# A study of histopathological spectrum of endometrial lesions in abnormal uterine bleeding with analysis of expression pattern of Estrogen receptor, Progesterone receptor and Proliferative index (Ki-67) in endometrial hyperplasias and carcinomas

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## Abstract

**Background:** Studies show that 9-30% of women in reproductive age group suffer from Abnormal Uterine Bleeding (AUB). Excessive menstrual bleeding has many side effects, including iron deficiency anemia, reduced quality of life & increased healthcare costs. Detection of ER and PR expression on tissues can be used as an adjunct to histopathologic examination, offering the advantage of tissue localization and helps in analyzing tissue distribution and severity of the underlying pathology. Expression of ER, PR & Ki-67 markers can be used as potential prognostic markers in Endometrial hyperplasia (EH) and Endometrial carcinomas (EC) using IHC because ER, PR expression decreases and Ki67 expression increases with the progression of severity of endometrial lesion from hyperplasia to carcinoma.

**Aims and objectives:** 1. To study the histopathological spectrum of endometrial lesions in AUB

2. To Study expression pattern of ER,PR and Ki-67 in EH and EC.

**Material and Methods:** It is a prospective study of 400 cases of women having AUB, who underwent dilatation & curettage, endometrial biopsy or hysterectomy. The specimens were received at Department of Pathology, Dr.B.R. Ambedkar medical college, Bangalore. Study duration was 18 months. Routine tissue processing and staining with H & E was done. Immunohistochemistry with ER, PR and Ki-67 was done on cases diagnosed as EC and EH.

**Results:** Maximum cases of AUB were seen in the age group 36-45 years. Endometrial hyperplasia without atypia (EHWOA) and Atypical endometrial hyperplasia (AEH) were seen in 36-45 years where as 56-60 years age group in carcinomas. Age of patient and risk factors like patient's menopausal status and hypertension showed statistically significant correlation with progression to carcinoma. Proliferative phase was most common HPE finding. All cases of EHWOA were ER, PR positive and showed low Ki- 67 index. Majority of AEH were positive for ER,PR and showed moderate Ki-67 index. 84.6% and 77% EC cases were ER, PR positive respectively. Ki-67 positivity was observed in majority of EC. ER, PR positivity reduced and Ki 67positivity increased with tumor progression from Grade 1 to 2.

**Conclusion:** Histopathological examination (HPE) remains the most important diagnostic tool in AUB. Incorporation of ER, PR and Ki-67 along with HPE in AUB, helps understanding its biological behavior, early diagnosis, planning individual treatment strategies and prognostication.

**Keywords:** Abnormal uterine bleeding; ER; PR; Ki-67; Endometrial carcinoma

## Introduction

Abnormal uterine bleeding (AUB) is defined as any bleeding outside of normal menstrual pattern with excessive duration, frequency and amount of loss. It is estimated that 9-30% of women of reproductive age suffer from abnormal uterine bleeding. The prevalence

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increases with age, peaking just prior to menopause. The term dysfunctional uterine bleeding is used to describe abnormal uterine bleeding for which no specific organic cause has been found[1,2]. Excessive menstrual bleeding has many side effects, including iron deficiency anemia, reduced quality of life, and increased healthcare costs because it is a major indication for referral to gynaecological outpatient clinics. About 25.30% of abdominal hysterectomies are done for abnormal uterine bleeding[3]. AUB accounts for 70% of the complaints among premenopausal women[1]. It is caused by a wide variety of organic or non-organic causes and endometrial carcinoma is one of them and its precursor-endometrial hyperplasia usually presents with AUB.

Endometrial carcinoma is the second most common gynecologic malignancy with an incidence of 5.9 per 100,000 women in the developing countries. In India, the incidence is 4.3 per 100,000 women[4]. An estimated 61,380 women were diagnosed with endometrial cancer in the year 2017 as per SEER data, forming 3.6% of all cancers. There have been about 10,920 deaths due to endometrial cancer, accounting for 1.8% of all cancer deaths. In the India, endometrial cancer lags behind cervical cancer but is slowly rising in incidence, especially amongst pre-menopausal women. The incidence of endometrial hyperplasia and atypical endometrial hyperplasia is estimated to be 355/100,000 woman-years and 56/100,000 woman-years. As hyperplasia precedes all cases of atypical hyperplasia and endometrial carcinoma, it highlights the potential advantages of identifying women at risk of developing endometrial carcinoma by possible analysis of histopathological features supplemented by a pattern of expression by molecular markers involved in the carcinogenesis[5].

