

# HISTO-PATHOLOGICAL AND IMMUNO-HISTOCHEMICAL STUDY OF ER, PR RECEPTOR EXPRESSION IN ENDOMETRIAL CURETTING'S OF WOMEN WITH ABNORMAL UTERINE BLEEDING IN TERTIARY CARE HOSPITAL, JAIPUR, RAJASTHAN

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## Abstract

**Introduction:** Abnormal uterine bleeding (AUB) is defined as any uterine bleeding that is more than the normal volume, of longer duration and varies in regularity or frequency". Nearly 30% of all gynecological outpatient attendants are for AUB. An endometrial biopsy is usually done for AUB to rule out organic pathology. Age and menstrual history are particularly important. **Materials & Methods:** The present study included 244 endometrial curetting's samples, received in the Department of pathology presenting with complain of AUB in the outpatient department of Gynaecology at JNUIMSR, JAIPUR from 01 January 2021 to 30 June 2022. Hysterectomy specimens were not included in study. The tissue sample were fixed in 10% Neutral buffered formalin. Routine tissue processing done. Sections were stained with Hematoxylin and Eosin. Slides were examined under the microscope by pathologists. Further according to their histomorphology representative sections were utilized for IHC. **Results:** Among patients with AUB, the majorities (43.85) were between the ages of 18 and 40, and another 43.44% were between the ages of 41 and 50. Menorrhagia were the most prevalent form of AUB in reproductive and perimenopausal women, followed by metrorrhagia. The commonest histopathological diagnosis in reproductive age (18-40) group was proliferative pattern (36.45%), followed by secretory pattern (24.3%) and disordered proliferative pattern (10.28%). In perimenopausal age(41-50) group, diagnoses were proliferative pattern (37.74%), secretory pattern (20.75%), followed by disordered proliferative pattern (15.09%). IHC was done on 30 cases out of 244 cases of endometrial curetting's. The results of the IHC showed that 03(60%) malignant cases were diffusely intensely positive for both ER and PR, whereas 02(40%) malignant cases were negative for both. In contrast, 72% of non-malignant patients tested positive for ER and 80% were positive for PR. This distribution was statistically significant (p value <0.05). **Conclusion:** The expression of ER and PR diminishes from proliferative to the hyperplastic to the adenocarcinoma stage, as interpreted by IHC. The expression of ER and PR in endometrial cancer has prognostic value. We analysed 244 cases of endometrial curetting's in which 30 cases were chosen for ER, PR expression and compared them with the morphology on H&E sections which was used as the gold standard.

## INTRODUCTION

"Abnormal uterine bleeding (AUB) is defined as any uterine bleeding that is more than the normal volume, of longer duration and varies in regularity or frequency". Nearly 30% of all gynecological outpatient attendants are for AUB.<sup>[1]</sup> Abnormal uterine bleeding (AUB) is a collective terminology that encompasses both organic and non-organic causes. Dysfunctional uterine bleeding (DUB) is a subgroup of AUB that includes abnormal bleeding due to non-organic causes. It is present in 50% of

women with AUB. An endometrial biopsy is usually done for abnormal or irregular uterine bleeding to rule out organic pathology. Age and menstrual history are particularly important, because the etiologies of abnormal uterine bleeding differ according to the age and menstrual pattern.<sup>[2]</sup> In women of reproductive age group, pregnancy complications, including abortion are more common, whereas in postmenopausal women atrophy and organic pathologies are common.<sup>[3]</sup> A diagnosis of Dysfunctional uterine bleeding can only be made after the histopathological examination has ruled out

organic causes.<sup>[4]</sup> The cyclical release of estrogen and progesterone from the ovaries controls the normal cyclical physiological changes that occur in the endometrium of women during the reproductive period.<sup>[5]</sup> Hormonal imbalance is the main factor involved in the pathogenesis of Dysfunctional Uterine Bleeding. This alteration is better studied by a combination of histopathological and immunohistochemical evaluation of the endometrium.<sup>[6]</sup>

