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## Original Research Article

## Immunohistochemical study of hormone receptors in endometrium with abnormal uterine bleeding

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## ABSTRACT

**Background:** In the case of AUB, estrogen and progesterone exert their effects by acting on specific nuclei. Receptor proteins such as estrogen receptor (ER) and progesterone receptor (PR). And periodic changes in its expression have been shown in human uterine tissue. Immunohistochemistry (IHC). Immunohistochemistry using specific monoclonal antibodies estimate the content of the receptor at the cellular level and interpret their exact location and distribution.

**Materials and Methods:** A two-year descriptive study was conducted. There were a total of 50 cases clinically diagnosed with AUB who underwent hysterectomy and endometrial curettage, and 20 non-AUB controls were included in this study. IHC was performed for ER and PR receptors in all cases and controls.

**Result:** The age range was 29 to 65 years and the average age of the patients was 43.21 years. Out of 50 patients, 26 were pre-menopausal and 24 were post-menopausal, and the mean hemoglobin, fertility and bleeding duration were 10.34 g/dL, 2.8 and 8.4 days per cycle. The average thickness of the endometrium was 10.5 mm in the cases and 6.25 mm in the control group. Increasing endometrial thickness was statistically significantly related to AUB and increased ER and PR HSCORE in stroma and glands ( $p < 0.001$ ). In terms of histomorphology, the highest number of cases was endometrial hyperplasia without atypia (27 cases), followed by early proliferative phase (7 cases). ER expression was more abundant in glands and PR expression was more in stroma. In endometrial hyperplasia with atypia, the expression of ER and PR was higher than in other endometrial stages.

**Conclusion:** In addition to pelvic ultrasound and histology, immunohistochemistry. ER and PR have the advantage of allowing these hormone receptors to localize to the tissue.

Endometrial aspiration samples and auxiliary tests in patient treatment along with AUB. Endometrial hyperplasia is more common in patients with AUB. The concentration of estrogen and progesterone receptors is significantly higher. Therefore, the location and concentration of ER and PR receptors increases and this makes it possible for targeted therapies and avoid invasive surgical procedures using selective progesterone receptor modulators and progesterone antagonists in the treatment of patients with AUB and endometrial hyperplasia.

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## 1. Introduction

Abnormal uterine bleeding (AUB) is essentially a diagnosis of exclusion that cannot be attributed to any recognizable

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anatomic pathology or systemic disease. It is one of the most common gynecological problems, it affects 33% of patients in gynecological practice and about 11-14% of women of reproductive age. More than 50% of patients undergoing hysterectomy for menorrhagia have abnormal uterine bleeding. Abnormal uterine bleeding leads to severe anemia and infertility due to anovulation, affecting health status and quality of life<sup>1</sup> Abnormal uterine bleeding (AUB) occurs during both ovulation and anovulation. Regular cyclical bleeding in the ovulatory cycle and irregular, prolonged heavy bleeding in the anovulatory cycle.<sup>1</sup>

The pathogenesis of AUB is complex. The underlying mechanism is not opposed estrogen stimulation of the endometrium leading to excessive endometrial proliferation and hyperplasia regardless of serum estradiol concentrations. Endometrial hyperplasia is a known risk factor for endometrial cancer. A comprehensive understanding of the mechanism of AUB is therefore essential for its management. Endometrial sampling is chosen for the evaluation of abnormal uterine bleeding because it has several advantages over diagnostic methods. Hormone testing is very expensive and labs are not available in rural areas.

More than transvaginal ultrasound, histological examination of endometrial curettage is the treatment of choice for abnormal uterine bleeding. Transvaginal ultrasonography can be used to diagnose abnormal uterine bleeding along with histopathological examination.<sup>1,2</sup> Only a few studies on steroid receptors have been performed in patients with abnormal uterine bleeding.<sup>3</sup>

Estrogen and progesterone exert their effect by acting on specific nuclear receptor proteins such as the estrogen receptor (ER) and the progesterone receptor (PR).<sup>2</sup> ER and PR receptors play an important role in the etiopathogenesis of AUB and further treatment of patients with AUB with drugs acting on these receptors. The steroid receptor test gained interest, leading to better guidelines for the treatment of various diseases. Steroid receptors can be tested either quantitatively in tissue homogenates or qualitatively by localizing receptors in tissue structures using immunohistochemistry.<sup>1,2</sup> Radiolabel steroid binding assays quantify occupied and unoccupied forms of steroid receptors. Estrogen receptors (ER), progesterone receptors (PR) and their cyclical change in expression have been demonstrated in human uterine tissues by immunohistochemical technique (IHC).<sup>4</sup> Immunohistochemistry using specific monoclonal antibodies estimates the contents of receptors at the cellular level and thus their exact localization and distribution.<sup>3</sup> Therefore, the study was designed to determine the role of hormone receptors in the etiopathogenesis of abnormal uterine bleeding and to contribute to the management of abnormal uterine bleeding.

