Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

Original research article

Clinicopathological and immunohistochemical evaluation of abnormal uterine bleeding

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Abstract

Aim and Objectives: The majority of perimenopausal women's health issues are related to abnormal uterine bleeding. AUB is defined as uterine corpus bleeding that is abnormal in regularity, volume, frequency, or duration and takes place when there is no pregnancy. Perimenopause occurs 2-8 years before menopause and 1 year after the last period. Study the range of histopathological findings in endometrial samples with a clinical history of abnormal uterine bleeding in the 40-51 age group. To assess the endometrium's hormone receptor markers, ER, PR, and HER-2/neu.

Methods: A prospective study was conducted over a period of 24 months on 200 patients aged between 40-51 years with AUB excluding adenomyosis and leiomyoma patients. Of these, 74 cases which included all the cases of benign endometrial hyperplasia (36), endometrial intraepithelial neoplasia (29) and endometrial carcinoma (09) were evaluated for ER, PR and HER-2/NEU expression.

Results: The most common histopathological pattern was benign endometrial hyperplasia (18%). Expression of PR receptors was high in glands and stroma compared to ER in benign hyperplasia, EIN and endometrial carcinoma. Expression of ER and PR receptors was high in glands compared to stroma. A significant difference was noted in ER and PR expression in stroma between EIN and Endometrial carcinoma cases. Her2-neu was positive only in one case of endometrial carcinoma.

Conclusion: In order to identify patients with elevated levels of ER and PR receptors who might benefit from medications that target these receptors and potentially stop the progression of hyperplasia to EIN, invasive surgical procedures are avoided by using immunohistochemical estimation of estrogen and progesterone receptors in endometrial aspirate samples.

Keywords: Abnormal uterine bleeding, ER, HER-2NEU, PR, Peri-menopause

Introduction

The majority of women in the perimenopausal age group experience abnormal uterine bleeding (AUB) [1]. In the absence of pregnancy, AUB is defined as uterine corpus bleeding that is abnormal in regularity, volume, frequency or duration [2]. Perimenopause lasts for two to eight years before menopause and lasts for one year after the last period [2]. It is the transitional period a woman's body naturally goes through from relatively regular cycles of ovulation and menstruation toward menopause or permanent infertility. This phase typically starts when a person is 40 to 50 years old [3Based on the PALM-COEIN classification developed by the working group of the International Federation of Gynecology and Obstetrics, AUB in non-gravid women can be categorized. Adenomyosis, polyp, leiomyoma, malignancy, and hyperplasia are all referred to collectively as PALM. The acronym COEIN stands for coagulopathy, ovulatory dysfunction, endometrial, iatrogenic, and not yet classified. The PALM part of the classification consists of structural causes that may be assessed by imaging techniques and/or histopathology.

The endometrium is under the action of two hormones estrogen and progesterone. They exert their effect by acting on specific nuclear receptor proteins, Estrogen receptor (ER) and the stromal and glandular cells of the endometrium contain progesterone receptors (PR) [4]. The proliferative phase is when ER concentrations are at their highest, and the secretory phase is when they are at their lowest in both glands and stroma. As a result of an increase in progesterone production after ovulation, PR concentrations fall in the glandular epithelium from their high levels during the proliferative phase under the influence of estrogen. In both the pathogenesis of endometrial polyps, hyperplasia, and endometrial carcinoma as well as normal endometrial function, the expression and distribution pattern of ER and PR may be significant. The ERBB2 gene, which is found on chromosome 17q12, encodes the proto-oncogene HER-2/neu. Recently, several studies have turned their attention to the function of HER-2/neu in the pathogenesis

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and the importance of its prognostic significance in endometrial carcinomas [5, 6]. After HER2 was successfully targeted for the treatment of breast cancer and gastric/gastroesophageal cancer, its role in the management of endometrial carcinoma has generated a great deal of interest [6, 71].

Investigating the underlying medical disturbances allows for the evaluation of the COEIN portion of the classification [2]. One-third of the patients who visit the gynecology OPD complain of unusual uterine bleeding [8]. It performs two-thirds of all hysterectomies performed globally, making it a significant contributor to morbidity and mortality in the perimenopausal age group [1]. Between menarche and menopause, AUB is said to affect 9 to 14% of women. Every nation has a different prevalence. The prevalence in India is said to be around 17.9% [9]. Ages 40 to 50 make up about 50% of those who are affected.

AUB may be a reflection of underlying pathology and is connected to significant social and physical morbidities in all societies [2]. It negatively impacts women's psychological well-being and quality of life. Work, productivity and quality of life disruption caused by AUB can range from minor to severe globally [10, 11]. Because it adds to the causes of anaemia that are already common in women, it is especially concerning in developing nations [12].

AUB in women of the perimenopausal age group needs special attention due to the increased risk of endometrial carcinoma in this age group. Along with pelvic ultrasound and histopathology of endometrial biopsies, IHC may be a useful investigation in the management of AUB (AUB-E). Understanding the pattern of ER and PR in endometrial tissue is crucial because it could open up a new area of hormone therapy for endometrial cancer. Both medical treatments and surgical procedures are available for the treatment of AUB. Patients who are clinically unstable, unmanageable medically, or who have unsuitable reactions to medical therapy should receive surgical treatment.

Aims & Objectives of the study

This study is a Clinicopathological and Immunohistochemical evaluation of endometrial biopsies in Abnormal Uterine Bleeding.

1. To study the spectrum of histopathological findings in endometrial samples with a clinical history of abnormal uterine bleeding in the age group of 40-51 years.
2. To evaluate hormone receptor markers ER, PR and markers of oncogene HER-2/neu on Endometrium.

Materials and Methods

A Prospective Study was done at our hospital for a period of 2 years from October 2019 to September 2021. A total of 200 cases were taken up for the Clinicopathological study. Of these 74 cases which included all the cases of benign endometrial hyperplasia (B.E.H;36), endometrial intraepithelial neoplasia (E.I.N;29) and endometrial carcinoma (E.C;09) were evaluated for immunohistochemical expression of ER, PR and HER-2/NEU. Remaining 126 cases which had histological patterns as proliferative endometrium (P.E;33), secretory endometrium (S.E;31), menstrual endometrium (M.E;11), endometrial polyp (E.P;20) and disordered proliferative endometrium (D.P.E;31) were evaluated only clinicopathologically.

Inclusion criteria

- All cases with a clinical diagnosis of AUB and who were between the ages of 40 and 51 were perimenopausal.

Exclusion criteria

- Postmenopausal bleeding cases and endometrial curettings from patients of other age groups.
- Endometrial curettings from patients with adenomyosis and uterine leiomyoma.

Patients were asked to complete a questionnaire (Annexure) in order to provide the pertinent clinical data. Patients with clinically confirmed AUB and perimenopausal ages of 40 to 51 years had their endometrial curettage and hysterectomy specimens taken. Hematoxylin and eosin was used to stain the samples, which were then processed as usual and paraffin-embedded sections. The full range of benign hyperplasia, EIN, and endometrial carcinoma cases were chosen, and their representative formalin-fixed, paraffin-embedded tissue samples were subjected to immunohistochemistry for ER, PR, and HER-2/neu status.