## MATERIALS AND METHODS

The present study included all the endometrial curetting's samples, received in the Department of pathology at JNUIMSRC, JAIPUR from 01 January 2021 to 30 June 2022. Patients presenting with complaints of abnormal uterine bleeding in the outpatient department of Gynecology are incorporated in this study. Patients with systemic diseases, genital TB, in-situ IUCDs, an incomplete medical history, bleeding or coagulation problems, pregnancy complications (abortion, molar pregnancy, or ectopic pregnancy), or who are on antiplatelet drugs were not included. Specimen received other than formalin, Insufficient or autolysed samples, Hysterectomy specimens were not included in study. The tissue samples were collected by dilatation and curettage in gynecology department were sent to pathology department, were fixed in 10 % neutral buffered formalin. Routine tissue processing done. Sections of 4-6 micron were taken with the help of microtome and further stained

with Hematoxylin and Eosin. Slides were mounted with DPX and examined under the microscope by pathologists. Further according to their histomorphology representative sections were utilized for Immunohistochemistry. Perimenopausal women's endometrial tissue samples were examined immunohistochemically for ER and PR expression based on their histological diagnosis of secretory, proliferative, simple hyperplasia, atypical hyperplasia, endometrial adenocarcinoma and malignancies.

### Statistical Methods

Statistics transferred to Excel for evaluation. Data were analyzed using SSPS 20 software. Data were presented in the form of percentages, proportions and interpretation is being done in the form of mean, tables and graphs. Comparison of qualitative variables were done using chi square or fisher exact test whenever necessary. Comparison of quantitative variables were done using t-test. Significance was considered if p value is less than 0.05.

### ER and PR status

RESULT	CRITERIA
Positive	Immuno reactive tumor cells present ( $\geq 1\%$ )
Negative	Immuno reactive tumor cells present ( $< 1\%$ )

**Table 1: Age wise distribution of patients with AUB.**

Age Group (years)	Number of Participants	Percentage
18-40 (Reproductive)	107	43.85
41-50 (Perimenopausal)	106	43.44
>50 (Postmenopausal)	31	12.7
Total	244	100

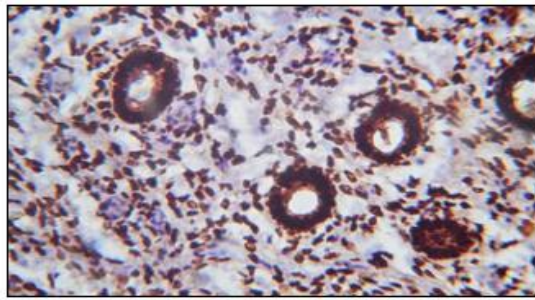
**Table 2: Immunohistochemical staining of ER and PR in the glandular epithelium of perimenopausal women presenting with AUB.**

Immunostaining	ER				PR			
	Positive		Negative		Positive		Negative	
Endometrial changes	N	%	N	%	N	%	N	%
Non-Malignant	18	72%	7	28%	20	80%	5	20%
Malignant	03	60%	2	40%	03	60%	2	40%
Total	21	70	9	30	23	76.67	7	23.33

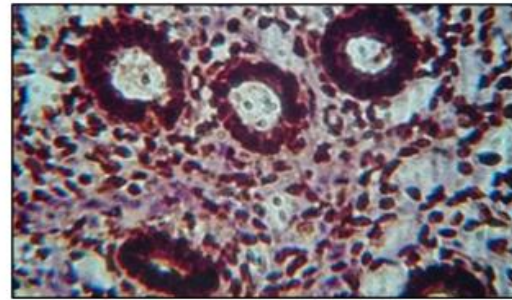
**Table 3: Correlation between immunostaining for ER, PR and histopathological findings.**