Estrogen (ER) and progesterone (PR) receptors are present in endometrial stroma as well as endometrial glands. They belong to the nuclear steroid receptor super family. Detection of ER and PR on tissues can be used as an adjunct to histopathologic examination of AUB. They offer the advantage of tissue localisation and helps in analysing tissue distribution and intensity in stromal and glandular cells. Loss of ER and PR are noted in cases of endometrial carcinoma with decreased expression as lesion progressed from endometrial hyperplasia to endometrial carcinoma. Ki-67 is a proliferation marker which is an independent prognostic marker in endometrial carcinomas and its positivity is shown to increase as the severity of endometrial lesion progresses from endometrial hyperplasia to endometrial carcinoma[6].

This study has been undertaken with the aim to study the various histomorphological patterns of endometrium with AUB in patients of different age group and to evaluate the expression of ER,PR and Ki-67 markers as potential prognostic markers in Endometrial hyperplasia and Endometrial carcinoma using Immunohistochemistry[7,8].Hence, this study emphasizes the potential use of the hormone receptors ER,PR and the proliferative marker Ki-67 as markers of severity of endometrial lesion[9].

**Objectives/Aims:** 1. To study the histopathological spectrum of endometrial lesions in abnormal uterine bleeding. 2. To study expression pattern of ER, PR AND Ki-67 in endometrial hyperplasias and carcinomas.

## Materials & Methods

This prospective, observational study of 400 cases was done in the Department of Pathology from January 2021 to June 2022. Ethical clearance was taken from Instituitional ethics committee. The study included patients with clinical history of AUB. All patients diagnosed clinically as having abnormal uterine bleeding and who underwent, Dilatation and curettage, Endometrial biopsy, Hysterectomy were included. Autolyzed samples, Inadequate samples, Patients below the age of 18 yrs were excluded.

The received specimens were fixed in 10% formalin. After fixation, gross appearance of the specimen was noted. The endometrial curettage samples were processed entirely. Multiple bits from endometrium were processed from the hysterectomy specimens. The standard protocol for surgical grossing of resection specimens were followed. Detailed specimen description was noted and multiple sections were taken as per the standard protocol from the viable sections of the tumor including surgical margins for carcinomas. Specimens were processed in the conventional method and embedded in paraffin wax. Sections of 4-5µm thickness were cut using Leica RM 2125 and stained using haematoxylin and eosin for histopathological study. In addition, 4µm sections were cut from paraffin blocks of tissue and taken on 2 poly L lysine coated glass slides for further Immunohistochemical analysis to detect ER, PR and Ki67 expression. The H&E stained slides were studied for detailed microscopic features. The nuclear staining pattern with ER, PR and Ki67 were observed and noted as per standard protocol. Datas collected were entered in MS Excel spreadsheet 2017 and statistical analysis was done and Chi-square test (P-value at the significance level of <0.05) was calculated wherever required.

## Results

The age of patients ranged from 20 to 75 years with a mean age of 42 years. Maximum number of cases were seen in the age group of 36 to 45 years (54%)

[Table-1].The youngest patient was 20 years old and the oldest patient was 75 years old. The parity ranged from 0 to 7 in the present study. The incidence of AUB in our study was found to be highest in multiparous (82%) [Fig-1]and tubectomised women (280cases,72%). Proliferative phase endometrium was the most common histopathological finding in this study, which was seen in 164 patients(44%),followed by Secretory phase endometrium(89 patients, 22.25%) and Endometrial hyperplasia without atypia(50 patients,12.5%)[Table-2]. Amongst the 56 cases of hyperplasia there were 50 cases of Endometrial hyperplasia without atypia (EHWOA) and 6 cases of Atypical hyperplasia.