Primary antibodies used

- **ER:** Anti-Estrogen Receptor-Alpha (EP1)-Rabbit monoclonal Antibody (BioGenex).
- **PR:** Anti-Progesterone Receptor (EP2)-Rabbit monoclonal Antibody (BioGenex).
- **HER-2/neu:** Anti-Her2/ErbB2 (CB11) -Rabbit monoclonal Antibody (BioGenex).

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IHC Result

ER & PR: Brown nuclear staining of the endometrial glands and stroma was taken as positive immunoreactivity.

HER-2/neu: Brown membrane staining of the endometrial glands was taken as positive immunoreactivity.

Scoring and Evaluation

ER and PR Scoring (H-score): A total of 100 cells were counted under oil immersion at the highest stained area in the glands and stroma separately and semi-quantitative grading of staining was done. They were graded as 0-no staining, 1-mild staining, 2-moderate staining and 3- intense staining.

H-score = 3 x % of strongly stained nuclei + 2 x % of moderately stained nuclei+% of weakly stained nuclei. It gives a score range of 0-300 [30, 43].

H-score ≥ 75 is considered positive.

Statistical Analysis: Chi-square test was used to evaluate the correlation between clinicopathological features and the unpaired student-T test was used to calculate the statistical difference between the mean values of H-scores between endometrial hyperplasia, EIN and endometrial carcinoma.

Results

In the present study, the percentage of women presenting with AUB in the age group 40-45 years were 70.5% (141) and between 46-51 were 29.5% (59).

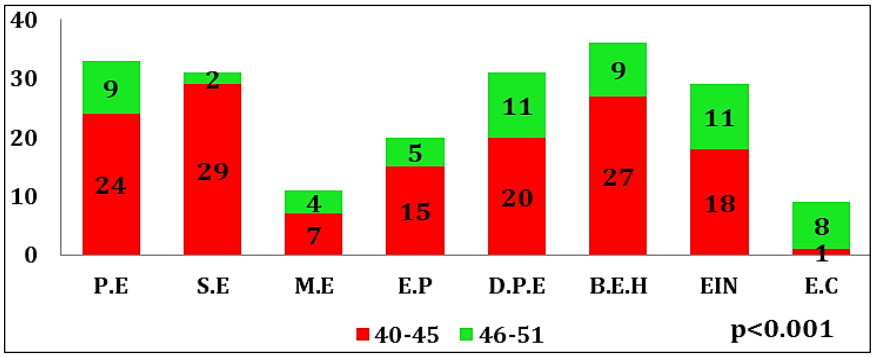

Chart 1: Showing Histopathological Distribution in Different age Groups of Peri-Menopausal Women with AUB

The majority of the patients were multiparous (154; 77%) and 6 (3%) of the women were nulliparous and there was a significant correlation between parity and histopathological patterns in AUB patients with a p-value of <0.0001.

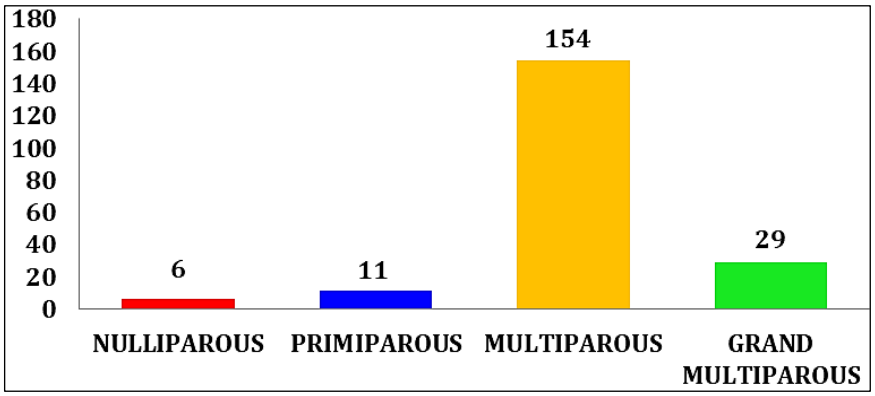

Chart 2: Showing Parity Distribution of Peri-Menopausal Women with AUB

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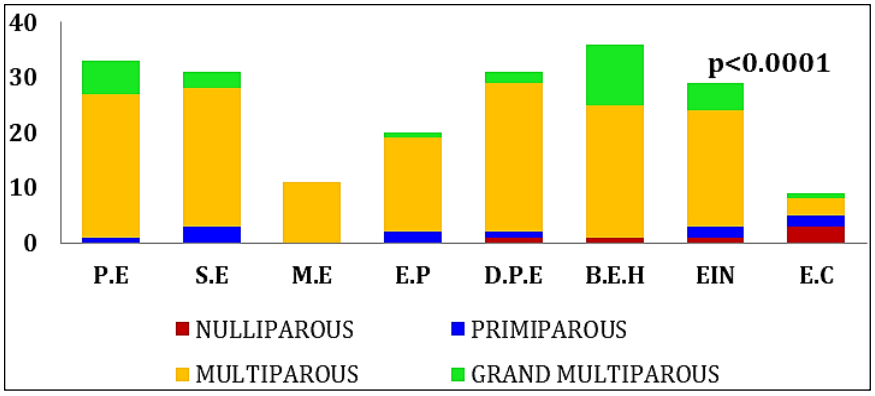

Chart 3: Showing Parity Distribution in Different Histopathological Patterns of Peri-Menopausal AUB

The most common histopathological pattern was benign endometrial hyperplasia (B.E.H) (18%) followed by proliferative endometrium (16.5%), secretory endometrium (15.5%), disordered proliferative endometrium (D.P.E) (15.5%), endometrioid intraepithelial neoplasia (E.I.N) (14.5%), endometrial polyp (10%), menstruating emdometrium (5.5%) and endometrial carcinoma (4.5%).

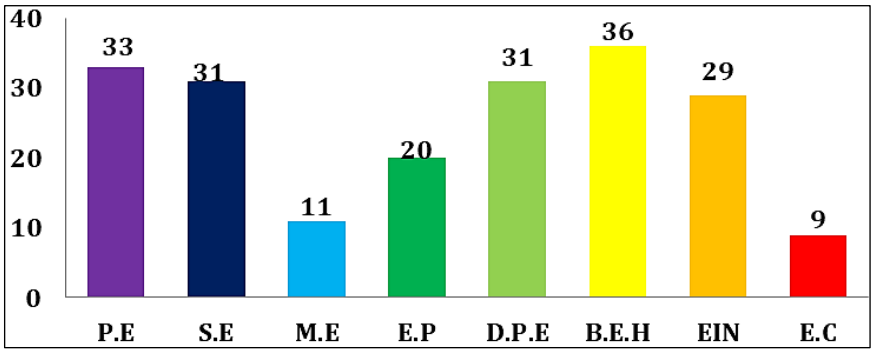

Chart 4: Showing Histopathological Distribution of Peri-Menopausal Women with AUB

35.5% of the patients were overweight while 28.5% of the patients were obese and there was a significant correlation between BMI and histopathological pattern with a p-value < 0.01.