Immunostaining	ER		PR	
	N	% of Positive Cases	N	% of Positive Cases
Histopathological Findings				
Complex hyperplasia without atypia	1/2	50	1/2	50
Complex hyperplasia with atypia	1/1	100	1/1	100
Endometrial carcinoma	3/4	75	3/4	75
Simple hyperplasia with atypia	1/1	100	1/1	100
Endometrial polyp	1/1	100	1/1	100
Endometrial stromal tumor	0/1	0	0/1	0
Proliferative pattern	8/12	66.66	8/12	66.66
Proliferative phase with atypical glands	1/1	100	1/1	100
Simple hyperplasia without atypia	5/7	71.43	7/7	100
Total	21/30	70	23/30	76.67

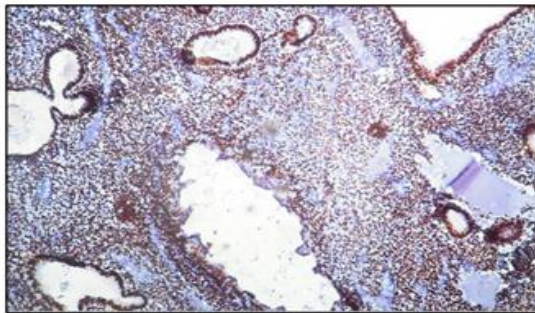




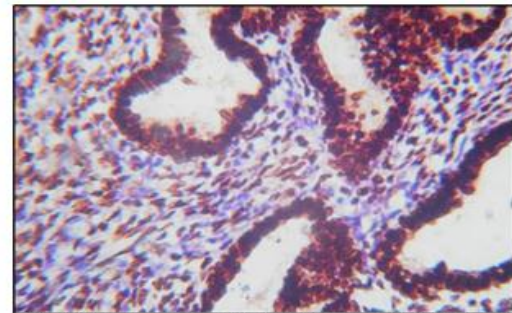
**Fig 1A: ER expression of proliferative endometrium-strong and intense nuclear positivity (40x)**



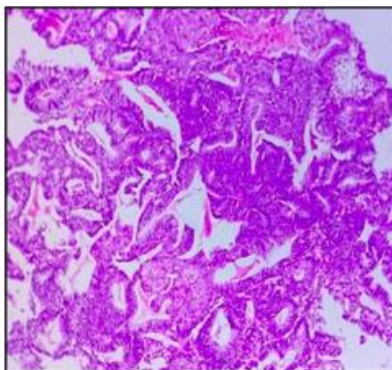
**Fig 1B: PR expression of proliferative endometrium-strong and intense nuclear positivity (40x magnification).**



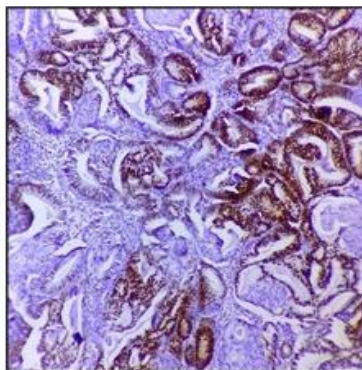
**Fig 2A: ER expression of simple hyperplasia-moderate nuclear positivity (400x magnification).**



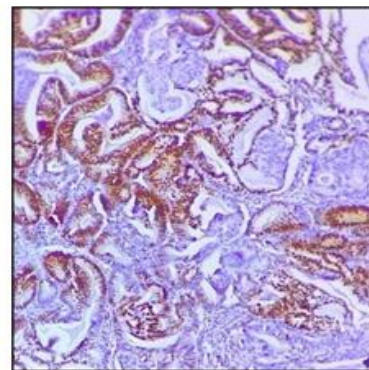
**Fig 2B: PR expression of simple hyperplasia-moderate nuclear positivity (400x magnification)**



