinogenesis. E cadherin is a calcium dependent transmembranous epithelial cell adhesion molecule which binds to cytoskeletal actin filaments through alpha and b catenins, therefore, B catenin helps in cell adhesion. Defect of this cause decrease in cell adhesions, increase in metastatic potential, and decrease in dedifferentiation. Secondly it takes part in Wnt pathway. Mutation of this leads to stabilization and nuclear accumulation of the molecule in about 10-30% in type 1 endometrial carcinomas [53]. Microsatellite instability – Microsatellites are polymorphic, short tandem repeats extending throughout the genome. Previous studies reported that MSI is common genetic abnormality detected in 20-45% of sporadic endometrioid endometrial carcinomas. The percentage of this abnormality is very less in type 2 endometrial carcinoma. [54,55]

WHO CLASSIFICATION OF TUMOURS OF THE UTERINE CORPUS (2014):

-EPITHELIAL TUMOURS AND PRECURSORS

a) Precursors

1) Hyperplasia without atypia

2) Atypical hyperplasia / Endometrioid intraepithelial neoplasia

b) Endometrial carcinomas

1) Endometrioid carcinoma

- Squamous differentiation
- Villoglandular
- Secretory

2) Mucinous carcinoma

3) Serous endometrial intraepithelial carcinoma

4) Serous carcinoma

5) Neuroendocrine tumours

- Low grade neuroendocrine tumour- Carcinoid
- High grade neuroendocrine carcinoma- small cell and large cell neuroendocrine carcinoma

6) Mixed cell adenocarcinoma

7) Undifferentiated carcinoma

8) Dedifferentiated carcinoma

c) Tumour like lesions- Polyp, metaplasias, Arias stella reaction, lymphoma like

-MESENCHYMAL TUMOURS

- MIXED EPITHELIAL AND MESENCHYMAL TUMOURS

- MISCELLANEOUS TUMOURS

- LYMPHOID AND MYELOID TUMOURS

- SECONDARY TUMOURS

HISTOLOGICAL TYPES

Endometrioid type or type 1 endometrial carcinoma:

This type is defined as an adenocarcinoma of the endometrium exhibiting glandular pattern[27]. It accounts for 70-80% of endometrial carcinoma compared to serous type. The average age at the time of diagnosis is 63 years. Patients usually present with postmenopausal bleeding .They develop on a hyperplastic endometrium. An atrophic endometrium is frequently associated with high grade lesions and has a great potential to metastasize.

Gross appearance :

All types of endometrial carcinoma have almost similar gross picture. The endometrial surface is shaggy, tan in colour and glistening with focal hemorrhagic areas. They have an exophytic growth pattern even though there is deep invasion. They may also present as a polypoidal mass, or they form one or more discrete nodules. Necrosis is evident only in poorly differentiated forms. The size of the uterus is enlarged and myometrial invasion is appreciated by well demarcated, firm, gray white tissue with linear extensions into the myometrium. In advanced cases extrauterine involvement is quite evident. [23]

Microscopic findings:

They have a typical glandular or villoglandular pattern. The neoplastic glands are lined by stratified columnar epithelium. These glands are crowded and show complex branchings. The neoplastic cells are columnar with uniform apical border giving a smooth glandular contour at the luminal aspect and have eosinophilic, granular cytoplasm with mild to moderate nuclear atypia and inconspicuous nucleoli. The presence of mitotic figures is quite variable[29].

The differentiating feature between well differentiated endometrioid carcinoma and atypical hyperplasia is the presence of stromal invasion. It is defined by loss of intervening stroma, confluent glandular pattern and presence of desmoplasia. Grading is primarily made by the architectural pattern and nuclear features. [23]

Architectural grading of endometrial carcinoma :

GRADES	**ARCHITECTURE**
Grade 1	**Well differentiated .** **Mostly glandular with solid elements less than 5 %**
Grade 2	**Moderately differentiated.** **6-50% of the tumor is composed of solid elements**
Grade 3	**Poorly differentiated.** **More than 50% of tumor is composed of solid elements**

(The FIGO system divides into 3 grades depending upon glands to solid elements. The presence of solid elements signifies poorer differentiation. [22,23]

%	Grade 1	Grade 2	Grade 3
Grade type	**Well differentiated**	**Moderately differentiated**	**Poorly differentiated**
Glands %	**>95%**	**>50%**	**</=50%**
Solid elements %	**<5%**	**</=50%**	**>50%**

Nuclear grading is determined by variation in nuclear size and shape, distribution of chromatin and the nucleolar size.

Grade 1- Nuclei are mildly enlarged, oval in shape with evenly distributed chromatin.

Grade 2- Nuclei are having feature in between grade 1 and grade 3.

Grade 3- Nuclei are markedly enlarged, pleomorphic with irregular borders, has coarse chromatin and prominent eosinophilic nucleoli. The mitotic activity is an independent feature but generally increases with increase in nuclear grading.

The grade can be upgraded by 1 grade when the nuclear grade is higher than the architectural grade. [27]

Variants of endometrioid carcinoma are: [29]

a) With squamous differentiation- About 10-25% of the endometrioid carcinoma have a foci of squamous differentiation which are identified with the presence of keratin pearl formation, intercellular bridging or solid nest of large polygonal cells having abundant eosinophilic cytoplasm with distinct cell border. These may be present as morules or seen at the stromal interface. It is important to recognize this feature, as it

is not included in the estimation of solid elements for grading. Grading is done only based on glandular component.

The presence of squamous component can either be metaplastic or neoplastic. In tumours where the squamous component is well differentiated, is called as Adenoacanthoma. But when both glandular as well as the squamous components are poorly differentiated then the neoplasm is called as Adenosquamous carcinoma.

b) With secretory differentiation- Here the endometrioid carcinoma are lined by columnar cells which have single, large, sub/supra nuclear vacuolation of glycogen instead of eosinophilic cytoplasm. They constitute less than 2% of the typical endometriod carcinoma. This variant resembles the secretory endometrium of reproductive age group. Most of them are found in non treated postmenopausal women and are almost always well differentiated.

b) Villoglandular variant is a rare subtype and has a papillary architecture. These papillary fronds are composed of delicate fibrovascular core covered by columnar cells having bland nuclei. Median age of the patients for this variant is 61 years.

Microscopic findings show thin, delicate fronds covered by stratified columnar epithelial cells. The nuclei are oval in shape, exhibiting mild to moderate pleomorphism with nuclear grading of 1 or 2.Abnormal mitotic figures are rare in this variant. Myometrial invasion is mostly superficial. This variant has to be differentiated from serous carcinoma of the endometrium where the papillae are mostly arranged in tufts and nuclei exhibiting severe pleomorphism.

d) Ciliated carcinoma- This is a rare entity and is hardly present in malignant tumours. If 75% of the tumour has ciliated cells, then only it can be termed as ciliated cell carcinoma. The diagnosis of ciliated cell carcinoma has to be made with extra caution as the hyperplastic endometrium with complex proliferation may also exhibit cilia, which is a benign lesion. The only features supporting the diagnosis has to be presence of myometrial or lymphatic invasion.

e) Sertoliform differentiation- This variant of endometrioid carcinoma showing areas composed of glands which resemble sex cord-stromal tumours. These glands are arranged in closely packed tubules /trabeculae, and the cells have basally located nuclei and clear cytoplasm. The adjacent area (non sertoliform) is representing typical endometrioid carcinoma.

Genetic profile of endometrioid carcinoma shows most frequent alterations in PTEN (inactivation/mutation) comprising > 50% of carcinomas, mutations in PIK3CA (30%), ARID1A (40% of low grade tumours), KRas (20-26%), and TP53(30% of grade 3 endometrioid carcinoma).

Microsatellite instability is accounted in 35% of the tumours and mostly seen in the sporadic type of endometrioid carcinoma due to hypermethylation of the MLH1 gene promoter.

LYNCH SYNDROME

It is been reported, that about 5% of endometrial carcinoma patients are attributed to an inherited predisposition. Hereditary non polyposis colorectal cancer (HNPCC) now known as Lynch syndrome comprises majority of the inherited cases. Women, who are affected, have 40-60% and 10-12% predicted lifetime risk of developing endometrial carcinoma and ovarian carcinoma respectively. Lynch syndrome is an Autosomal dominant disease There is an incomplete penetrance due to germline transmission of DNA mismatch repair genes (MSH2, MLH1,MSH6 and PMS2).The median age of onset of Lynch associated endometrial carcinoma is younger than that of sporadic. Most common form of familial cases is associated with colonic carcinoma.

There are two primary guidelines used to identify the families who are at risk of developing carcinoma are :

(1) Amsterdam criteria and Modified Amsterdam 2

(2) Bethesda guideline (screening procedure to select patients who are eligible for MSI testing). [56]

AMSTERDAM CRITERIA
1) 3 or more relatives with an associated cancer like colorectal or endometrial cancer, cancer of small intestine, ureter or renal pelvis
2) 2 or more successive generations affected
3) 1or more relatives diagnosed before the age of 50 years
4) 1 should be a first degree relative of the other two
5) Familial adenomatous polyposis should be excluded in cases of colorectal carcinoma
6) Pathologist should verify the tumours accurately

Women have to undergo endometrial biopsies every year between the ages of 30-35 years or a decade before first diagnosis of endometrial cancer.

Serous adenocarcinoma/ type 2 endometrial adenocarcinoma

This is an aggressive form of endometrial carcinoma characterized by complex papillary and/or glandular architecture with diffuse and marked nuclear pleomorphism. Patients mostly belong to post-menopausal age group, present with postmenopausal bleeding. Most often they are multiparous and obese .Tumour has a tendency to develop on an atrophic endometrium. Serous carcinoma causes an increase in CA125 like ovarian carcinoma . The uterus is generally small as the tumour most often arises from atrophic endometrium of old women .Otherwise rest of the features in gross, is almost similar to other endometrial carcinomas. Sometimes this tumour arises in an endometrial polyp.

Serous endometrial intraepithelial carcinoma (SEIC) either develop in atrophic or directly on a polyp. The lesion in SEIC is confined to the surface epithelium. Even though myometrial invasion is absent, there is always a chance for extra uterine metastasis due to shedding of the tumour cells. Hence intraperitoneal spread such as omental cake is often seen even though myometrial invasion is absent. They exhibit complex papillary architecture. The papillae are of various size and width ranging from short and stout to long and thin, with few of the papillae exhibiting branching and features of hyalinization. The papillae have a fibro vascular core and are lined by large atypical nuclei with prominent eosinophilic macronucleoli and scanty cytoplasm. The luminal surface appears scalloped or frayed. Numerous mitotic figures are seen. Myometrial invasion is evident by the presence of gaping glands and shows higher incidence of lower uterine segment involvement. 25% of the cases show psammoma

bodies. Most of the patients diagnosed with serous carcinoma show striking vascular invasion. [29]

80 to 90 % cases show T P53 mutation. There may be a germ line BRCA1/2 mutations present in association with this serous carcinoma. The patients have unfavourable prognosis, therefore comprehensive staging has to be done to determine the risk of recurrence of the tumour.