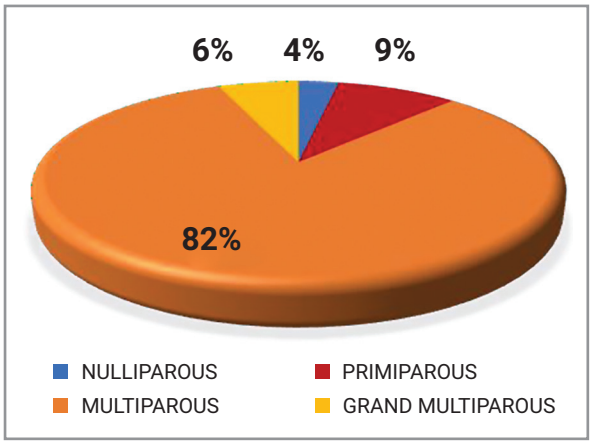


**Figure 1: Parity distribution in AUB**

**Table-1: Age distribution in AUB**

| AGE | NUMBER | PERCENTAGE |
|---|---|---|
| 20-35yrs | 70 | 17% |
| 36-45yrs | 216 | 54% |
| 46-55yrs | 99 | 25% |
| 56-65 yrs | 10 | 3% |
| 66-75yrs | 5 | 1% |
| TOTAL | 400 | 100% |

**Table-2: Analysis of various histopathological findings**

| Histopathological finding | Number | Percentage |
|---|---|---|
| Proliferative phase | 164 | 44% |
| Secretory phase | 89 | 22.25% |
| Disordered proliferative endometrium | 8 | 2% |
| Menstrual endometrium | 6 | 1.50% |
| Endometrial polyp | 16 | 4% |
| Atrophic endometrium | 6 | 1.50% |
| Lytic endometrium | 19 | 4.75% |
| Senile cystic atrophy | 12 | 3% |
| Chronic granulomatous inflammation | 1 | 0.25% |
| Pill endometrium | 4 | 1% |
| Mixed endometrium | 4 | 1% |
| Scar endometrium | 1 | 0.25% |
| Endometrial hyperplasia without atypia | 50 | 12.50% |
| Atypical hyperplasia | 6 | 1.50% |
| Non-specific endometritis | 1 | 0.25% |
| EndometrialCarcinoma | 13 | 3.25% |
| TOTAL | 400 | 100% |

Endometrial hyperplasia both without atypia and with atypia was most commonly seen in the age group of 36-45 years. Among 13 cases of endometrial carcinoma the most commonly seen age group was 56-65 years. So age of patient showed statistically significant correlation with endometrial carcinoma[Table-3].

**Table-3:Age distribution of patients with hyperplasias and endometrial carcinoma.**

(EHWOA-Endometrail hyperplasia without atypia, AEH-Atypical endometrial hyperplasia, EC-Endometrial hyperplasia)

*p-value<0.05 was considered statistically significant

| Age range | EHWOA | AEH | EC | |
|---|---|---|---|---|
| 25-35 yrs | 8(14.8%) | 0 | 0 | |
| 36-45 yrs | 24 (53.1%) | 5(83.3%) | 4(30.7%) | **P-value =0.0001** |
| 46-55 yrs | 16 (32.1%) | 1(16.7%) | 4(30.7%) | |
| 56-65 yrs | 0 | 0 | 5(38.6%) | |
| TOTAL | 50(100%) | 6(100%) | 13(100%) | |

Endometrioid type (92.3%) of endometrial carcinoma was found to be the most common type of carcinoma in this study. Patient's menopausal status was seen to have statistically significant association with endometrial carcinomas (p value <0.001).

All cases of EHWOA showed positivity (100%) to ER and PR immune stain [Fig-3,4]. Except one case, all other cases of atypical hyperplasia were positive for ER and PR immune stain [Fig-5,6] and 11 out 13 (84.6%) cases of endometrial carcinomas were ER and PR positive [Table-4,5, Fig-7]. Among carcinomas majority were Grade 1 endometrioid carcinoma (69.2%) and it

**Table-4: Estrogen receptor status in hyperplasia and endometrial carcinoma**

| ER | EHWOA (n=50) | AEH (n=6) | EC (n=13) |
|---|---|---|---|
| Category I (Immuno-negative) | 0 | 1(16.7%) | 2(15.4%) |
| Category II (Immuno-positive) | 5(10%) | 5(83.3%) | 9(69.2%) |
| Category III (Immuno-positive) | 45(90%) | 0 | 2(15.4%) |