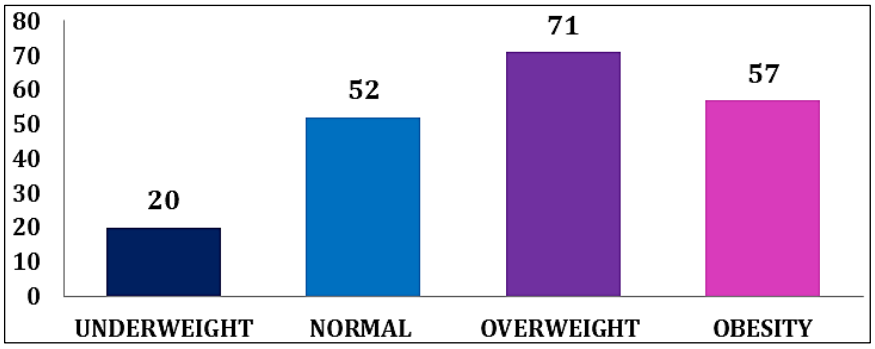

Chart 5: Showing BMI Distribution of Peri-Menopausal Women with AUB

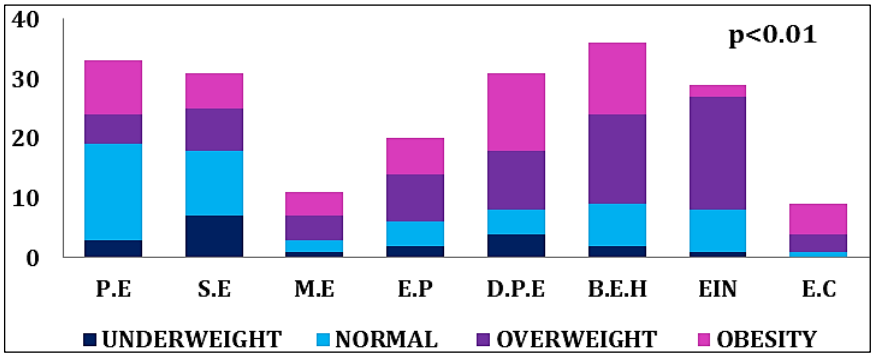

Chart 6: Showing BMI Distribution in Different Histopathological Patterns of Peri-Menopausal AUB

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28% of the patients were diabetic, 38.5% were hypertensive and 37% of them were hypothyroid. A significant association was seen between diabetes vs. histological pattern and hypothyroidism vs. histological pattern, while no such association was found between hypertension and histological pattern.

Table 1: Showing Distribution of Co-morbidities among Peri-Menopausal women with AUB

S. No.	Co-morbidity	Total
1.	Diabetes	56 (28%)
2.	Hypertension	77 (38.5%)
3.	Hypothyroidism	74 (37%)

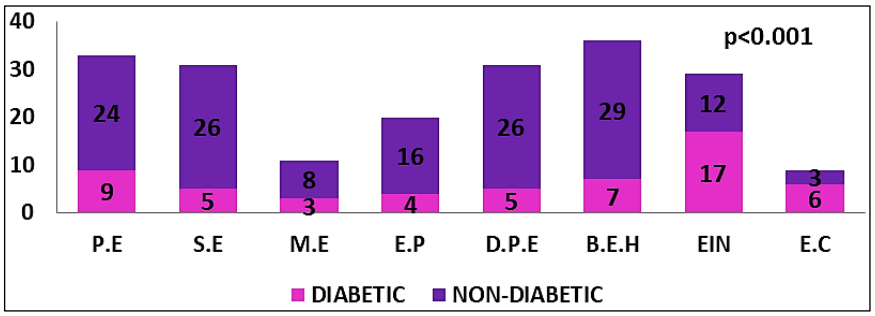

Chart 7: Showing Distribution of Diabetes in Different Histopathological Patterns of Peri-Menopausal Women with AUB

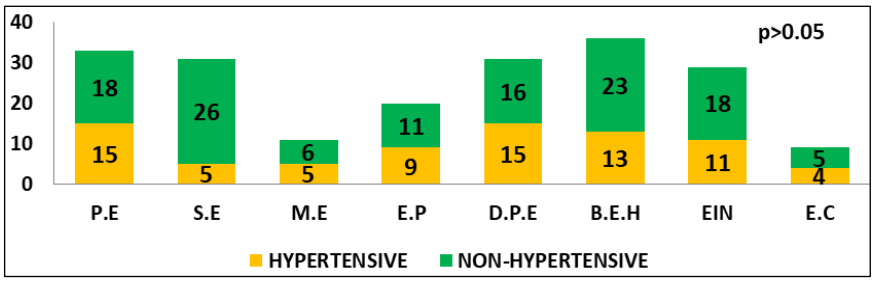

Chart 8: Showing Distribution of Hypertensives in Different Histopathological Patterns of Peri-Menopausal Women with AUB

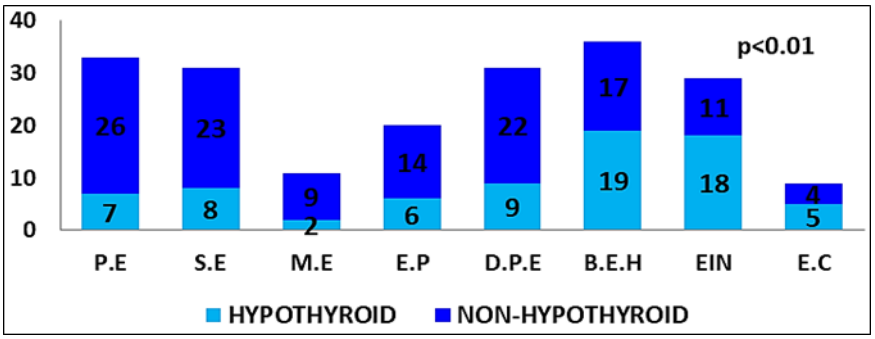

Chart 9: Showing Distribution of Hypothyroidism in Different Histopathological Patterns of Peri-Menopausal women with AUB

The present study compared the immunohistochemical staining of ER, PR and HER-2/neu in the endometrium of patients with benign endometrial hyperplasia, Endometrioid intraepithelial neoplasia and endometrial carcinoma. IHC was done on 74 cases which included 36 cases of benign endometrial hyperplasia, 29 cases of EIN and 09 cases of endometrial carcinoma. Mean H-scores were calculated and an unpaired student-t-test was done to compare the results of ER and PR staining. HER-2/neu was positive in only 1 case of grade-2 endometrial adenocarcinoma and hence it was not included in the comparison studies.

In endometrial hyperplasia, ER was positive in glands and stroma in 91.6% of cases. PR was positive in endometrial glands in 91.6% of cases and stroma in 83.3% of cases. In EIN, ER was positive in glands in 100% of cases, while it was positive in the stroma in 82.75% of cases. PR was positive in glands and stroma in 100% of the cases.

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Table 2: Showing ER and PR Positivity in B.E.H, EIN, E.C.