Mucinous carcinoma

It is a type of endometrial carcinoma composed of cells containing prominent intracytoplasmic mucin. More than 50% of the neoplasm must contain mucinous cells to call it as a mucinous carcinoma. It accounts for 1-9% of all endometrial carcinoma. Women diagnosed with this carcinoma present with vaginal bleeding similar to the clinical presentation of typical endometrioid carcinoma. These are mostly well differentiated and present as stage 1, therefore the prognosis is favourable. The most frequently occurring mutation in this tumour is the KRAS mutations (2). Treatment for this type of endometrial carcinoma is surgery.

Clear cell carcinoma

It is characterised by papillary and tubulocystic architecture. The cells are polygonal or exhibiting hobnail appearance, with clear or eosinophilic cytoplasm and at least focal high grade nuclear atypia. 2/3rd of this tumour contain densely eosinophilic extracellular globules / hyaline bodies. These tumours accounts for 3-6% of all endometrial carcinoma. The mean age of the women diagnosed with this carcinoma is 65years. Ki 67 labelling index is 25- 30% in clear cell carcinoma. Somatic mutations

in PTEN, P53 have been reported in 30 to 40 % of this carcinoma. Prognosis is poor with 5 year and 10 years disease free survival reported in this type of carcinoma are 43-68% and 39% respectively.[29]

Neuroendocrine tumours

They are a diverse group of tumours which share a morphological neuroendocrine phenotype. The incidence is rare and accounts for less than 1% of all endometrial carcinoma. Patients with this neoplasm present with postmenopausal bleeding. On gross, the tumour is bulky, exophytic, polpoidal masses with variable myometrial invasion. Prognosis of small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma is poor but a study reported that these tumours may have favourable prognosis if the lesion is confined to the endometrial polyp. [27]

Mixed carcinomas

Endometrial carcinomas composed of two or more histological types with one component should be serous carcinoma. . The prognosis is poor if 25% or more of the aggressive component of the tumour present in mixed carcinoma. [57]

Undifferentiated carcinoma

It is an endometrial carcinoma that lacks proper architecture. The neoplasm is composed of solid sheets of medium sized, monotonous epithelial cells which has no pattern. Overall prognosis is poor. Stroma is mostly absent / scant.

OTHER RARE FORMS

1) Signet ring type

2) Transitional type

3) Glassy cell type

4) Mucinous adenocarcinoma of intestinal type

5) Lymphoepithelioma like carcinoma

Prognostic factors in endometrial carcinoma:[23]

1.Histological type

Type 1/ Endometrioid type endometrial carcinoma and its variations have good prognosis whereas, non endometrioid type, clear cell, carcinosarcoma and undifferentiated have unfavourable prognosis. Pure squamous cell carcinoma of endometrium have poor prognosis .

2) Histological Grade

FIGO (International Federation of Gynaecology and obstetrics) is the most common grading system which is used to grade the tumour according to the degree of architectural and nuclear pleomorphism.

Nuclear grading of endometrial carcinoma

1) Round nuclei
2) Variable size and shape of the nuclei
3) Hyperchromatic nuclei
4) Coarse and clumped chromatin
5) Prominent nucleoli
The grade of the tumour increases with significant nuclear atypia.

3) Stage and depth of invasion

The tumour staging system was recommended by FIGO, which determines the outcome of the women with endometrial carcinoma.

FIGO CLASSIFICATION 1988

STAGE	GRADE	CHARACTERISTICS
I A	1,2,3	Tumour limited to the endometrium
I B	1,2,3	Tumour invasion to less than half of the myometrium
I C	1,2,3	Tumour invasion to more than half of the myometrium
2 A	1,2,3	Endocervical glandular involvement only
2 B	1,2,3	Cervical stromal invasion only
3 A	1,2,3	Tumour invades serosa or adenexa or positive peritoneal cytology
3 B	1,2,3	Vaginal metastasis, metastasis to pelvic and para aortic lymph nodes
3 C	1,2,3	Vaginal metastasis, metastasis to pelvic and para aortic lymph nodes
4 A	1,2,3	Involving the bladder mucosa
4 B	1,2,3	Distant metastasis including abdominal/inguinal lymph node

FIGO CLASSIFICATION 2009

Stage	Characteristics
I	Tumour confined to corpus uteri
I A	No or less than half of myometrial invasion
I B	Invasion equal to or more than half of the myometrium
2	Tumour invades the cervical stroma but does not extend beyond the uterus
3	Local/ Regional spread of tumour
3 A	Invades the serosa of corpus uteri/adenexa
3 B	Vaginal and parametrial involvement
3 C	Metastasis to pelvis/ para aortic lymph nodes
3 C1	Positive pelvic lymph nodes
3 C2	Positive para aortic lymph nodes with/without positive pelvic lymph node
4	Tumour invades bladder/distant metastasis
4 A	Tumour invasion of bladder/bowel mucosa
4 B	Distant metastasis includes inta abdominal metastasis/inguinal lymph node

Patient with more than 50% of myometrial invasion are at high risk for extra uterine metastasis and may require aggressive surgical staging.

4) Myometrial invasion

Previously, myometrial invasion was reported as proportion of the uterine wall invaded by the tumour and is expressed in thirds. Recent classification includes the feature wherein the maximum depth of invasion is measured in mm and further expressed in % of invasion.

Myometrial invasion independent of tumour grade is a crucial parameter for prognostic depiction. The frequency of nodal metastasis is related to the depth of invasion.

When grading was analyzed against myometrial invasion, it was seen that Grade 1 tumours invading inner one third do not show metastasis, whereas when invasion up to outer third causes pelvic node metastasis in 25% of the cases.[58]

Cervical involvement:

Cervical involvement can determine the risk of recurrence as well as the possibility of extra uterine metastasis of the tumour. Hence presence of cervical involvement is associated with increasing grade, depth of invasion, and recurrences, thereby determining the prognosis of the patient. [59]

Peritoneal cytology: Only 5-15% of patients have positive peritoneal cytology and therefore signifies the manifestation of extrauterine spread of the tumour and generally these patients are staged as 3A. Larger studies with multivariate analysis support the presence of malignant cells in peritoneal washing as indicative of poor prognosis.[60]

Vascular invasion and lymph node invasion:

Vascular invasion is most uncommon in type1 but there is increase in frequency when there is evident deep myometrial invasion, aggressive cell type, and decrease in histological differentiation. Therefore presence of positive aortic lymph nodes is the most important factor in predicting the prognosis of the patient . [61]

Atypical endometrial hyperplasia shows favourable prognosis as it is correlated with low grade tumours. Atrophic endometrium is always associated with high grade tumours therefore shows unfavourable prognosis.

Steroid receptors: The presence of steroid receptors, its quantity and their distribution have been correlated with histological differentiation, FIGO stage and survival outcome. Early stage tumours and well differentiated tumours are having association with positive hormone receptor status (i.e. ER and PR positive).[62]

Bcl2 is the marker of apoptosis. It inhibits programmed cell death. The expression varies with menstrual cycle. In endometrial carcinoma it has an antiapoptotic effect. Therefore overexpression of BCL2 will have poor prognosis.[63]

In a previous study conducted in our institute, Estrogen Receptor, Progesterone receptors were present in Grade 1 and Stage I tumours, BCL-2 expression was directly proportional to ER/PR expression. Her 2 neu receptors were seen in high grade and stage tumours.

Angiogenesis and vascular endothelial growth factor permits tumour growth. This neovascularization could result from many factors that are released from the tumour itself or from the surrounding matrix. The tumour vascularity is associated with the increasing stage, decreased differentiation, lymphovascular invasion and increase in micro vessel count. Hence micro vessel density has been proved to be one of the prognostic markers of high potential utility.

MATERIALS AND METHODS

All the specimens of endometrial hyperplasia without atypia lesions from patients admitted to KLE'S DR. PRABHAKAR KORE HOSPITAL received in the Department of Pathology from January 2019 to December 2020 were considered for our study.

For prospective cases data and H&E slides and unstained slides were obtained for immunohistochemistry.

For retrospective cases data as well as tissue blocks were retrieved from storage and slides were cut and H&E slides were made and immunohistochemistry was done.

Retrospective cases were taken from January 2019-May 2019 which were 20 in number.

Prospective cases were taken from June 2019- December 2020 which were 40 in number.

Inclusion criteria:

1. Endometrial hyperplasia without atypia cases

Exclusion criteria:

1. Frank malignancies
2. Infective conditions eg. Endometritis
3. EH with atypia

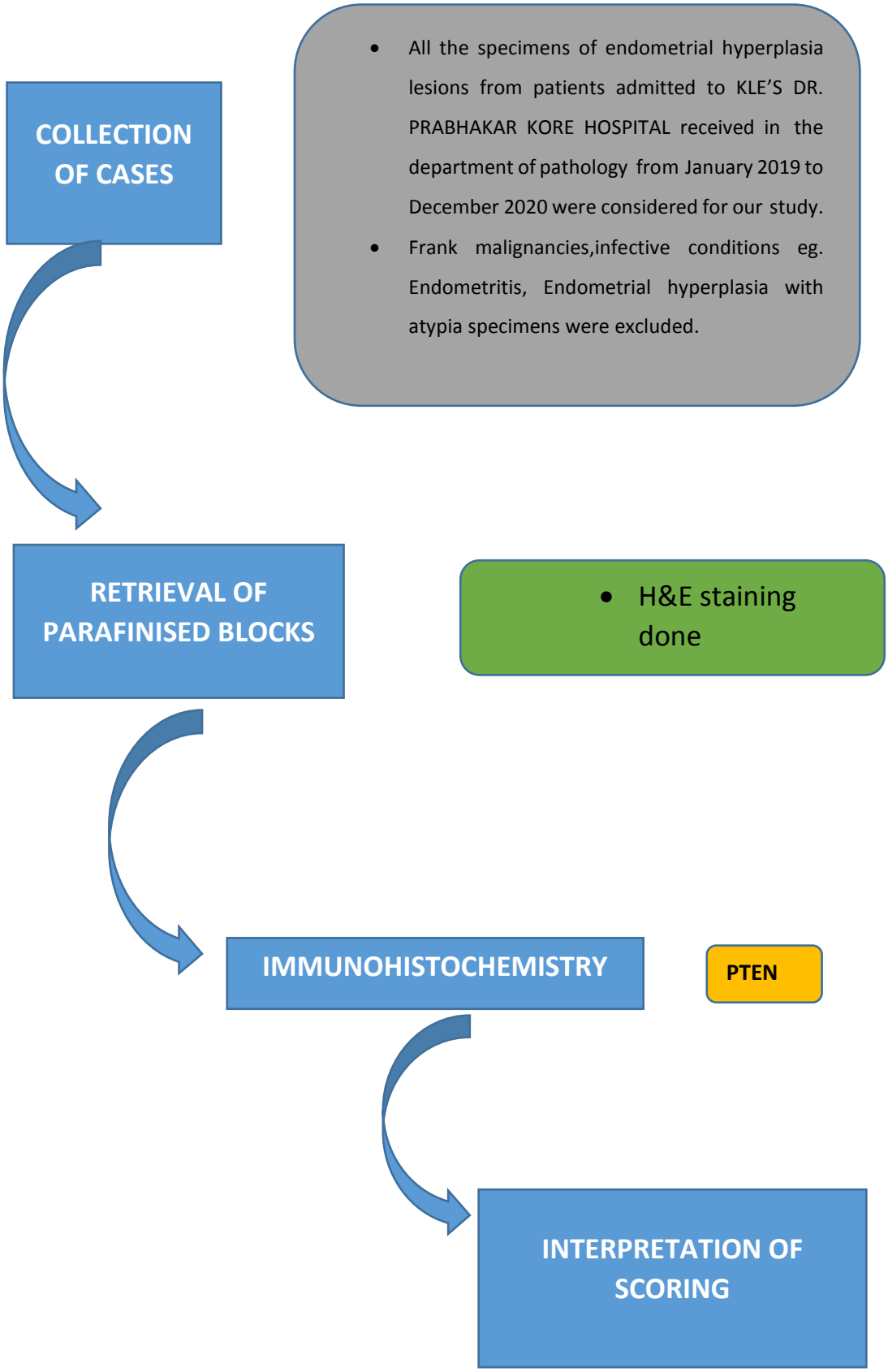
Sample size: 60

Sampling procedure: Universal Sampling

Number of specimens of the included lesions obtained at the Histopathology laboratory at JN Medical College and DR. PRABHAKAR KORE HOSPITAL, BELAGAVI in a year is around 30.