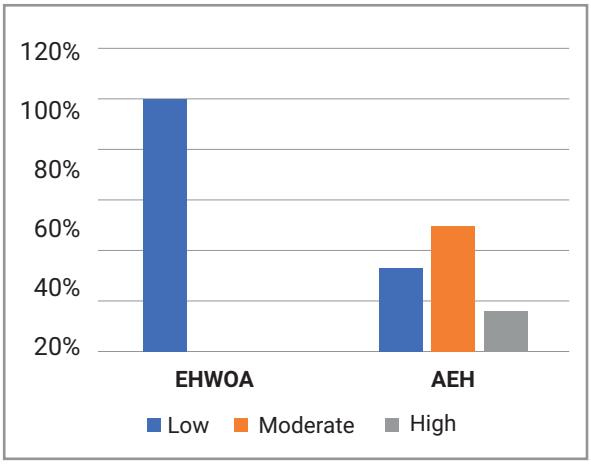


**Figure 2: Ki-67 receptor status in hyperplasias**

**Table-5: Progesterone receptor Status in hyperplasias and endometrial carcinoma**

| PR | EHWOA (n=50) | AEH (n=6) | EC (n=13) |
|---|---|---|---|
| CategoryI (Immuno-negative) | 0 | 1(16.7%) | 2(15.4%) |
| CategoryII (Immuno-positive) | 3(6%) | 5(83.3%) | 8(61.5%) |
| CategoryIII (Immuno-positive) | 47(94%) | 0 | 3(23.1%) |

**Table-6:ER, PR status in Endometrial carcinoma**

| | ER, PR positive | ER,PR negative |
|---|---|---|
| GRADE1 (n=9) | 9(69.2%) | 0 |
| GRADE2 (n=4) | 2(15.4%) | 2(15.4%) |
| GRADE3 (n=0) | 0 | 0 |
| TOTAL(n=13) | 11 (84.6%) | 2(15.4%) |

was observed that the ER and PR positivity reduced as the tumor progressed from Grade 1 to Grade 2 [Table-6].In this study out of 50 cases of EHWOA all cases (100%) showed nil to low Ki-67 index and out of 6 cases of Atypical hyperplasia maximum cases (50%) showed moderate Ki-67 index [Fig-2].7 out of 9, Grade 1 endometrioid carcinomas showed high Ki-67 index and all 4 cases (100%) of moderately differentiated Endometrioid carcinoma showed high Ki 67 index suggesting that Ki 67 index increases with tumor progression.[Fig-8]

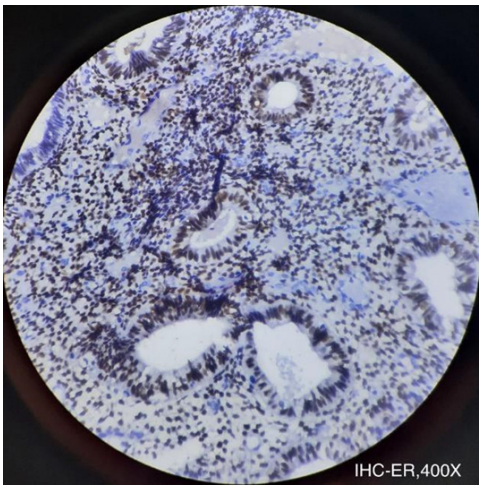


**Figure 3: Microphotograph showing strongly positive nuclear immuno expression of ER in endometrial hyperplasia without atypia (IHC,400X)**

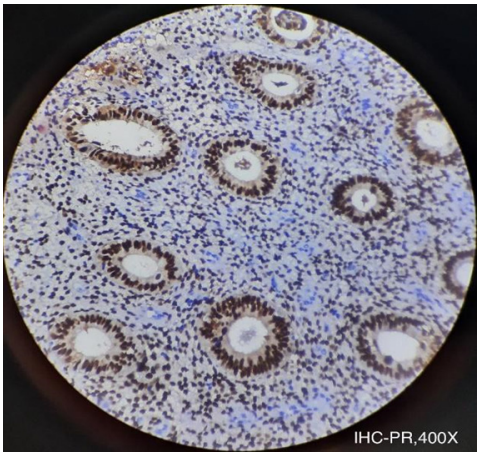


**Figure 4: Microphotograph showing strongly positive nuclear immuno expression of PR in endometrial hyperplasia without atypia (IHC,400X)**