Total	E.R		P.R	
	Glands	Stroma	Glands	Stroma
B.E.H (36)	33 (91.6%)	33 (91.6%)	33 (91.6%)	30 (83.3%)
E.I.N (29)	29 (100%)	24 (82.75%)	29 (100%)	29 (100%)
E.C. (9)	6 (66.6%)	3 (33.3%)	7 (77.7%)	4 (44.4%)

In endometrial carcinoma, we observed a mixed pattern with 02 cases showing no expression of ER and PR in both glands and stroma. 03 cases showed Positive expression of ER and PR in both glands and stroma. 01 case showed positive expression of ER in only glands and PR was positive in both glands and stroma. 02 cases showed expression of both ER and PR limited only to the glands. 01 case showed only positive expression of PR limited to glands. On a whole, ER was positive in glands in 66.6% of cases of endometrial carcinoma, while it was positive in the stroma in 33.3% of cases. PR was positive in glands in 77.7% of cases while it was positive in the stroma in 44.4% of cases. Her-2neu was positive in only 01 case of endometrial carcinoma.

Table 3: Showing expression of ER and PR in Endometrial Carcinoma

ER		PR		Endometrial carcinoma
Glands	Stroma	Glands	Stroma	Fequency (Total=09)
Negative	Negative	Negative	Negative	02
Positive	Positive	Positive	Positive	03
Positive	Negative	Positive	Positive	01
Positive	Negative	Positive	Negative	02
Negative	Negative	Positive	Negative	01

In benign endometrial hyperplasia (Table: 4 Chart: 10) there was a significant difference between ER and PR expression in glands compared to stroma with P-value < 0.05. A significant difference was also noted between glandular and stromal expression of ER compared to PR with stromal expression particularly showing a p-value of < 0.01.

Table 4: Showing Mean H-Scores of ER and PR in BEH

	ERG	ERS	PRG	PRS
Mean	152.47	125	195.38	157.47
SD	70.75	36.41	81.88	55.77
Observations	36	36	36	36
P-Value	ERG vs. ERS= 0.0420		PRG vs. PRS= 0.0247	
	ERG vs. PRG= 0.0201		ERS vs. PRS= 0.0046	

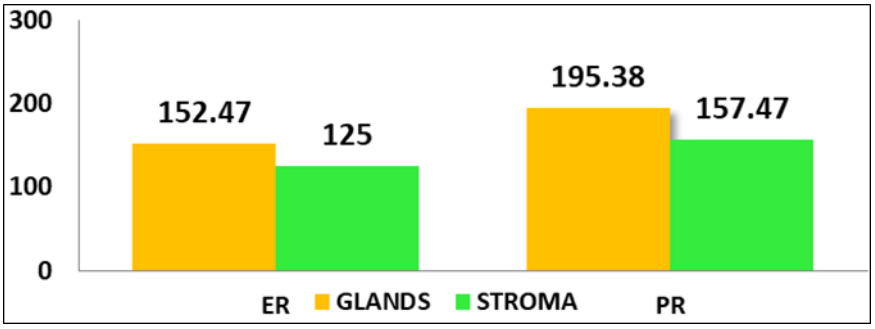

Chart 10: Showing Mean H-Scores of ER and PR in BEH

In EIN (Table: 5, Chart: 11) significant difference was seen only between the stromal expression of ER and PR with a P- value < 0.05.

Table 5: Showing Mean H-Scores of ER and PR in EIN

	ERG	ERS	PRG	PRS
Mean	133.79	116.20	160.51	146.62
SD	66.56	57.68	69.87	51.60
Observations	29	29	29	29
P-Value	ERG vs. ERS = 0.2868		PRG vs. PRS = 0.3928	
	ERG vs. PRG = 0.1415		ERS vs. PRS = 0.0387	

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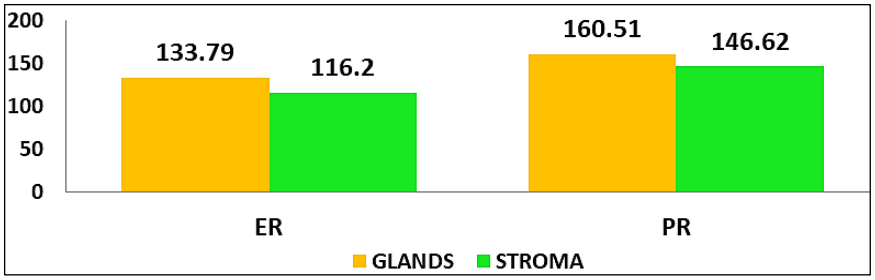

Chart 11: Showing Mean H-Scores of ER and PR in EIN

In endometrial carcinoma (Table: 06, Chart: 12) significant difference was seen only between the expression of ER and PR in glands compared to the stroma. (P-value < 0.05).

Table 6: Showing Mean H-Scores of ER and PR in Endometrial Carcinoma

	ERG	ERS	PRG	PRS
Mean	112.7	48.6	131.6	62.2
SD	80.51	38.35	68.7	46.57
Observations	09	09	09	09
P-Value	ERG vs. ERS = 0.0466		PRG vs. PRS = 0.0233	
	ERG vs. PRG = 0.5995		ERS vs. PRS = 0.5085	

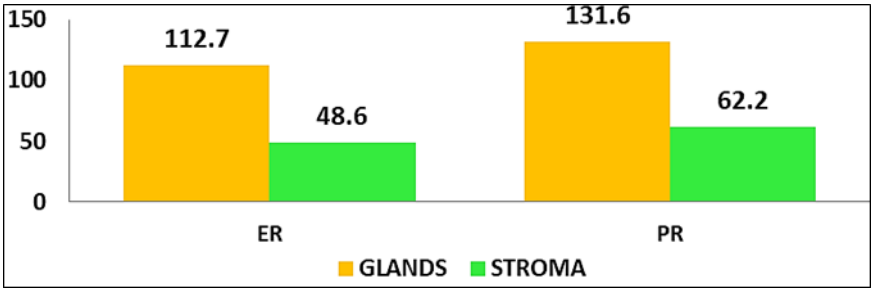

Chart 12: Showing Mean H-Scores of ER and PR in Endometrial Carcinoma

There was no significant difference in ER and PR expression in glands and stroma between endometrial hyperplasia and EIN (Table: 07, Chart: 13).