The clinical data required was collected from medical records after obtaining permission from the concerned authorities. The entire study was carried after getting the clearance from Institutional Ethics Committee.

PLAN OF STUDY



- The routine H&E staining was done using the regular protocol followed in the Department oF Pathology,JNMC ,KLE Hospital. (Annexure 1).
- The H&E stained slides of the cases were analysed .
- The paraffin blocks retrieved were then subjected to IHC marker PTEN.
- Immunohistochemistry procedure in (Annexure 2).

Immunohistochemistry for PTEN was interpreted based on Memorial Sloan Kettering Cancer Centre (MSKCC) .[49]

Interpretation	**Staining Pattern**
Positive	Strong positive staining in the entire tumor or cast majority of the tumor
Negative	No staining in entire/most tumour with strong positive staining of adjacent normal endometrium or stromal cells
Heterogenous	Tumour with convincingly positive staining and convincingly negative staining

RESULTS AND OBSERVATIONS

A total number of 60 patients having endometrial hyperplasia without atypia were considered in this study. The mean age of the patients was 43.77 ± 7.59 years. The age distribution of patients is shown in Table 1 and chart 1. It was noted that there was a high prevalence of cases between 31- 50 years of age, with the peak incidence in the 41 - 50 years age group.

Table 1: Distribution of study population according to age groups

SL no	Age Group	No of Subjects	Percentage
1	18 - 30 years	1	1.7 %
2	31 - 40 years	22	36.7 %
3	41 - 50 years	31	51.7 %
4	51 - 60 years	4	6.7 %
5	61 - 70 years	2	3.2 %
	Total	60	100.0 %

Graph 1: Distribution of study population according to age groups

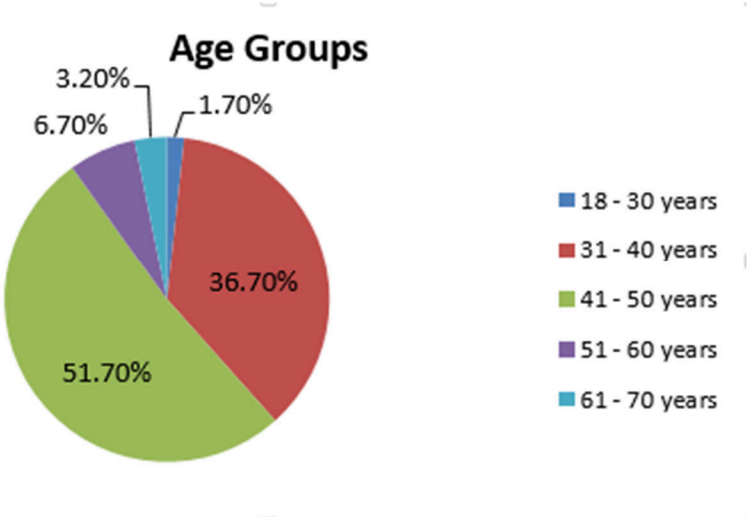
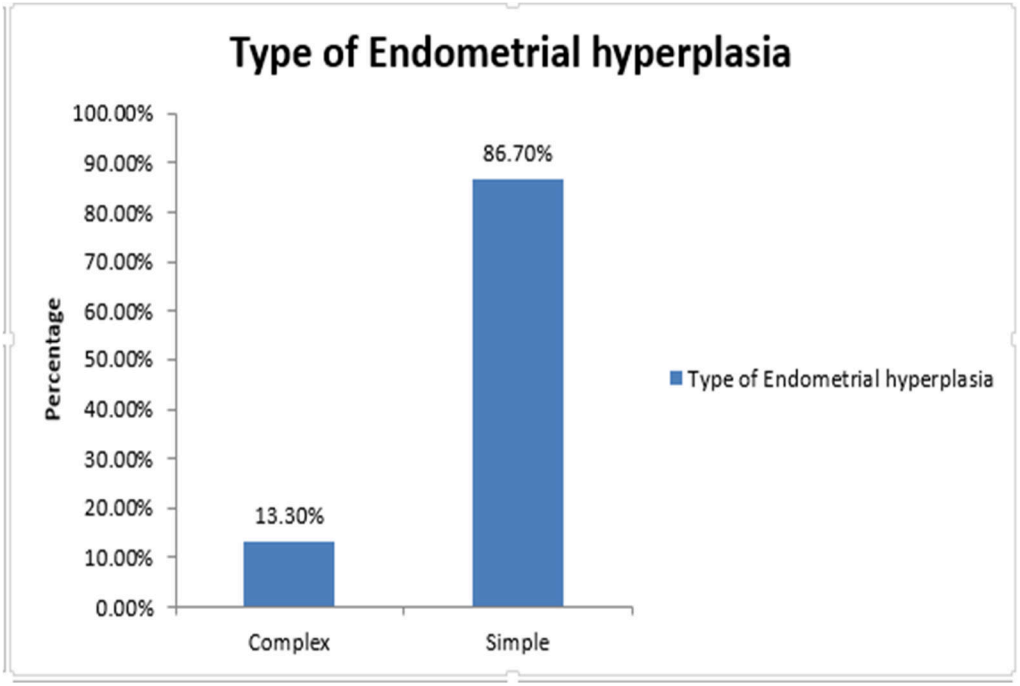


Table 2: Distribution of Study population based on the type of Endometrial Hyperplasia

SL no	Type of Endometrial Hyperplasia	No of Subjects	Percentage
1	Simple	52	86.7 %
2	Complex	8	13.3%
	Total	60	100.0 %

Graph 2 : Distribution of Study population based on the type of Endometrial Hyperplasia



The study population comprised of 8 subjects (13.30%) with complex type of Endometrial hyperplasia and 52 subjects (86.70%) with Simple type of Endometrial hyperplasia respectively as indicated in Table 2 and Graph 2.

Table 3: Distribution of Study population based on the Proportion score (Area of Staining) of Specimen

SL no	Proportion Score	No of Subjects	Percentage
1	> 50% area stained	41	68.30 %
2	10 – 50% area stained	17	28.40%
3	0% area stained	2	3.30 %
	Total	60	100.0 %

Graph 3: Distribution of Study population based on the Proportion score (Area of Staining) of Specimen

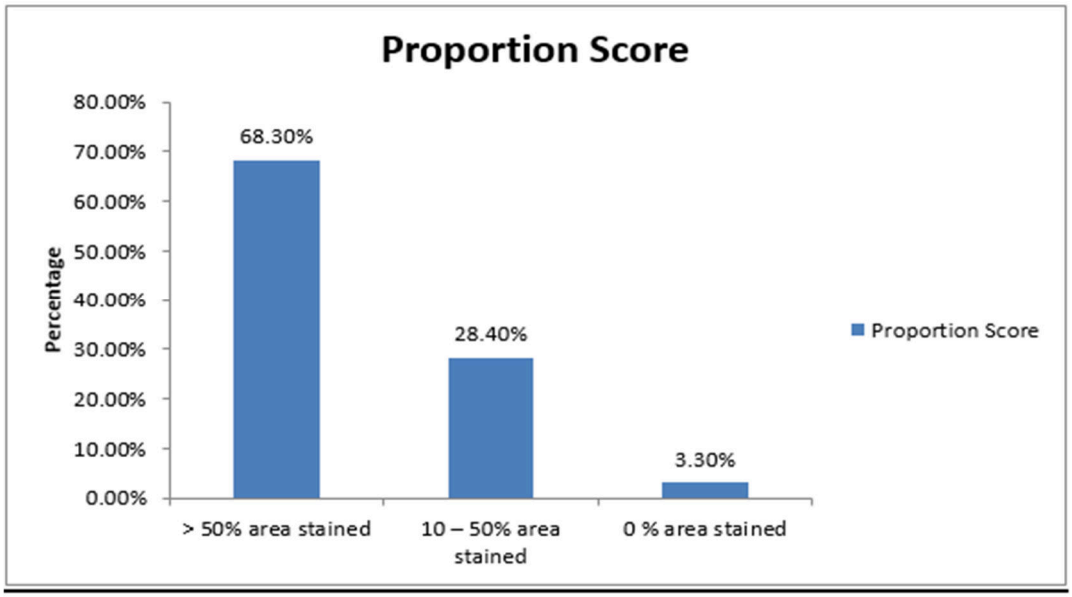


Table 3 and Graph 3 indicate the distribution of study population based on the proportion scores as determined by the area of staining in the specimen. It was noted that among 60 subjects, specimens obtained from 41 subjects (68.30%) exhibited >50% of the area being stained, 17 subjects (28.4%) exhibited 10 – 50% area being stained and 2 subjects showed none of the areas being stained respectively.

Table 4: Distribution of Study population based on the Colour intensity as exhibited from their specimens

SL no	Colour Intensity	No of Subjects	Percentage
1	Dark Brown (2+)	45	75.00%
2	Light Brown (2+)	13	21.7%
3	No Colour (0)	2	3.30%
	Total	60	100.0 %

Graph 4: Distribution of Study population based on the Colour intensity as exhibited from their specimens

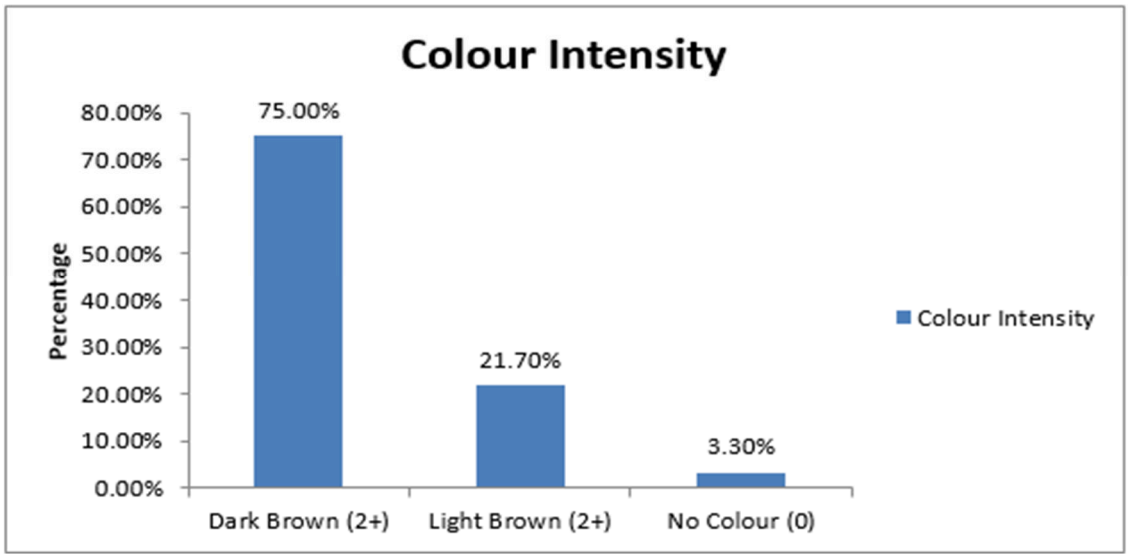


Table 4 and Graph 4 indicate the distribution of study population based on the colour intensities of staining in their specimen. It was noted that among 60 subjects, specimens obtained from; 45 subjects (75%) showed Dark Brown (2+) staining, 13 subjects (21.7%) showed Light brown (1+) staining and 2 subjects showed no staining respectively.