## 2. Materials and Methods

The present descriptive type study was conducted at Basaveshwar Teaching and General Hospital attached to Mahadevappa Rampure Medical College, Kalaburagi. A total of two years of study including 6 months retrospective and 18 months prospective (from 1<sup>st</sup> September 2020 to 31<sup>st</sup> August 2022). Detailed history along with menstrual history was recorded and general physical examinations were done. For retrospective cases, data were collected from medical records. A total of 50 cases of clinically diagnosed AUB who underwent hysterectomy and endometrial curettage and met all the study criteria and 20 controls without AUB were included in this study. For the control group, patients without complaints of AUB and who underwent vaginal hysterectomy for prolapsed uterus, leiomyoma uterus, and cervical lesions were taken for the study. None of these patients had menstrual irregularities and there was no microscopic evidence of any other pathology in the endometrium of these specimens.

A total of 50 cases of AUB were included according to inclusion criteria which were women of more than 18 years of age, confirmation the of diagnosis of AUB done by clinical examination and ultrasonography, and specimens or endometrial biopsy with adequate endometrial samples taken for IHC, and exclusion criteria were women in immediate puerperal and post-abortal periods, women with pelvic inflammatory diseases and histological evidence of endometrial malignancy. Hence total of 70 endometrial samples including 50 AUB cases and 20 controls were studied for histomorphology and ER (estrogen receptor) and PR (progesterone receptor) immunohistochemistry.

## 3. Results

In the present study 50 AUB cases and 20 controls from September 2020 to august 2022 were included in this study. In AUB cases Parity (Cross the age of viability in India > 20 weeks of gestational age) was 2 (42%) in majority of the cases followed by 3(32%) and controls were of parity 2 (40%) followed by 4 (30%). The mean parity among cases was 2.8 and controls 3.0. The majority of AUB cases (19) had Hemoglobin levels between 10.1-12gm/dl followed by (17 cases) 7-10 gm/dl and the majority of (9) control hemoglobin levels were between 10.1-12gm/dl. The mean Hemoglobin in cases was 10.3gm/dl and in control was 11.2gm/.

Maximum 26 (52%) AUB cases had endometrial thickness between 8.1-15mm followed by 4.1-8mm in 17 (34%) cases.

Ultrasonographically, 15/24cases of postmenopausal women and 4/26 of premenopausal showed endometrial hyperplasia.

In controls, the majority of 16 (80%) cases showed endometrial thickness between 4.1-8mm.

**Table 1:** Ultrasonographical distribution of endometrial thickness in pre-menopausal and post-menopausal women

Endometrial thickness	Cases				Control			
	Pre-menopausal		Post-menopausal		Pre-menopausal		Post-menopausal	
≤4	00	00	01	02%	01	05%	01	05%
4.1-8	09	18%	08	16%	09	45%	07	35%
8.1-15	13	26%	13	26%	02	10%	00	00
>15	04	08%	02	04%	00	00	00	00
Total	26	52%	24	48%	12	60%	08	40%

**Table 2:** One sample t-test to compare the endometrial thickness of AUB with controls

Endometrial thickness (mm)	Test value = 6.25 mm (controls)			95% Confidence Interval of the Difference	
	t	Sig. (2-tailed)	Mean difference	Lower	Upper
	10.5	p<0.001	6	3.15	9.85

**Table 3:** Histomorphological spectrum in AUB cases

Histopathological pattern in endometrium	Cases	Percentage	No of control	Percentage
EP: early proliferative	7	14%	6	30%
LP: late proliferative	6	12%	4	20%
ES: early secretory	3	6%	2	10%
LS: late secretory	2	4%	8	40%
Endometrial hyperplasia without atypia.	27	54%	0	0
Endometrial hyperplasia with atypia	5	10%	0	0
Total	50	100%	20	100%

**Table 4:** Distribution of ER/ PR receptor expression in AUB

HSCORE (0-300)	Estrogen receptor (ER)		Progesterone receptor (PR)		Total
	Glands	Stroma	Glands	Stroma	
1-50	00	00	00	00	00
51-100	02	12	19	03	36
101-150	12	11	09	12	44
151-200	09	16	11	11	47
201-250	16	06	08	15	45
251-300	11	05	03	09	28
Total	50	50	50	50	200