Table 7: Showing Comparison of Mean H-Scores of BEH vs. EIN

	ERG		ERS		PRG		PRS	
	BEH	EIN	BEH	EIN	BEH	EIN	BEH	EIN
Mean	152.47	133.79	125	116.20	195.38	160.51	157.47	146.62
SD	70.75	66.56	36.41	57.68	81.88	69.87	55.77	51.60
Observations	36	29	36	29	36	29	36	29
P-Value	0.2815		0.4564		0.0735		0.4233	

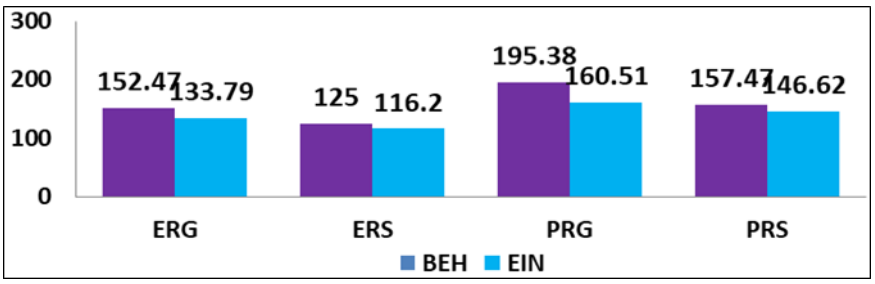

Chart 13: Showing Comparison of Mean H-Scores of BEH vs. EIN

A significant difference was seen in ER expression in stroma between endometrial hyperplasia and endometrial carcinoma with a p-value < 0.001. There was also a significant difference in PR expression in glands and stroma between endometrial hyperplasia compared to endometrial carcinoma with p-value < 0.05 and < 0.001 respectively (Table: 8, Chart: 14).

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Table 8: Showing Comparison of Mean H-Scores of BEH vs. EC

	ERG		ERS		PRG		PRS	
	BEH	EC	BEH	EC	BEH	EC	BEH	EC
Mean	152.47	112.7	125	48.6	195.38	131.6	157.47	62.2
SD	70.75	80.51	36.41	38.35	81.88	68.7	55.77	46.57
Observations	36	09	36	09	36	09	36	09
P-Value	0.1492		0.0001		0.0372		0.0001	

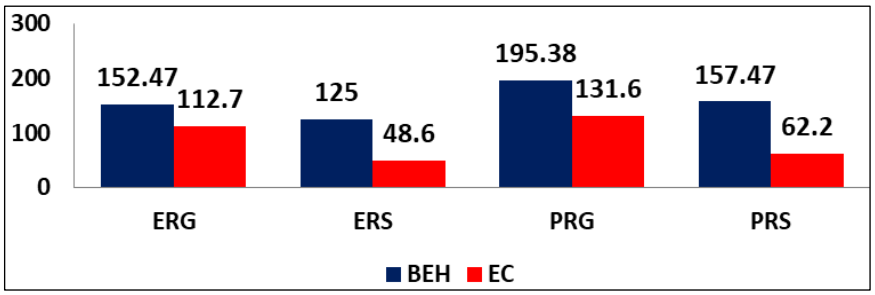

Chart 14: Showing Comparison of Mean H-Scores of BEH vs. EC

A significant difference was noted in the expression of ER and PR receptors in stroma between EIN and endometrial carcinoma cases. Stromal expression of ER and PR was very less compared to glandular expression of ER and PR (Table: 9, Chart: 15).

Table 9: Showing Comparison of Mean H-Scores of EIN vs. EC

	ERG		ERS		PRG		PRS	
	EIN	EC	EIN	EC	EIN	EC	EIN	EC
Mean	133.79	112.7	116.20	48.6	160.51	131.6	146.62	62.2
SD	66.56	80.51	57.68	38.35	69.87	68.7	51.60	46.57
Observations	29	09	29	09	29	09	29	09
P-Value	0.4343		0.0023		0.2836		0.0001	

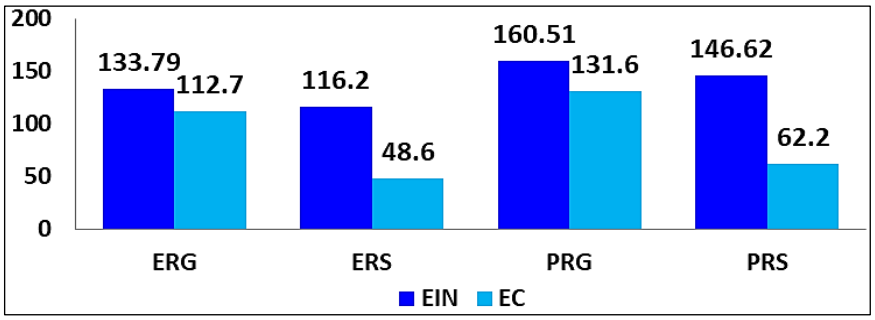

Chart 15: Showing Comparison of Mean H-Scores of EIN vs. EC

Discussion

Abnormal uterine bleeding continues to be one of the most frequently encountered health problems in gynaecological practice, especially in the perimenopausal age group. Nearly 50% of all the cases seen in the gynaecology outpatient department are of AUB.

While abnormal uterine bleeding can be due to a lot of structural causes like polyps, adenomyosis, leiomyoma and malignancy, the most common cause in the perimenopausal age group is due to anovulatory cycles leading to prolonged estrogen effect. The perimenopausal transition represents a special "window of risk" for unopposed estrogen action. This along with low and irregular levels of progesterone leads to an increased risk of endometrial carcinoma in perimenopausal women.

Parity

Nulliparity is a strong independent risk factor for endometrial carcinoma. In the present study, 77% (154) of the women were multiparous and 3% (06) of the women were nulliparous. There was a significant correlation between parity and histopathological patterns in AUB patients with a p-value of <0.0001. (Chart: 3). 3 of the 6 (50%) nulliparous women had endometrial carcinoma. Anupamasuresh Y *et al.* [12] conducted a prospective study on 359 women aged 46 & 73 years who presented with abnormal uterine bleeding. 53.5% of the patients were multiparous and 6.4% of women were nulliparous. A similar trend was also seen in a study done by Lotha L *et al.* [13] on 148 perimenopausal women with AUB with a majority of the women (64.9%) being multiparous and 6.1% being nulliparous.

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B.M.I

Increased BMI is one of the risk factors for the development of endometrial hyperplasia with atypia which is a precursor for endometrial carcinoma in perimenopausal women with AUB. In the present study, 35.5% of the patients were overweight while 28.5% of the patients were obese. (Chart: 5) there was a significant correlation between BMI and histopathological pattern with a p-value < 0.01. 5 of the 9 (55.5%) endometrial carcinoma cases were obese while 3 of them (33.3%) were overweight. In the study by Anupmasuresh Y *et al.* [12] 32.5% of patients were obese, and 27% of patients were overweight. In their study endometrial hyperplasia was more common in obese patients. Similarly in the study by Sharma AS *et al*. [55]. 56.25% of the patients were overweight while 25% of the patients were obese.

Diabetes

In the present study, 28% of the patients were diabetic while in the study by Anupamasuresh Y *et al.* [12] 9.2% of the patients were diabetic. There was a significant association between diabetes and histopathology patterns both in the present study (Chart: 7) and in the study by Anupamasuresh Y *et al.* [12] with a p-value <0.01. In our study diabetes was more common in patients with EIN, proliferative endometrium and benign endometrial hyperplasia with 17 out of 29, 9 out of 33 and 7 out of 36 cases being diabetic respectively.

Hypertension

In the present study, 38.5% of the patients were hypertensive. In the study by Anupamasuresh Y *et al.* [12] 32.3% were hypertensive. There was no significant association between the pattern of histopathology and hypertension in the present study (Chart: 8) while in the study by Anupamasuresh Y *et al.* [12] there was a significant association between histopathology and hypertension, with hypertension being more common in patients with atrophic endometrium, endometrial hyperplasia and endometrial malignancy.