Table 5: Distribution of Study population based on the type of Endometrial Hyperplasia in association to the staining Colour intensity of their specimens

Serial Number	Type of Endometrial Hyperplasia (EH)	Colour intensity			Total	Chi square value	P value
		Dark brown(2+)	Light brown(1+)	NO COLOUR(0)			
1	Simple EH	41 (68.3%)		0	52 (86.7%)	13.817	0.001 (sig)
2	Complex EH	4 (6.7%)	2 (3.3%)	2 (3.3%)	8 (13.3%)		
	Total	45 (75.0%)	13 (21.7%)	2 (3.3%)	60 (100.0%)		

Graph 5: Distribution of Study population based on the type of Endometrial Hyperplasia in association to the staining Colour intensity of their specimens

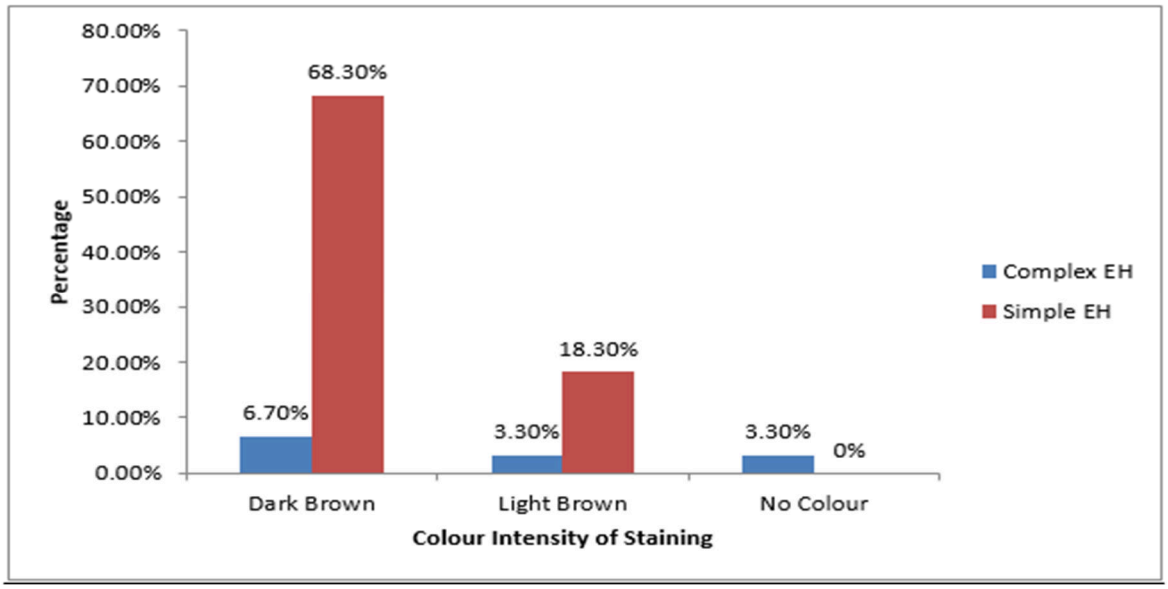


Table 5 and Graph 5 depict the distribution of Study population based on the type of Endometrial Hyperplasia in association to the Colour staining intensity of their specimens. It was noted that specimens of 45 (75.0%) subjects showed dark brown staining, 13 (21.7%) showed light brown staining and 2 (3.3%) showed no coour. Among the specimen which showed dark brown staining, 4 (6.7%) subjects had complex endometrial hyperplasia. and 41 (68..3%) had simple endometrial hyperplasia. Among the specimen

which showed light brown staining, 2 (3.3%) subjects had complex endometrial hyperplasia. and 11 (18.3%) had simple endometrial hyperplasia. Among the specimen which showed no colour in staining, 2 (3.3%) subjects had complex endometrial hyperplasia. and none of them had simple endometrial hyperplasia.. The associations between these two components were found to be highly statistically significant (p=0.001) as determined by chi-square test.

Table 6: Distribution of Study population based on the type of Endometrial Hyperplasia in association to the Proportion scores (Area of staining) of their specimens

Serial Number	Type of Endometrial Hyperplasia (EH)	Proportion score			Total	Chi square Value	P value
		>50% area stained	10-50% area stained	0% area stained			
1	Simple EH	36 (60.0%)	16 (26.7%)	0	52 (86.7%)	13.863	0.001 (sig)
2	Complex EH	5 (8.3%)	1 (1.7%)	2 (3.3%)	8 (13.3%)		
	Total	41 (68.3%)	17 (28.3%)	2 (3.3%)	60 (100.0%)		

Graph 6: Distribution of Study population based on the type of Endometrial Hyperplasia in association to the Proportion scores (Area of staining) of their specimens

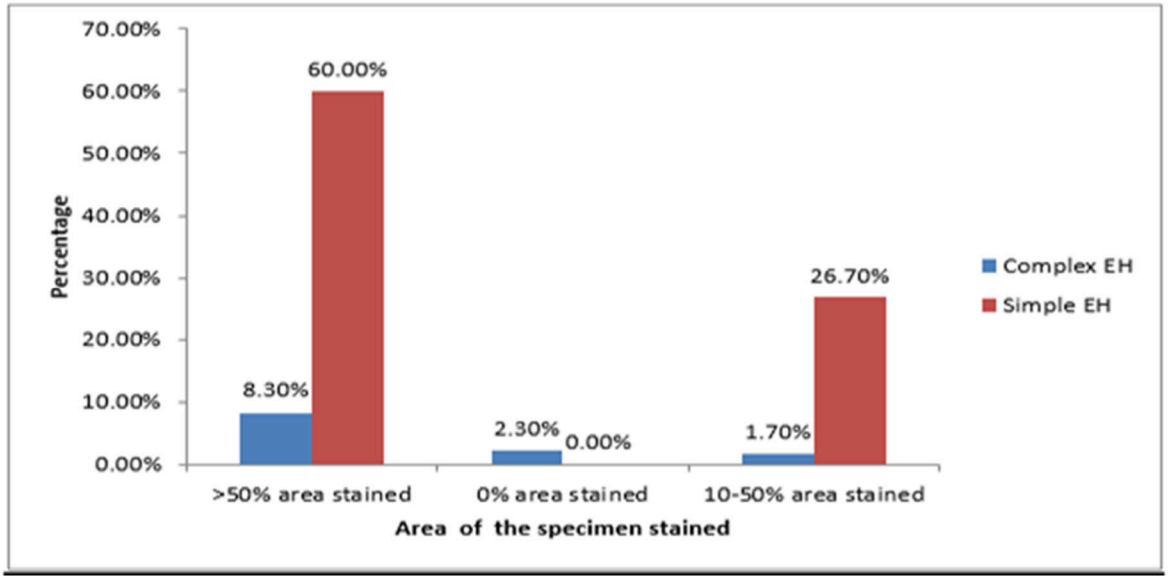


Table 6 and Graph 6 depict the distribution of Study population based on the type of Endometrial Hyperplasia in assciation to the Proportion scores (Area of staining). It was noted that specimens of 41 (68.30%) subjects showed >50% area being stained, 2 (3.3%) showed none of the area being stained and 17 (28.3%) showed 10-50% area being stained.

Among the specimen which showed >50% area staining, 5 (8.3%) subjects had complex endometrial hyperplasia. and 36 (60.0%) had simple endometrial hyperplasia. Among the specimen with no areas of staining, 2 (3.3%) subjects had complex endometrial hyperplasia and none had simple endometrial hyperplasia. Among the specimen which showed 10-50% area of staining, 1(1.7%) subject had complex endometrial hyperplasia. and 16 (26.7%) had simple endometrial hyperplasia. The associations between these two components were found to be highly statistically significant (p=0.001) as determined by chi-square test.

DISCUSSION

Endometrial hyperplasia is classified by the WHO (2014) into 2 groups:

1. Endometrial Hyperplasia with atypia
2. Endometrial Hyperplasia without atypia

Endometrial hyperplasia with atypia is involved in development of endometrial cancer (EC) of endometrioid type, which is most common malignancy in Western world. Since EH can become precancerous due to unopposed action of estrogens, its malignant potential is highly variable , with rates of progression of Endometrial carcinoma ranging from less than 1% to over 40%.

Previous WHO systems had also considered the complexity of glandular architecture for EH classification, although its impact on the malignant potential was not well defined .[4,5,6]

Only a minority of atypical EH progress to Endometrial carcinoma (8.2–27.5%), while a little percentage of EH without atypia still progress (1.2–4.6%)[7] .

EECA has a variety of genetic alternations including microsatellite instability (MI) and mutations of PTEN, PIK3CA, k-ras and â-catenin genes.

PTEN is the most frequently altered gene in EECA [(27, 34)] and several studies have found that PTEN inactivation is correlated with clonal growth patterns detected in endometrial hyperplasia with atypia and carcinoma.[(35)]

PTEN-null glands (i.e. Loss of PTEN expression) are shown in a diffuse pattern in Endometrial carcinoma but may also be detected in morphologically normal endometrial tissue, in atypical and non-atypical hyperplasias. These findings suggest that PTEN alternation occurs in the earliest phase of endometrial carcinogenesis [(21,22)]. Accurate

diagnosis of premalignant lesions in routine endometrial biopsies has a great clinical value in patient management.

Evaluation of PTEN loss by immunohistochemistry is highly reproducible. This has led to the suggestion that PTEN immunostaining may be an effective tool for screening of malignant and pre malignant endometrial lesions .[16,17,29,30]

In present study , 60 patients diagnosed with Endometrial hyperplasia without atypia over a period of one year were considered. The age of these patients ranged between 18 to 70 years. The mean age of these patients was 43.77 ± 7.59 years. Majority patients were in between age group of 41-60 years. One patient was of 28 years.

There results are in agreement with those obtained by Samar A. et al. [64], who stated that PTEN positive cases with strong intensity was significantly higher in normal proliferative endometrium and SH than in CAH.

Also in agreement with the study done by Kapucuoglu et al.[64], who stated that PTEN expression was significantly higher in cyclical endometrium and non-atypical hyperplasia than in atypical hyperplasia but there were no differences between SH and cyclical endometrium.