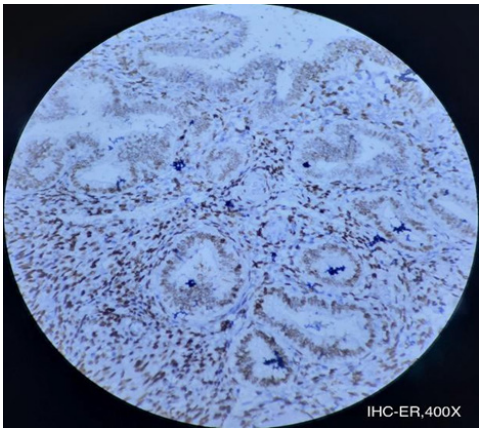


**Figure 5: Microphotograph moderate positive nuclear immuno expression of ER in Atypical hyperplasia (IHC, 400X)**

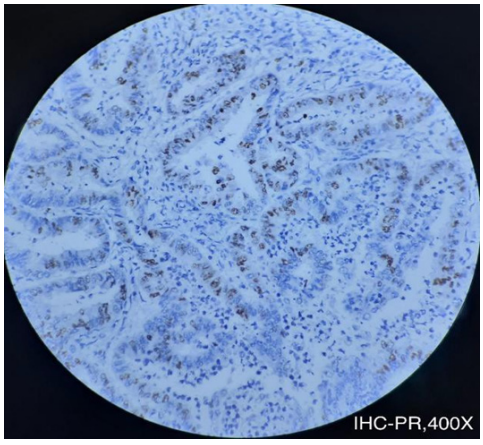


**Figure 6: Microphotograph moderate positive nuclear immuno expression of PR in Atypical hyperplasia (IHC,400X)**

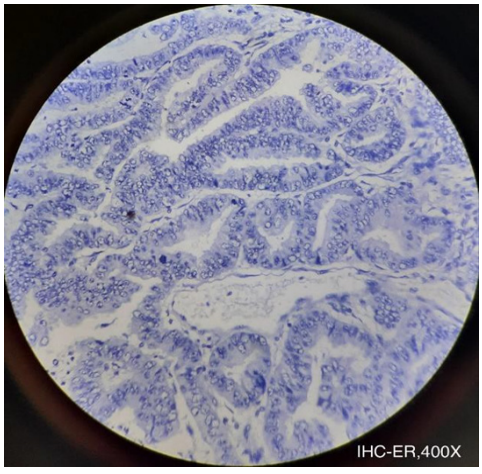


**Figure 7: Microphotograph showing negative nuclear immuno expression of ER in one case of Moderately differentiated Endometrioid Carcinoma (IHC,400X)**

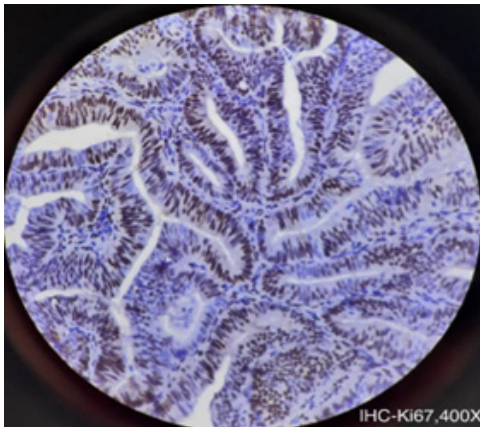


**Figure 8: Microphotograph showing strong nuclear immuno expression of Ki-67 in a case of Moderately differentiated Endometrioid Carcinoma (IHC,400X)**

## Discussion

In the present study, the most common histopathological finding in AUB in this study was Proliferative endometrium (41%) which is similar to the findings seen in studies by Pillai SS[10], Sreedhar et al.[2] and Zimik T et al[6]. The maximum number of cases of AUB were seen in the age group (36-45 years) with a mean age 42 years which was similar to the observation made by Madhu Chaturvedi et al[11]. [Table-7].