Hypothyroidism

Thyroid dysfunction is an important causative aetiology of menstrual abnormalities. In the present study, 37% of the patients were hypothyroid. There was a significant association between hypothyroidism and histopathological pattern with a p-value <0.01 (Chart: 9). Hypothyroidism was more common in patients with Endometrial hyperplasia and EIN.

Histopathological pattern

The proliferative pattern is one of the most common endometrial histopathological diagnoses seen in AUB cases in most of the studies. Proliferative endometrium cases were seen in 16.5% of cases in the present study which was second only to benign endometrial hyperplasia cases which were seen in 18% of cases.

The study by Lotha L *et al.* [13] also showed similar distribution with 48.6% of benign hyperplasia cases and 41.4% proliferative endometrium cases. Studies by Sudhamani S *et al.* [14], Kumari A *et al.* [1] and Katke RD *et al.* [15] had the highest number of cases of proliferative endometrium at 48.7%, 43.3% and 46.9% respectively.

Secretory endometrium was seen in 15.5% of patients in the present study. In the studies by Sudhamani S *et al.* [14], Kumari A *et al.* [1] and Katke RD *et al.* [16] Secretory endometrium was seen in 17%, 28.3% and 40.9% cases respectively. Secretory endometrium was the most common pattern in the study by Jetley S *et al.* [8] (32.4%), while it was seen in only 5.4% of cases in the study by Lotha L *et al.* [13].

Disordered proliferative endometrium is an intermediate step between normal proliferative endometrium and benign hyperplastic endometrium. It was seen in 15.5% of cases in the present study while it was seen in 6.8% of cases in the study by Jetley S *et al.* [8].

Benign endometrial hyperplasia develops from disordered proliferative endometrium under the continued influence of unopposed estrogens. It was the most common pattern seen in the present study, i.e. it was seen in 18% of cases in the present study. A similar trend was seen in the study by Lotha L *et al.* [13] where it was seen in 48.6% of cases. In the studies by Sudhamani S *et al.* [14], Kumari A *et al.* [1], Katke RD *et al.* [15] and Jetley S *et al.* [8] it was seen in 20.7%. 21.1%, 4.5% and 10.4% cases respectively.

EIN is a precursor lesion to adenocarcinoma of the endometrium. The previous terminologies like simple hyperplasia with atypia and complex hyperplasia with atypia were replaced by Endometrioid intraepithelial neoplasia (EIN) in the 2003 W.H.O Classification. EIN cases constituted 14.5% of the present study. In the studies by Katke RD *et al.* [15] and Jetley S *et al.* [8] atypical hyperplasia cases constituted 1.5% and 0.4% cases only.

Endometrial adenocarcinoma is the final malignant stage in D.P.E, BEH, EIN, and EC spectrum. Malignancies are less common, but an important cause of abnormal uterine bleeding. In the present study Endometrial carcinoma cases accounted for 4.5% of cases. In the study by Sudhamani S *et al.* [8] endometrial carcinoma accounted for 7.32% of cases. While in the study by Lotha L *et al.* [13] and Kumari A *et al.* [1] they accounted for 1.3% and 1.1% respectively.

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Expresion of ER, PR and HER-2NEU in hyperplastic and neoplastic endometrium

Estrogen and progesterone are cyclically produced by the ovaries. The endometrium is a highly sensitive indicator of the hypothalamic-pituitary-ovarian axis. Estrogen and progesterone receptors are present in both endometrial glandular and stromal cells. The concentrations of both estrogen receptor and progesterone receptor undergo variations in the endometrium throughout the menstrual cycle, leading to variable expression of the steroid receptors in the epithelium and stroma. Estradiol increases both Estrogen and progesterone receptors, while progesterone decreases both receptors. Unopposed estrogen is one of the predominant stimuli behind the development of both endometrial hyperplasia and endometrial carcinoma. Progesterones have a role in mitigating the carcinogenic effect of estrogens.

The present study was done to understand the steroid hormonal status and evaluate ER and PR status in hyperplastic and neoplastic endometrium. We compared the immunohistochemical staining of ER, PR and Her-2neu in the endometrium of patients with benign endometrial hyperplasia, Endometrioid intraepithelial neoplasia and endometrial carcinoma.

The expression of ER and PR was more in glands compared to stroma except in case of benign endometrial hyperplasia where ER expression was equal in both glands and stroma and in EIN where both glands and stroma had equal PR expression. Endometrial carcinoma cases had a significant decrease in the expression of ER and PR compared to benign endometrial hyperplasia and EIN. It is also noted that stromal expression of ER and PR was very less compared to glandular expression in endometrial carcinoma. Her-2neu was positive in only 01 case of endometrial carcinoma.

Srijaipracharoen S *et al*. [17] tried to determine the association between ER, PR and HER-2/neu expression and clinicopathological features including survival in endometrial carcinoma patients. In their study ER, PR, and HER-2/neu expression was positive in 59.3%, 65.7% and 2.8% cases respectively. A similar trend was also seen in our study where positivity of PR (77.7% in glands and 44.4% in stroma) was more compared to ER (66.6% in glands and 33.3% in stroma) and HER-2 neu was positive in only 01 patient with endometrial carcinoma, while in the study by Wang C *et al*. [18] the overall positive expression of PR (75%) and HER-2/neu (71.1%) was greater than that of ER (59.8%).

A different result was seen in the studies done by Suthipintawong C *et al*. [19] and Mohapatra K *et al*. [20] where the positive expression of ER (76.9% & 82.9%) was greater compared to PR(72.3% & 62.9%) and HER-2/neu expression (1.5% & 2.8%).

HER-2/neu over-expression in endometrial carcinoma is associated with poor prognosis. Mean H-scores of ER and PR in endometrial glands in endometrial hyperplasia, EIN and endometrial carcinoma (Table: 4-6, Chart:10-12) showed that mean H-Score of PR was greater compared to ER both in glands and stroma i.e. expression of PR receptors was high in both glands and stroma irrespective of the case being, benign hyperplasia, EIN or endometrial carcinoma. Similarly, the mean expression of ER and PR receptors was high in glands compared to the stroma.

Kumari PR *et al*. [21] has done a study on the expression of estrogen and progesterone receptor in atrophic, hyperplastic and malignant endometrium. In this study mean H-score of ER in hyperplasia is 169.2, while that of PR was 208 and the mean H-score of ER in endometrial carcinoma was 55.6 and PR was 177.4. This was comparable to the present study which also showed increased expression of ER and PR in endometrial hyperplasia compared to endometrial carcinoma and also increased expression of PR compared to ER in both endometrial hyperplasia and endometrial carcinoma.

As Progesterones have a role in mitigating the carcinogenic effect of estrogens, these receptors can be targeted in patients with benign endometrial hyperplasia and EIN in preventing these cases from progressing to carcinoma and since expression of PR has been positively correlated with a good prognosis and response to progestin therapy and that of HER-2/neu with a poor prognosis immunohistochemical evaluation of these receptors helps in prognostication.