Also in agreement with the study by Tantbirojn et al. found a significant statistical difference of PTEN immunoreactivity among proliferative endometrium and EH on one side and atypical hyperplasia group. They reported that complete loss of PTEN expression was most commonly found in hyperplasia with cytological atypia and Endometrial carcinoma. In their study , they found they found complete absence of PTEN expression in 60% of CAH.

As regard the intensity, similar to our study , the majority of EH without atyia group , revealed moderate to strong PTEN expression.

Sarmadi et al, performed a study to evaluate te expression pattern of PTEN gene in normal and hyperplastic endometrium and neoplastic endometrium. PTEN immunoreactivity was present in all normal proliferative endometrium, all SH and 75% of CAH and the intensity of PTEN reaction were significantly higher in group with proliferative endometrium that hyperplastic endometrium.

Also the present results are in agreement with those obtained by Feng et al. they reported that the presence of PTEN protein was significantly decreased, as lesions progressed from normal endometrium to atypical hyperplasia. (64)

PTEN is a tumour suppressor gene which represents the most important genomic predisposing factor in the development of type1 endometrioid carcinoma. Inactivation of PTEN is the earliest event in carcinogenesis and the most frequent abnormality in type1 carcinoma. PTEN mutation and loss of heterogeneity (LOH) have been associated with advance stage and more aggressive behaviour.

Risinger et al have postulated that PTEN acts to inhibit invasion and metastasis through modulation of cytoskeleton. In addition, presence of phosphatase domain in PTEN suggests that PTEN opposes the activity of Tyrosine kinase oncogene product. Hence the targets for molecular therapy for endometrial carcinomas includes agents that inhibit AKT-PI3KPTEN pathway. (65,66)

Soheilia et al, documented that 100% of normal proliferative and 100% of endometrial carcinoma with simple hyperplasia exhibited PTEN immunoreactivity, whereas the immunoreactivity in atypical complex hyperplasias and early endometrial carcinomas, were75% and 45% respectively. (28)

The PTEN expression in normal proliferative endometrium is intracytoplasmic, and /or in the nuclei of glandular epithelial cells. In our study, the adjacent endometrium showed intense cytoplasmic/ nuclear positivity in the glandular epithelial cells.

Garg et al proposed a three tiered scoring system for PTEN immunostaining, which yielded significantly higher reproducibility rates. [49]

Leslie et al elaborated on the functions of PTEN and explained how normal cells control it and tumour cells lose it. [46]

PI3KCA are widely expressed lipid kinases and there is an inverse correlation with the loss of PTEN expression and AKT activation. Simultaneous mutation in PI3KCA and PTEN are thought to be mutually exclusive. Sahoo et al stated that 36% of endometrial carcinoma had PI3K/AKT mutations and PTEN double mutations.[67]

Kanamori et al, correlated loss of PTEN expression and AKT phosphorylation in endometrial carcinoma. They found that PTEN negative staining is seen in 37% of endometrial carcinoma and mixed pattern is seen in 16%of cases. AKT is significantly phosphorylated in tumour tissue with the loss of PTEN expression. This finding supported the evidence that AKT activation accompanied by PTEN inactivation is a key step in the development and progression of endometrial carcinoma. [68]

Mutter et al discovered that altered PTEN expression is a diagnostic marker for the endometrial pre cancers [69]. Hence, the immunostaining of PTEN may be informative in identifying premalignant lesions that are likely to progress to invasive carcinomas.

Hence, this study also shows that PTEN immunoreactivity was noted in 52 cases of Simple hyperplasia and 08 of Complex hyperplasia.

As regard the intensity of staining , 41 of the SH groups showed intense PTEN staining and 11 showed light staining. Among the PTEN-positive cases of CH, 04 cases showed strong staining and 02 showed light staining and 02 cases showed no staining depicting PTEN loss. Therefore, PTEN intensity was significantly higher in Simple Hyperplasia than in Complex Hyperplasia.

As regard to the Proportion scores (Area of staining) , 36 cases of SH showed >50% area stained, 16 cases with 10-50% of stained whereas in cases with Complex hyperplasia , 5 cases showed >50 % of stained area) , 01 case with 10-50% of stained area and 02 cases with no staining showing PTEN loss.

Hence, according to proportion score also , in Simple hyperplasia, more number of cases were in >50 % of area stained in comparison to complex hyperplasia cases.

SUMMARY AND CONCLUSION

- A total of 60 cases of Endometrial hyperplasia without atypia were considered in our study. It was noted that there was a high prevalence of cases between 41- 60 years of age, with the peak incidence in the **41 - 50 years** age group. The mean age of patients included in our study was 43.77 ± 7.59 years .
- According to **Intensity of staining**, 52 cases (86.7%) with Simple endometrial hyperplasia, **41 cases** (66.7%) showed strong positive staining **(2+)**, **11 cases** (18.3%) showed weak positive staining **(1+)**and none of the case showed negative staining.
- Among 8 cases (13.30%) with Complex endometrial hyperplasia, **04 cases** (8.3%) showed strong positive staining **(2+)**, **02** cases (1.7%) showed weak positive staining **(1+)**, and **02 cases** (3.3%)(0) showed **negative** intensities of staining of the specimens respectively.
- And according to **Proportion score**, In SH , **36 cases** showed >50% of area stained **(2+)** , **16 cases** with 10-50 % of area stained **(1+)** and in Complex hyperplasia cases, **5 cases** with 50 % area stained **(2+)** , **02 cases** with 10-50% are stained **(1+)** and **02** cases with **negative** staining (0).
- The association between these 2 components were found to be **highly statistically significant** (p=0.001) as determined by chi-square test.
- This validates the hypothesis that PTEN immunostaining may be an effective tool for screening of malignant and pre malignant endometrial lesions and inactivation of PTEN helps in predicting the response to targeted therapies against carcinogenic pathways .

LIMITATIONS OF THE STUDY

1. Less sample size
2. Follow up studies required for cases whether they progress into endometrial carcinoma or not.

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ANNEXURE I

INFORMED CONSENT

UTILITY OF PTEN MARKER IN ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA-ONE YEAR RETROSPECTIVE AND PROSPECTIVE OBSERVATIONAL STUDY

Purpose of the study: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo endometrial curettage, biopsy or hysterectomy for a endometrial hyperplasia you will be included in this study.

The purpose of this study is to determine the loss of PTEN as a prognostic marker.

Procedure: During this study , you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge . The principal investigator of the study is Dr. Anjali Lakhani under the guidance of Dr. Ganga S Pilli(guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better way to assess risk of endometrial cancers which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KLE University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator:
Guide :

Name of the participant:
(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator: (signature)

Date:
Address:

Phone no:

ANNEXURE II

ETHICAL CLEARANCE LETTER

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 417 Date: 14/09/2020

To,

REG No. BN0119001
PG student in Pathology,
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"TO STUDY THE UTILITY OF PTEN MARKER IN ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA – HOSPITAL BASED ONE YEAR OBSERVATIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

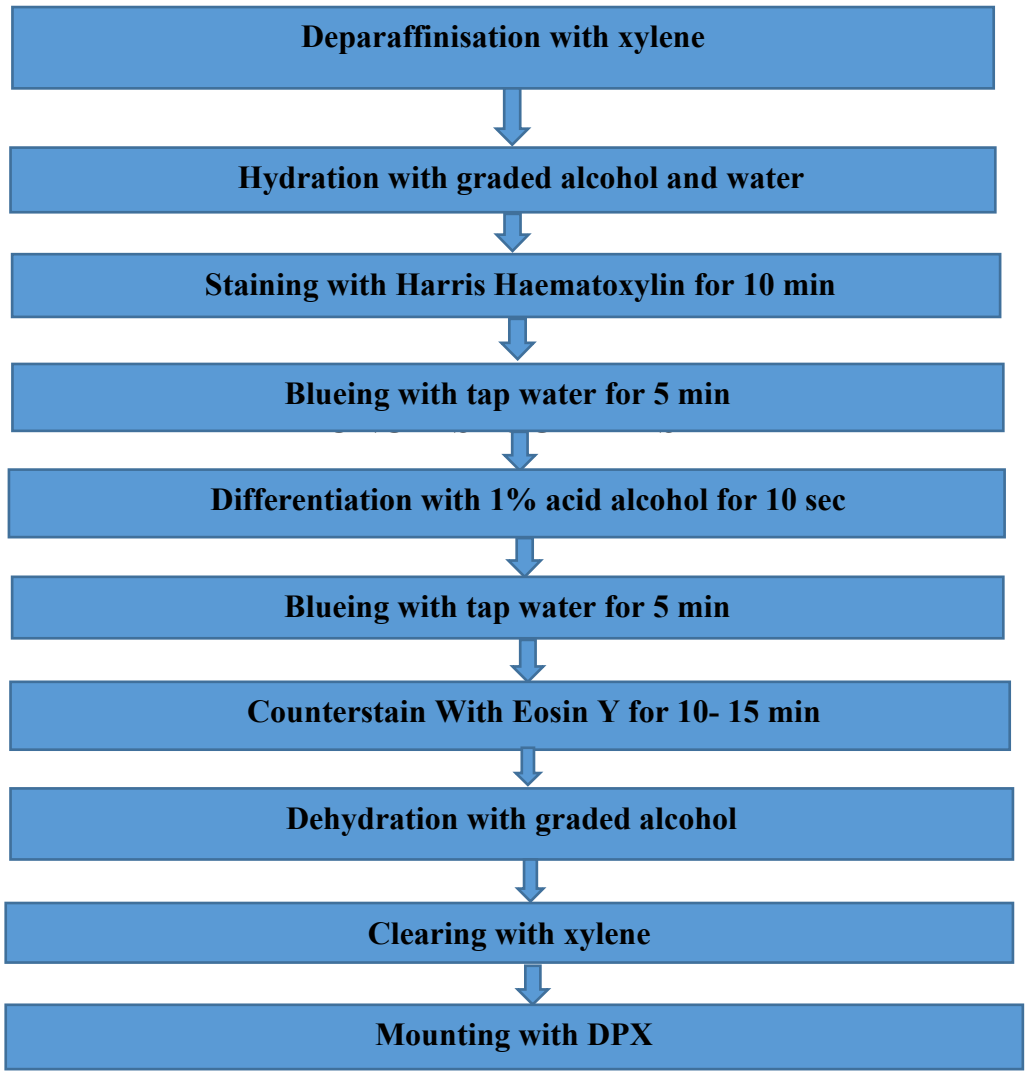
ANNEXURE III

HAEMATOXYLIN AND EOSIN STAINING

Reagents required are

- Harris haematoxylin ,Eosin, 1 % Acid alcohol, Graded concentration of iso-propyl alcohol, Xylene, DPX

Procedure:



ANNEXURE IV

IMMUNOHISTOCHEMISTRY STAINING PROTOCOL

PRINCIPLE- In this technique , an enzyme labelled antibody is used to link a cellular antigen specifically to a chromogen that can be more readily visualised under light microscope.

PROCEDURE- For the immunohistochemical technique we used routinely processed paraffin embedded tissue blocks. From each block we obtained histological sections of 3 micrometers of width.

Reagents required :

1.Antigen Retrieval: Tris Buffer-1.21 gms, EDTA-0.37 gms, Distilled water-1000ml, pH-8.5 to 9.0.