**Fig 3: Endometrial adenocarcinoma (H & E 40x magnification)**



**Fig 3A: Endometrial adenocarcinoma showing intense ER positivity (40x magnification)**



**Fig 3B: Endometrial adenocarcinoma showing intense PR positivity (40x magnification).**

## RESULTS

The current study included endometrial dilatation and curettage on AUB specimens from 244 patients who visited the gynaecology outpatient department complaining of the condition throughout the study period. Patients were categorised into those in their reproductive years (18-40), those in their perimenopausal years (41-50), and those beyond postmenopausal (>50). Among patients with abnormal uterine bleeding, the majorities (43.85) were between the ages of 18 and 40, and another 43.44% were between the ages of 41 and 50, which is considered the premenopausal years. (Table 1) Menorrhagia (49.18%) and metrorrhagia (27.87%) were the most common clinical diagnosis. The prevalence of meno-metrorrhagia is 16%, whereas postmenopausal bleeding affected 13.52 % of women. Only 7 individuals (2.87 %) were found to

have polymenorrhea. Menorrhagia is the most prevalent form of abnormal bleeding in reproductive and perimenopausal women, followed by metrorrhagia. Metrorrhagia, were the second most common complaint among women of reproductive age and were equally common among postmenopausal women.

The commonest histopathological diagnosis in reproductive age group was proliferative pattern (36.45%), followed by secretory pattern (24.3%) and disordered proliferative pattern (10.28%).

In perimenopausal age group, diagnoses were proliferative pattern (37.74%), secretory pattern (20.75%), followed by disordered proliferative pattern (15.09%).

Patients who presented with postmenopausal bleeding the commonest histopathological diagnosis was proliferative pattern (25.81%), followed by

secretory pattern and disordered proliferative pattern (16.13%) and endometrial carcinoma (6.46%).

Proliferative pattern (42.44%) is the most common non-organic cause (across all ages), followed by secretory endometrium (25-85%), disordered proliferative pattern (15.61%), early secretory pattern (7.32%), and atrophic endometrium (0.49%). (Table 3)

Immunohistochemical staining was done on 30 cases in women with histopathological diagnosis of proliferative endometrium, all types of hyperplasias & endometrial adenocarcinomas. The results of the immunohistochemical staining showed that 03(60%) malignant cases were diffusely intensely positive for both ER and PR, whereas 02(40%) malignant cases were negative for both. In contrast, 18(72%) of non-malignant patients tested positive for ER and 20(80%) came back positive for PR.(Table 2)

## DISCUSSION

In our research, AUB was most common among women of reproductive age (43.8%), followed closely by those in the perimenopausal age range (43.44%). Several studies, including those by Mariam Abidet al<sup>[7]</sup>, SupriyaSandeepa et al.<sup>[14]</sup>

The two most common symptoms were heavy bleeding during menstruation (menorrhagia, 48.18%) and cramping during the uterine period (27.13%). Menorrhagia has been reported as a common symptom in numerous studies, such as those conducted by Sadia Khan et al<sup>[10]</sup>, Sajitha K et al<sup>[11]</sup>, Shwetha Agrawal et al<sup>[8]</sup>, and Jagadale Kunda et al.<sup>[9]</sup> Studies such as those conducted by Bhatta S et al<sup>[12]</sup> and Naheed Moghal et al<sup>[13]</sup> have indicated that metrorrhagia is the most common symptom. Mariam abid et al<sup>[7]</sup> reported frequent cases of polymenorrhea, but this was not the case in the current study.

The most prevalent histological diagnoses are the proliferative and secretory patterns, which are also the Normal cyclical patterns, according to the majority of research. The current investigation exhibits the same occurrences. Together, the two events account for 57.38 percent of the data here. Similar results have been observed by Zeeba S. Jairajpuri et al<sup>[18]</sup>, Mahmoud Mohammed Mahmoud et al<sup>[19]</sup>, and Supriyasandeepa et al.<sup>[14]</sup>

13.11% cases show Disordered proliferative endometrium in present study.

Doraiswamisaraswathi et al<sup>[15]</sup> shows maximum incidence disorder proliferative pattern (20.53%).