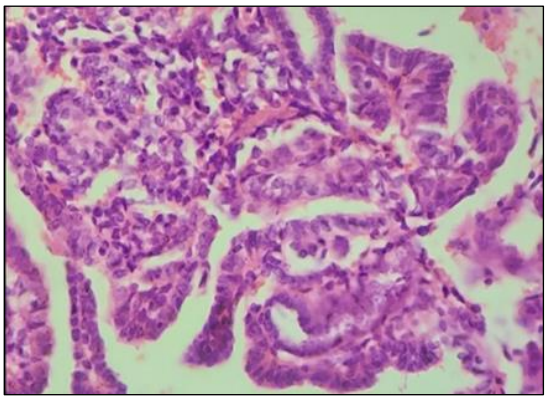

Fig 2: Benign Endometrial Hyperplasia (40x) H&E

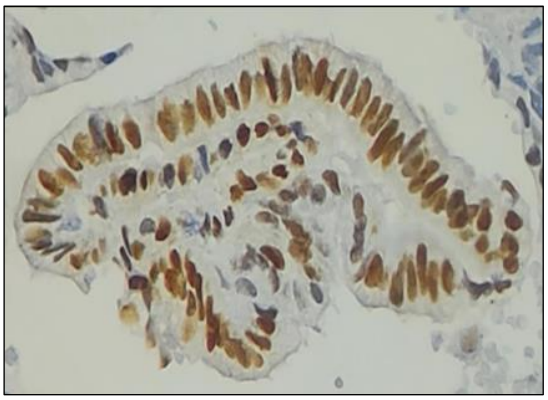

Fig 3: Benign Endometrial Hyperplasia ER 3+ Positive in Glands (40x)

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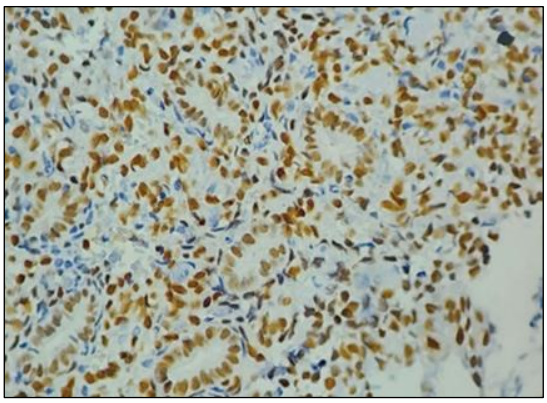

Fig 4: Benign Endometrial Hyperplasia PR 3+ Positive in glands and 1+ to 2+ positive in stroma(10x)

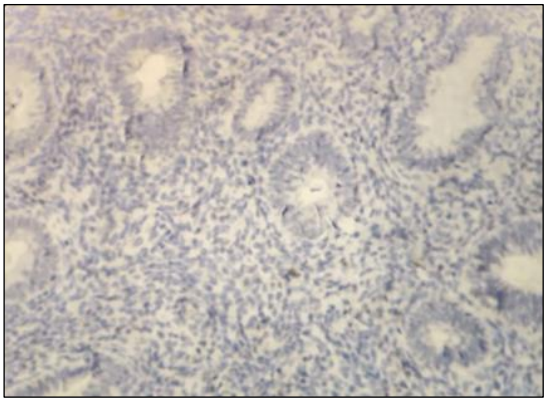

Fig 5: Benign Endometrial Hyperplasia Her-2lneu Negative(40x)

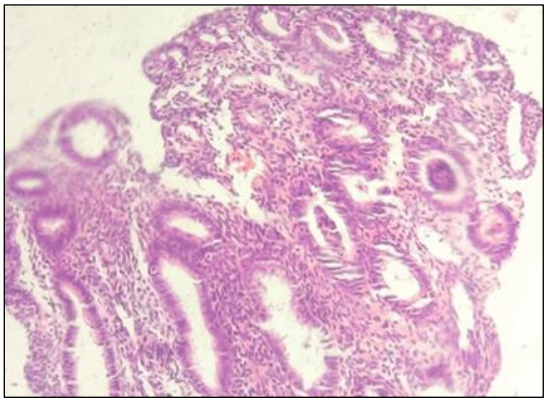

Fig 6: EIN (10x) H&E

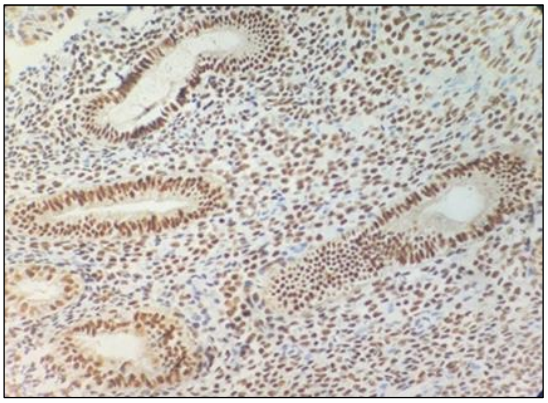

Fig 7: EIN ER 3+ Positive in Glands and stroma(40x)

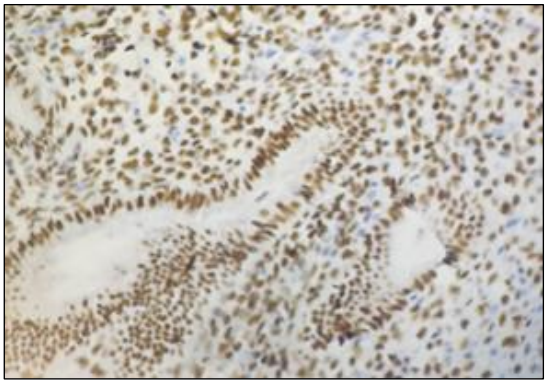

Fig 8: EIN PR 3+ Positive in Glands and stroma (40x)

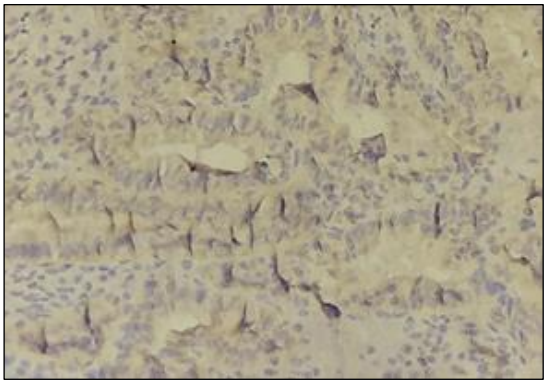

Fig 9: EIN HER-2/neu Negative (40x)