2. Wash buffer: Tris buffer- 8.6 gms, Nacl- 9.6 gms, pH- 7.4 to 7.6 (Adjust pH with concentrated HCL addind drop by drop) , Distilled water-1000ml.

3. 3% Hydrogen Peroxide (H_2O_2): H_2O_2-1 ml, Distilled water-100 ml.

4.DAB Solution: Substrate solution- 1 ml, Chromogen- 20 microlitre.

5. Coated slides: 1. Rinse the slides in water- 3 to 4 hours (Slowly in continuous tap water).

2.Rinse in distilled water.

3. Dry the slides and immerse in Poly L Lysine solution (10 ml distilled water+1 ml Poly L Lysine solution) for 2 to 3 hrs .

4.Dry the slides and a gain immerse in Poly L Lysine solution for 10 minutes. Dry the slides. Ready to use the slides.

METHODOLOGY

1. Cut the tissue sections on a microtome with thickness of 3 microns and collect them on coated slides.
2. Bake the sections at 37 deg C for overnight.
3. Before test bake it at 60 deg C for 1 hour.

Deparaffinize step

4. Xylene I – 10 minutes.

5. Xylene II-10 minutes.

6. Absolute alcohol I- 10 minutes

7. Absolute alcohol II- 10 minutes

8. Rinse in water- 5 minutes.

9. Rinse in distilled water- 1 minutes.

10. **Antigen retrieval – (Tris Buffer+EDTA)-** Buffer solution

11. Prepare the required amount of buffer and cook the slides in pressure cooker for 3 whistles.

12. Allow it to cool to room temperature for 15 minutes.

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

14. Apply 3% Hydrogen Peroxide – 8 to 10 minutes.

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

16. Primary Antibody incubate for 45 to 60 minutes . In closed chamber at room temperature. PTEN antibody used was rabbit monoclonal isotype IgG, Clone RM265 obtained from Bio SB.

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

18. Apply polymer HRP for 25 to 30 minutes in closed chamber at room temperature .

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

20. Apply DAB substrate for 10 minutes.

21. Wash with water- 2 minutes.

22. Wash with distilled water – 1 minute.

23. Counterstain with Haematoxylin – 3 minutes.

24. Blueing in warm water- 1 minute.

25. Clear in xylene and mount with DPX.

ANNEXURE V

PHOTOMICROGRAPHS

Fig.1. SIMPLE HYPERPLASIA WITHOUT ATYPIA (H&E) (10X)

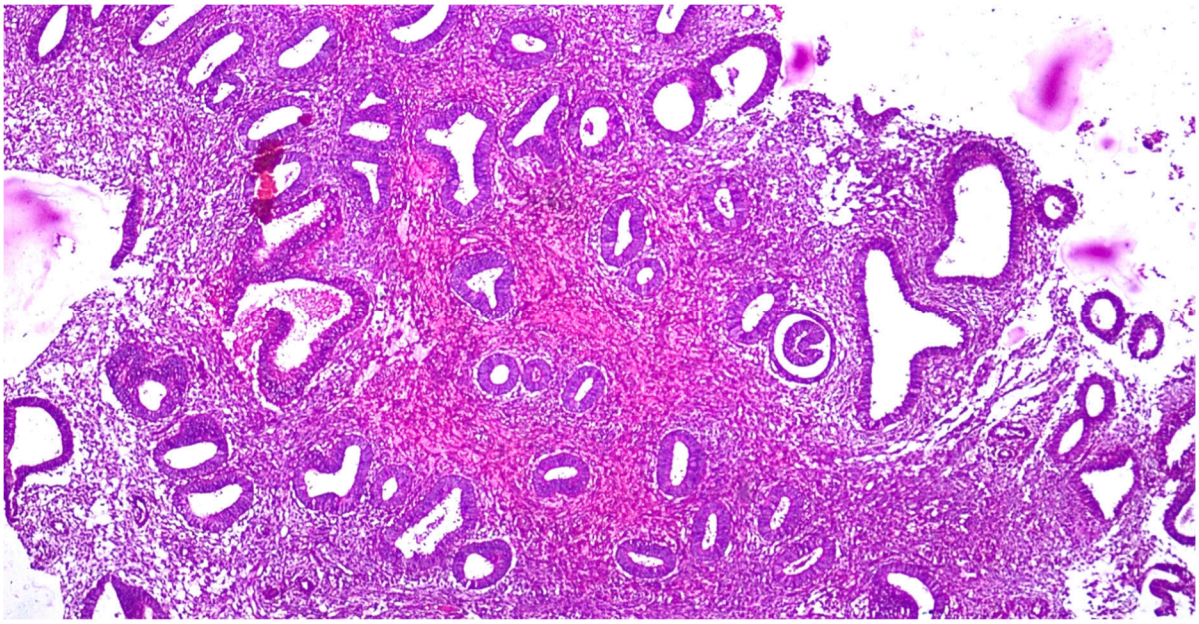


Fig.2. COMPLEX HYPERPLASIA WITHOUT ATYPIA (H&E)(10X)

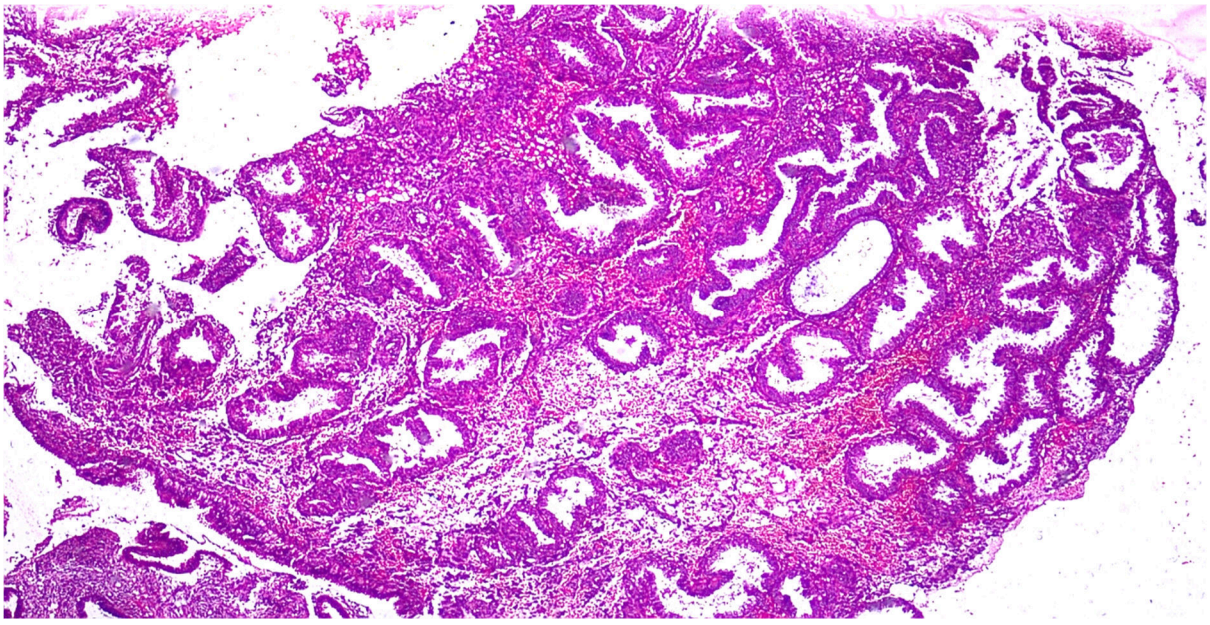
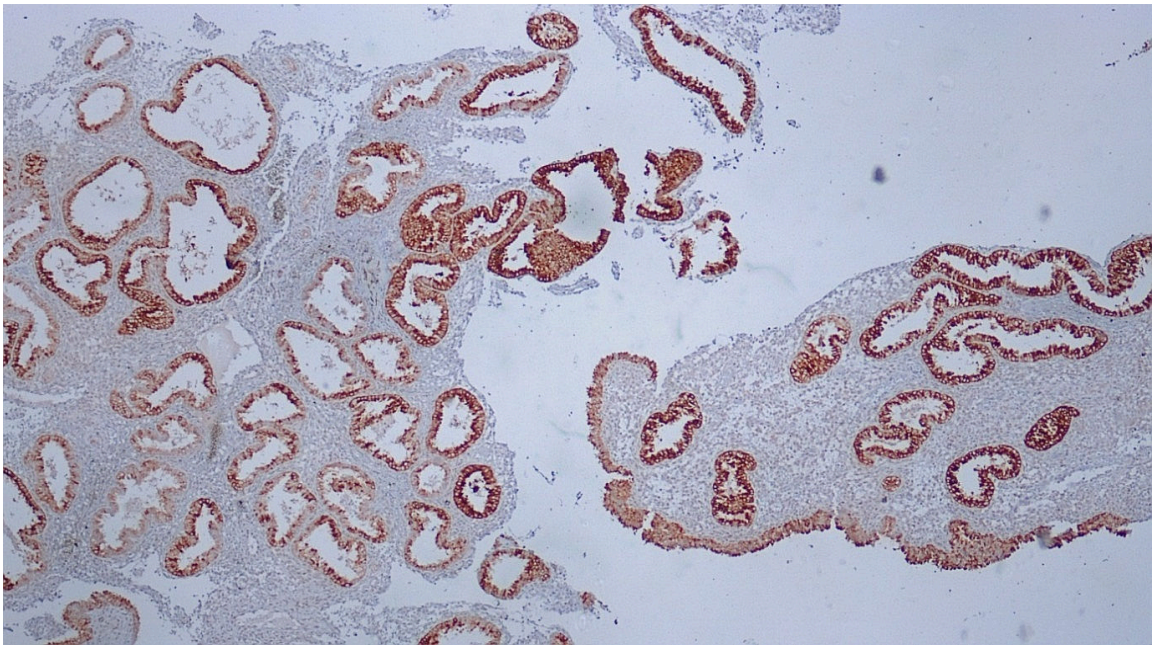
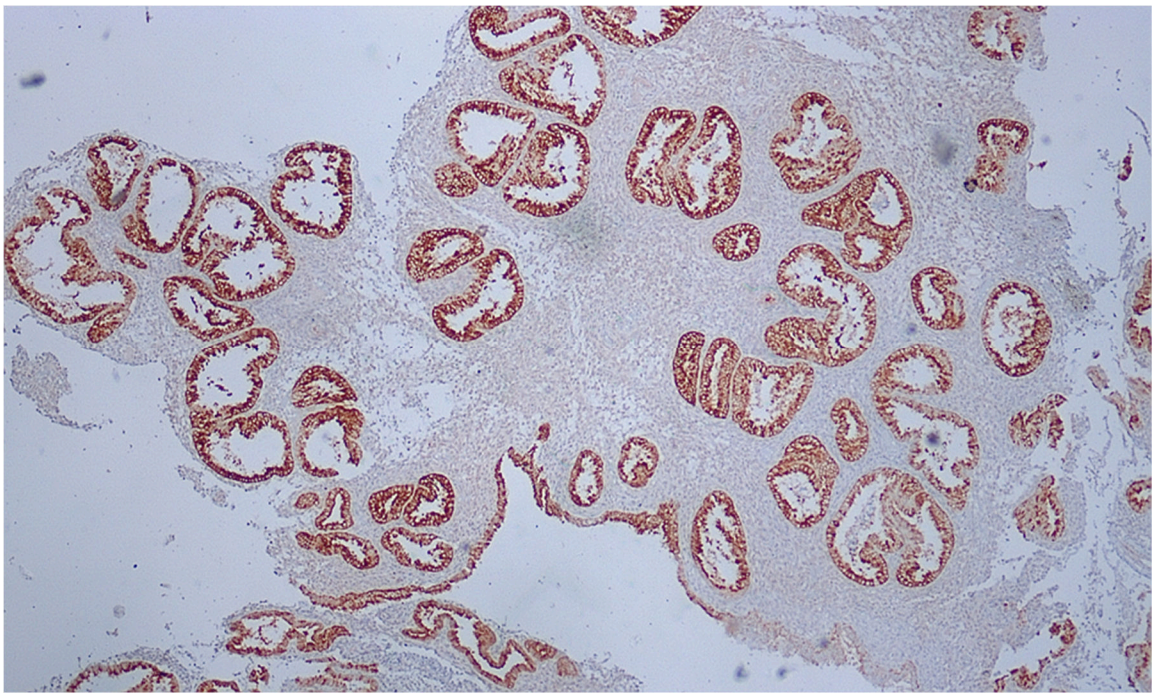