In the current study, hyperplasia's was found in 8.61 percent of patients with AUB. Simple hyperplasia is reported in 6.15% instances. Three different studies (by Layla S. Abdullah et al<sup>[20]</sup>, Doraiswamisaraswathi et al<sup>[15]</sup>, and Bhoomika Saera Afghan et al.<sup>[17]</sup>

Our results are consistent with those of S. Jairajpuri et al<sup>[18]</sup>, Saera Afghan et al<sup>[17]</sup>, Mariam abid et al<sup>[7]</sup> and Soleymani E et al<sup>[16]</sup>, who all reported a similarly low rate of hyperplasia. Women with hyperplasia of the endometrium often have moderate to severe vaginal bleeding, whereas those with atrophic

endometrium typically experience very mild bleeding.

Ovaries consistently release Estrogen and progesterone. Estrogen and progesterone receptors are present in both endometrial stroma and Glands. These receptors mediate the hormonal control. ER & PR both are increased by Estradiol, while progesterone reduces both ER& PR.

In This study Immunohistochemical staining was done on 30 cases in women with histopathological diagnosis of proliferative endometrium, all types of hyperplasias & endometrial adenocarcinomas. immunohistochemical staining showed that out of 5 malignant cases, 03(60%) malignant cases were diffusely intensely positive for both ER and PR, whereas 02(40%) malignant cases were negative for both. In contrast, out of 25 non-malignant cases, 18 cases (72 %) of non-malignant patients tested positive for ER and 20 cases (80 %) were positive for PR.

In their study, Mylonas et al.<sup>[21]</sup> compared the expression of ER and PR in normal (proliferative and secretory) human endometrium to that in malignant endometrial. Glandular epithelium ER and PR expression were shown to decrease considerably between the proliferative (high expression) and secretory (low expression) phases of the research. The hormone receptors were least expressed in adenocarcinoma.

Sahar Aly Daoud, Hala Naguib, and colleagues<sup>[22]</sup> looked at the difference in ER and PR immune staining expression between normal and hyperplastic endometrium and malignant endometrial. From a total of 28 cases of non-malignant endometrium, the researchers found that 22 (78.57%) were ER positive and 19 (67.85%) were PR positive. The researchers in this qualitative study were interested in finding out how many people tested positive for ER and PR in each group. The percentage of ER-positive and PR-positive cases among 20 cases of endometrial cancer was 70% and 60%, respectively.

## CONCLUSION

In present research, the reproductive age group were the demographic where AUB was most prevalent (18-40 years). Proliferative and secretory patterns were the most prevalent types of histological changes, followed by disordered proliferative changes.

The expression of ER and PR diminishes from the hyperplastic to the adenocarcinoma stage, as interpreted by immunohistochemistry. The expression of ER and PR in endometrial cancer has prognostic value. Women who want to keep their fertility and those who do not want surgery may benefit from hormone treatment if their tumours are PR positive. We analysed 244 cases of endometrial curetting's in which 30 cases were chosen for ER, PR expression and compared them with the morphology on H&E sections which was used as the gold standard.



Our conclusions are as follows:

1. Combination of ER, PR, IHC markers can be used to distinguish type1 and type2 endometrial cancers.
2. PR expression is more specific than ER in endometrioid carcinomas.
3. Individually these markers cannot be used to distinguish grade 3 endometrioid carcinomas from serous carcinomas.
4. There is heterogeneity of ER&PR expression within tumor cells. Hence, it is better not to divide the tissue between two different laboratories as it has important therapeutic implications.
5. Accurate diagnosis of tumor type in endometrial curettings is critical for patient management which can be done by using the above markers.
6. A substantial degree of immunohistochemistry overlap exists between FIGO grade 3 endometrioid carcinomas and serous carcinomas. So, in difficult cases, a panel of markers and close attention to morphologic detail should be used in conjunction to arrive at a diagnosis.

In difficult cases, a broad spectrum of IHC panel markers and close examination of morphology should be done to avoid erroneous diagnosis.

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