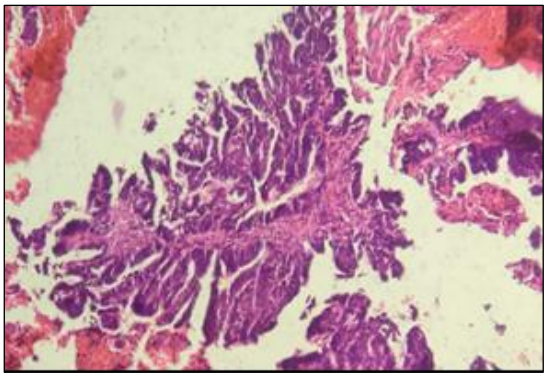

Fig 10: Endometrial Adenocarcinoma Grade 2 (10x) H&E

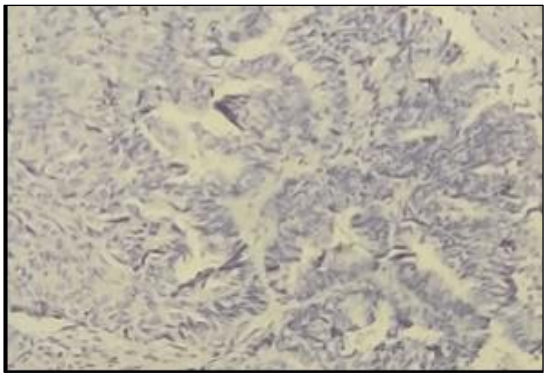

Fig 11: Endometrial Adenocarcinoma Grade 2 ER Negative (40x)

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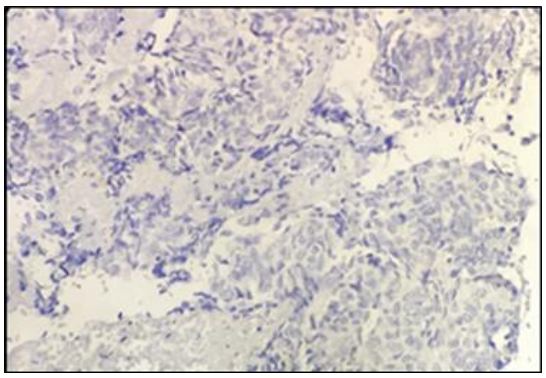

Fig 12: Endometrial Adenocarcinoma Grade 2 PR Negative (40x)

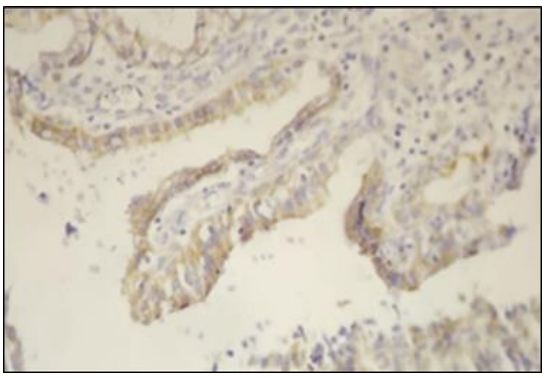

Fig 13: Endometrial Adenocarcinoma Grade 2 HER-2/neu Membrane positive in glands(40x)

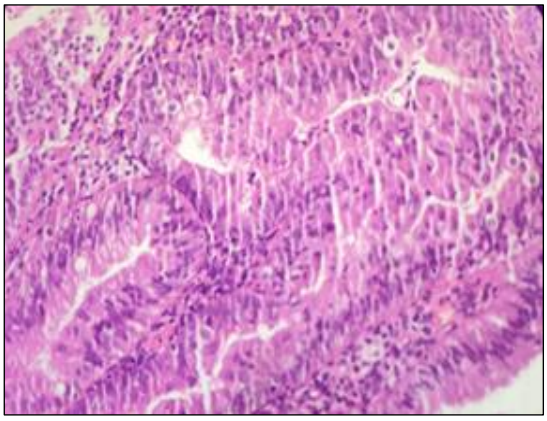

Fig 14: Endometrial Adenocarcinoma-Mucinous type (40x) H&E

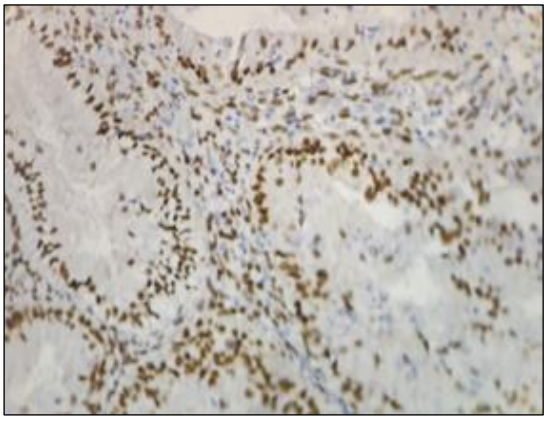

Fig 15: Endometrial Adenocarcinoma-Mucinous type ER 3+ Positive in glands and variable 2+ to 3+ positive in stroma (40x)

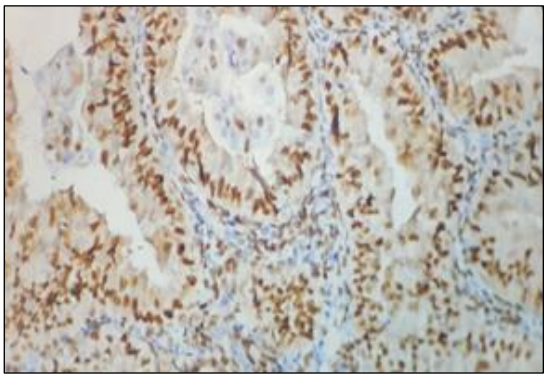

Fig 16: Endometrial Adenocarcinoma-Mucinous type PR 3+ Positive in glands and variable 2+ to 3+ positive in stroma (40x)

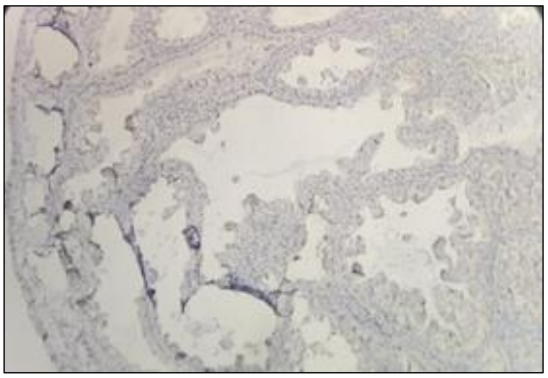

Fig 17: Endometrial adenocarcinoma-Mucinous type HER-2/neu Negative (10x)

Conclusion

One of the most prevalent issues affecting women in the perimenopausal age group is AUB. Endometrial biopsies from patients with AUB that underwent histopathological analysis revealed a wide range of changes, from malignancy to normal endometrium in different phases of the menstrual cycle. Along with ultrasound and histopathological examination of endometrial aspirate samples, immunohistochemical estimation of estrogen and progesterone receptors in endometrial aspirate samples is a very significant and helpful tool that can be used as an adjuvant investigation in the management of patients with AUB. Immunohistochemistry allows for the tissue localization of hormonal receptors in the endometrium and provides information on the distribution and intensity of these receptors in both glands and stroma. Invasive surgical procedures can be avoided by identifying patients with elevated levels of ER and PR receptors who might benefit from drugs that target these receptors and possibly stop the progression of hyperplasia to EIN.

Funding Source: None.

ISSN:0975 -3583,0976-2833 VOL13, ISSUE 05, 2022

Conflict of Interest: None

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