Fig.3 IHC-PTEN -Strong positive staining (2+) with > 50 % area stained (2+)





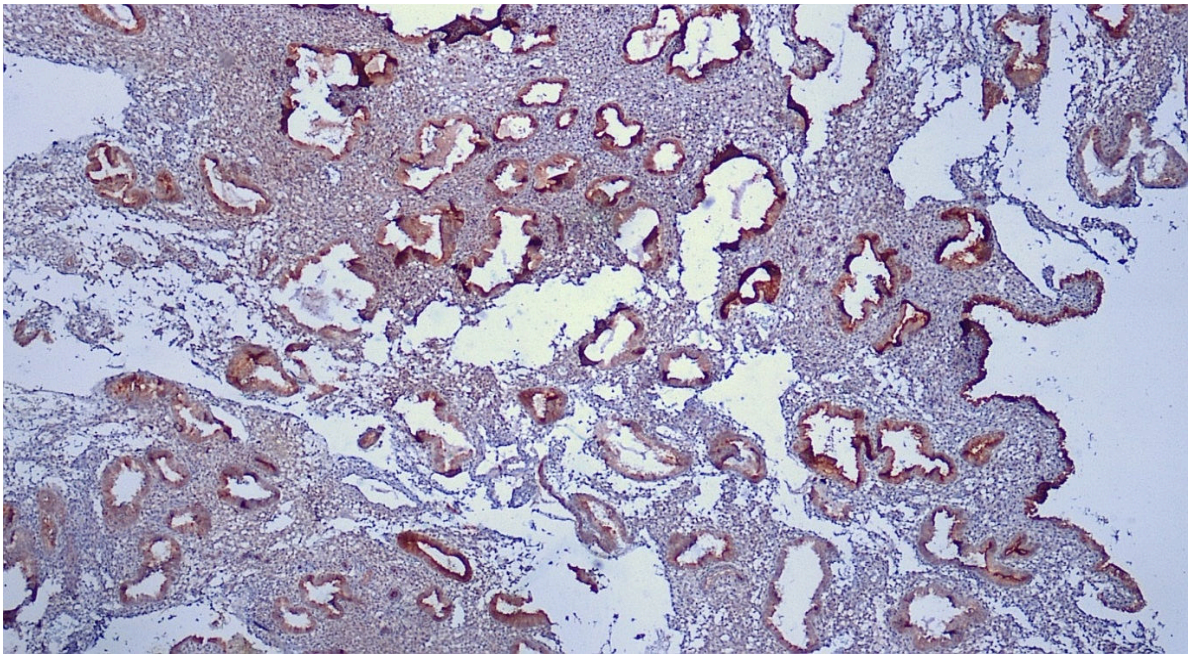


Fig.4.IHC-PTEN-Strong positive staining (Dark brown)(2+) with area covered 10-50% (1+)

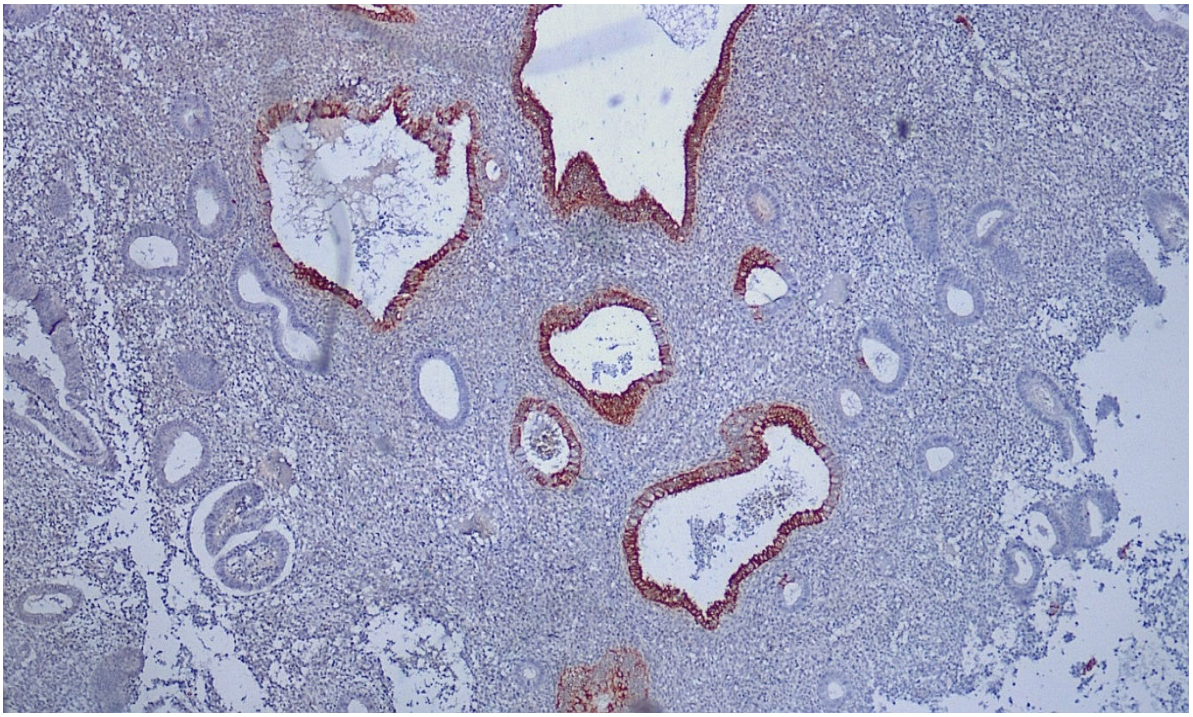


Fig.5. IHC- PTEN- Weak positive staining (1+) with area occupied >50% (2+)

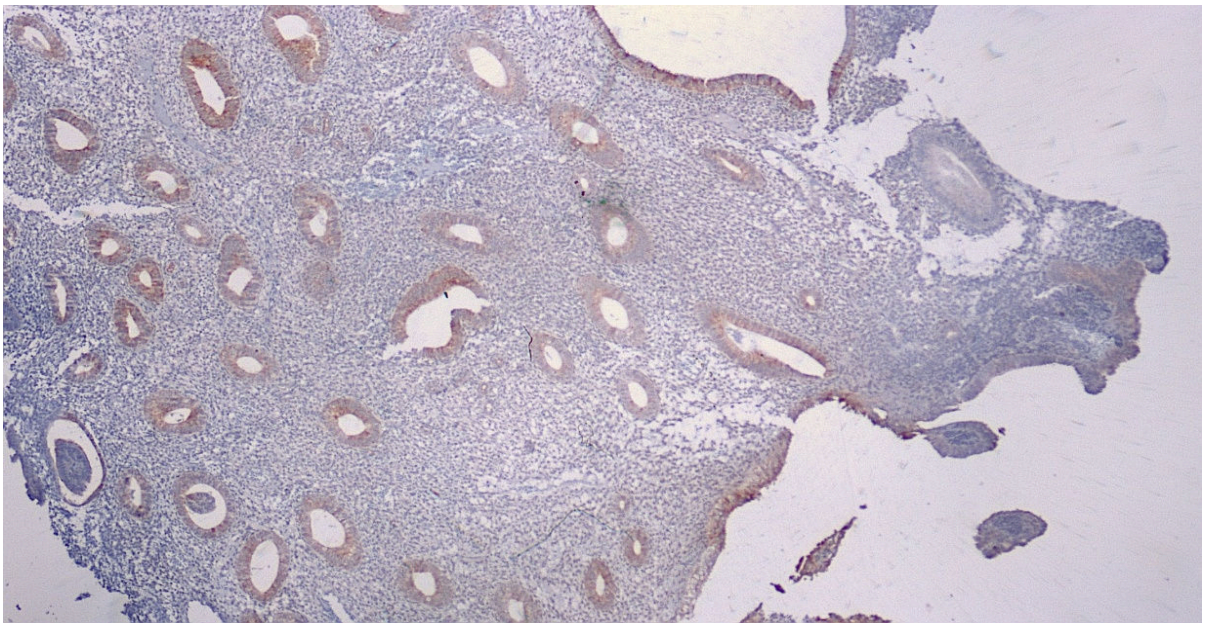
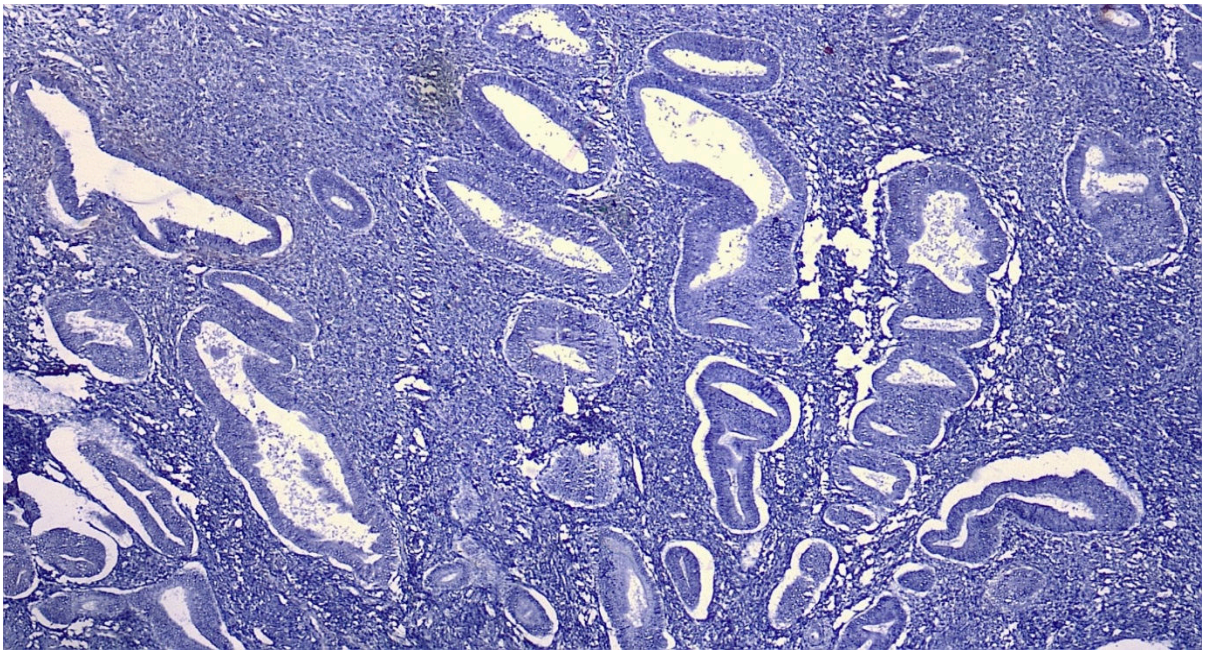
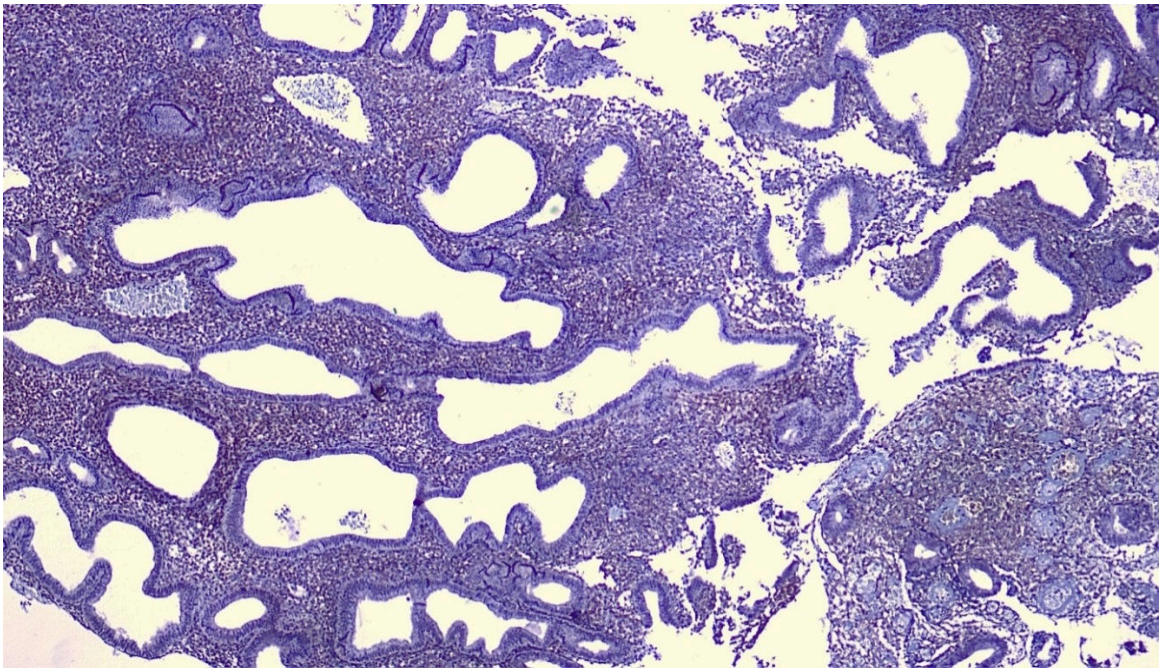