**Table-7: Age distribution in comparison with other studies**

| Authors | Zimik T et al[6] | Chaturvedi M et al[11] | Sreedhar V et al[2] | Present study |
|---|---|---|---|---|
| Mean age (Years) | 48 years | 42 years | 49.5 years | 42years |
| Age range (years) | 41 to 50 years | 36 -45 years | 40-49 years | 36-45 years |

In the present study, among the two types of endometrial hyperplasia, endometrial hyperplasia without atypia (EHWOA) was the most common type. Similar findings were seen in studies done by Tomar YS et al[12,13] and Coumary A et al[1].[Table-8]

The incidence of AUB in this study was found to be highest in multiparous (82%) women showing that the incidence of AUB increases with parity. Similar observation were made various studies by Sreedhar V et al[2], Coumary et al[1]and Chaturvedi M et al[11].

The present study showed that the incidence of endometrial hyperplasia and endometrial carcinoma was highest after 4th decade of life suggesting that the incidence of endometrial hyperplasia and endometrial carcinoma increases with age which is similar to the finding of Sreedhar V et al[2].

**Table-8: Comparison of frequency distribution of EHWOA and AEH with other studies**

| Authors | Year of study | Sample size | EHWOA | AEH |
|---|---|---|---|---|
| Tomar Y S et al.[13] | 2019 | 87 | 16(36.4%) | 2(4.5%) |
| Coumary A et al.[1] | 2017-2019 | 95 | 10 (10.5%) | 1(1.1%) |
| Present study | 2021-2022 | 400 | 50 (12.5%) | 6(1.5%) |

The predominant type of endometrial carcinoma in this study was Endometrioid type (92.7%) and majority of them belonged to Grade 1 endometrioid carcinoma which was in concordance with the study by Goswami S et al[14] and Watkins JC et al[12].

In the study by Goswami S et al. the mean age of the cases were found to be increasing with increase in histological grade. Hormonal receptor like ER, PRpositive tumors were found to be significantly associated with lower histological grades in their study. Type 1 tumors showed to be of lower histological grade and occurring in premenopausal females. They

also observed that ER/PR positivity was associated with lower grade of lesion and over expression of Ki67 with higher grade lesions. All these findings were similar to the findings in the present study.

In this study all cases (100%) of Simple hyperplasia without atypia showed ER and PR positivity and 4 out of 6 cases (98%) of Atypical hyperplasia showed ER positivity. In the study by Orejuela FJ et al[15] they observed that although 100% and 95% of both hyperplasia and normal endometrium samples expressed in estrogen and progesterone receptors, respectively, only 71% and 79% of endometrial cancers expressed estrogen and progesterone receptors (P = 0.01). ER and PR expression by the endometrium decreases as the lesion progresses from endometrial hyperplasia to endometrial carcinoma.

Stoian SC et al[16] observed decreased ER and PR expression in advanced stage cancers compared to well differentiated and earlystage cancer which was also similar to the findings in our study. Bozdogan O et al[17] observed the estrogen positivity in 96.1% of hyperplasia and 86.3% of carcinomas whereas all cases of hyperplasia (100%) and 90.9% of carcinomas were positive for progesterone receptors.

Ki-67 an important cell proliferation marker which has been widely used in analysing various endometrial lesions associated with AUB[18].In a study by Ghalib Farhood et al[9] the percentage of Ki67 positive staining results in simple hyperplasia was markedly lower than 40%. However, atypical hyperplasia demonstrated significant over expression of Ki-67 protein. Similarly, Ki-67 was expressed in 100% of cases with endometrial carcinoma which was in concordance with the present.

## Conclusion

Abnormal Uterine Bleeding (AUB) is a common gynaecological problem with significant patient morbidity and has a variety of causes according to the age. Clinical information regarding age, menstrual history, parity, risk factors and imaging studies are important to correlate but Histopathologic evaluation remains the most important diagnostic tool in AUB. Incorporation of ER, PR and Ki-67 immunostains in histopathological examination of endometrium in AUB patients will help in understanding its biological behaviour which in turn could help in individual treatment strategies and prognostication especially in developing countries for economical reasons. It will be helpful in providing evidence based treatment. Our study helps in adding details to the existing knowledge about the value of Ki-67 as a potential mitotic indicator and promising prognostic biomarker in carcinomas.

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