Fig. 6. IHC – PTEN negative staining seen in Complex Hyperplasia without atypia, showing loss of PTEN





ANNEXURE VI

KEY TO MASTER CHART

EH	-	Endometrial Hyperplasia
EC	-	Endometrial Curettage
D&C	-	Dilatation And Curettage
TAH&BSO	-	Total Abdominal Hysterectomy with Bilateral Salpingo-oophorectomy
AUB	-	Abnormal Uterine Bleeding
PMB	-	Post Menopausal Bleeding
PID	-	Pelvic Inflammatory Disease
DUB	-	Dysfunctional Uterine Bleeding
PV	-	Per Vaginal

ANNEXURE VII
MASTER CHART

S.no.	Name	Age	IP No.	Nature of Specimen	Clinical Diagnosis	Impression	Colour intensity	Proportion score
1	Yamannava Mahadev	37	958707	Endometrial tissue	Primary infertility	Simple EH	Dark brown(2+)	>50%(2+)
2	Seeta	35	1003163	EC	AUB	Simple EH	Dark brown(2+)	>50% (2+)
3	Shruthi	45	997678	EC	AUB with hypothyroidism	Simple EH	Dark brown(2+)	>50%(2+)
4	Jyothi	46	994996	EC	Metropathica haemmorhagica	Simple EH	Dark brown(2+)	>50%(2+)
5	Suman	60	3029355	EC	PMB	Simple EH	Dark brown(2+)	>50%(2+)
6	Saraswati Yallappa	48	992577	EC	AUB with mild anemia	Simple EH	Dark brown(2+)	10-50%(1+)
7	Sushma	45	3033304	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
8	Anasuya Piyar	40	3033575	D&C	PV bleeding	Simple EH	Dark brown(2+)	>50%(2+)
9	Geetha Rajendra	40	1007675	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
10	Pragati Patil	45	3031248	D&C	AUB	Simple EH	Dark brown(2+)	10-50%(1+)
11	Masabi nadaf	65	3033280	Endometrial biopsy	PMB	Simple EH	Light brown(1+)	10-50%(1+)
12	Shashi kala karki	47	1006384	TAH with BSO	AUB with PID	Simple EH	Dark brown(2+)	>50% (2+)
13	Urmilla Mahadevv Pote	45	1012498	Endometrial tissue	Fibroid uterus	Simple EH	Dark brown(2+)	>50%(2+)
14	Venkatamma Gudipathi	48	1012509	Endometrial tissue	Multiple fibroid	Simple EH	Dark brown(2+)	>50%(2+)
15	Bharathi Navadda	49	1014651	EC	AUB	Simple EH	Light brown(1+)	10-50%(1+)
16	Jiragi Shailla Dutta	49	3036517	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
17	Mallavva Pawadi	39	958863	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
18	Lakshmi Rajmane	44	966574	EC	AUB with adenomyosis	Simple EH	Dark brown(2+)	10-50%(1+)

19	Nagaratna Shrikant	45	968812	Endometrial tissue	AUB	Simple EH	Dark brown(2+)	>50%(2+)
20	Renuka kedari	45	1028118	TAH with BSO	AUB with cystocoele	Simple EH	Light brown(1+)	10-50%(1+)
21	Gauravva Chikkamath	70	972238	EC	PMB with cystocoele	Simple EH	Dark brown(2+)	>50%(2+)
22	Renuka kadam	33	973405	Endometrial sample	EH with infected polyp	Simple EH	Dark brown(2+)	>50% (2+)
23	Gangavva Guggaj	50	920832	EC	Endometrial hyperplasia	Simple EH	Dark brown(2+)	>50% (2+)
24	Sumangava Kudachi	40	924409	Endometrial sample	Chronic cervicitis	Complex EH	NO COLOUR(0)	<10% (0)
25	Sumitra M	40	990407	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
26	Parvati Shivanand	37	932954	Endometrial tissue	AUB	Simple EH	Dark brown(2+)	>50%(2+)
27	Asha	39	947565	EC	DUB	Simple EH	Dark brown(2+)	>50%(2+)
28	Prema Jagadole	50	998857	Endometrial sample	AUB	Simple EH	Dark brown(2+)	>50%(2+)
29	Sridevi Mulimani	52	3035733	Endometrial Biopsy	AUB with fibroid	Simple EH	Dark brown(2+)	10-50%(1+)
30	Nagavva Mohan	40	981754	Endometrial Biopsy	AUB	Simple EH	Light brown(1+)	10-50%(1+)
31	Chayya S.	48	3021182	EC	DUB	Simple EH	Dark brown(2+)	>50%(2+)
32	Gayatri Acharya	32	969452	EC	AUB	Simple EH	Dark brown(2+)	>50%(2+)
33	Mahadevi Dundappa	44	1107211	Endometrial tissue	Excessive PM bleeding	Complex EH	Light brown(1+)	>50% (2+)
34	Madhura Mahesh	44	1006689	Endometrial tissue	Heavy menstrual bleeding	Simple EH	Dark brown(2+)	>50%(2+)
35	Kasture Mathihoppa	45	957129	TAH with BSO	AUB	Simple EH	Light brown(1+)	10-50% (1+)
36	Swetha	36	1205643	D&C	AUB	Simple EH	Dark brown(2+)	>50% (2+)

37	Padmavati	38	9328895	D&C	DUB	Complex EH	Dark brown(2+)	>50% (2+)
38	Leela	40	3021182	D&C	Endometrial hyperplasia	Simple EH	Dark brown(2+)	>50% (2+)
39	Sushma	45	990534	D&C	AUB	Complex EH	Light brown (1+)	10-50% (1+)
40	Mahadevi Marath	36	970054	EC	Fibroid uterus	Simple EH	Light brown(1+)	10-50% (1+)
41	Shruti Shivanand	49	1047455	TAH with BSO	Endometrial hyperplasia	Simple EH	Dark brown(2+)	>50% (2+)
42	Kasturi Ramappa	51	996754	EC	AUB	Simple EH	Light brown(1+)	10-50% (1+)
43	Soniya Shrimath	19	1049704	EC	DUB	Simple EH	Dark brown(2+)	>50% (2+)
44	Vijaya Ramesh	39	1049345	EC	AUB	Simple EH	Dark brown(2+)	>50% (2+)
45	Monnawwa	48	998856	EC	Endometrial hyperplasia	Simple EH	Dark brown(2+)	>50% (2+)
46	Deepali Dinkar	54	1048175	EC	Endometrial hyperplasia	Complex Hyperplasia	Dark brown(2+)	>50% (2+)
47	Seeta	40	995807	EC	AUB	Simple EH	Dark brown(2+)	>50% (2+)
48	Supriya Dipak Shahapur	43	993311	EC	Menometrorrhagia	Simple hyperplasia	Dark brown(2+)	>50% (2+)
49	Sushila	41	3024874	EC	AUB	Complex hyperplasia	Dark brown(2+)	>50% (2+)
50	Bharathi Patil	48	959652	Endometrial tissue	AUB	Simple Hyperplasia	Light brown(1+)	10-50% (1+)
51	Ramavva Mallappa	42	972898	EC	PMB	Simple Hyperplasia	Dark brown(2+)	>50% (2+)
52	Radha Salogonkar	36	3022743	Endometrial sample	DUB	Simple Hyperplasia	Light brown(1+)	10-50% (1+)
53	Bhagyashree Patil	39	937520	EC	AUB	Simple EH	Dark brown(2+)	10-50% (1+)
54	Shubada Manokar	44	3019690	TAH & BSO	Endometrial hyperplasia	Simple EH	Dark brown(2+)	>50% (2+)
55	Sharada Mahesh	40	923799	EC	AUB	Complex EH	NO COLOUR (0)	<10%(0)
56	Champavva	42	932895	Endometrial tissue	AUB	Simple EH	Light brown(1+)	10-50% (1+)
57	Anita Pramod	44	3035733	EC	PMB	Simple EH	Dark brown(2+)	>50% (2+)
58	Rashmi	45	968505	D&C	AUB	Simple EH	Light brown(1+)	10-50% (1+)
	Kavita	40	3033745	EC	DUB	Simple EH	Dark brown(2+)	>50% (2+)
60	Seema	46	1098456	D&C	AUB	Complex EH	Dark brown(2+)	>50% (2+)