**Table 5:** Bivariate relationships between in AUB. (Minimum to maximum)

		Estrogen receptor (ER)		Progesterone receptor (PR)		P- value
		Gland	Stroma	Gland	Stroma	
Age (years)	30 (min)	286	206	106	262	0.15
	65(max)	222	84	162	170	
Parity	01(min)	165	156	86	147	0.27
	06(max)	222	84	162	170	
ET (mm)	4(min)	166	102	125	110	0.001
	16(max)	251	236	184	208	

**Table 6:** Distribution of cases and controls according to various histopathologic patterns

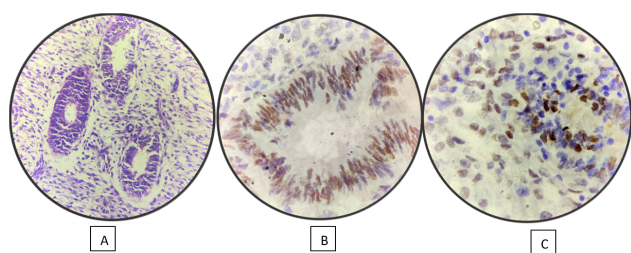
		EP	LP	ES	LS	E.H without atypia	E.H. with atypia.	Total
Control	No of control	4	4	2	8	2	00	20
	Expected count	2.6	3.4	4.6	6.2	2.8	0.4	20.0
AUB cases	No. of cases	07	06	03	02	27	05	50
	Expected count	6.9	8.8	11.2	15.6	6.9	0.6	50.0

**Table 7:** Comparison of ER receptor expression in various HP patterns

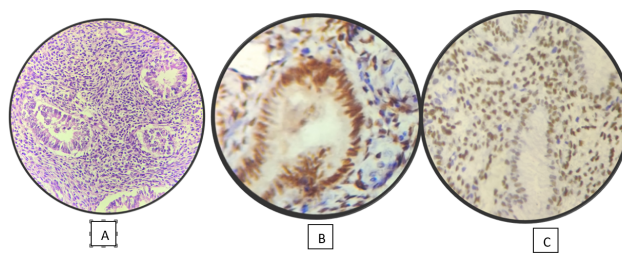
	ER glands		ER stroma	
	Control	Cases	Control	Cases
EP	102±14.1	197.42±24.83	73±17	179.42±22.54
LP	66±31.1	199.66±79.90	57±21	105.66±40.14
ES	62	148.66±30.55	54.2	98.33±21.73
LS	111.25±33.4	181±41.01	101.25±30.3	135±21.21
E.H without atypia	96.16	189.81±81.19	76.58	162.18±62.93
E.H with atypia	00	246.8±36.77	00	161±55.93
MEAN ±SD	102.7± 41.87	195.1±69.6	81.1+ 30.76	153.07±56.9

**Table 8:** Comparison of PR receptor expression in various HP patterns

	PR Gland		PR Stroma	
	Control	Cases	Control	Cases
EP	102±14.1	184.57±31.97	122±5.66	223.28±47.96
LP	66±31.1	125.5±87.21	91±32	191.66±74.07
ES	62	102±43.58	65.5	153±45.90
LS	111.25±33.4	129±38.18	150.25±73.6	184±13.432
E.H without atypia	96.16	143.4±81.80	117.66	180.48±71.43
E.H with atypia	00	174±92.43	00	191±108.55
MEAN ±SD	82.2±27.30	147.01±75.7	124.4±54.31	188.03±70.1



**Figure 1:** A): H & E 10X: Endometrium in the early proliferative phase; B): IHC 100x: ER Glands with 3+ strong nuclear staining and stromal nuclei with variable intensity (0 to 3+); C): IHC 100x: PR Strong nuclear staining of glands (3+) and weak and distinct nuclear staining of stroma (0, 1+,2+)



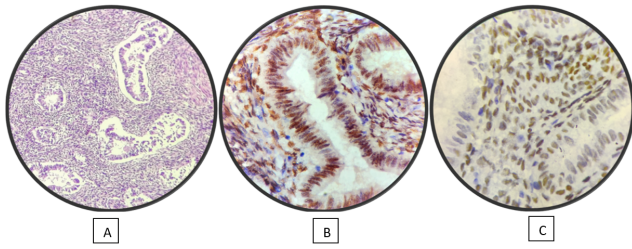
**Figure 2:** A): H & E 10X: Endometrium in late proliferative phase; B): IHC 100x: ER weak and distinct nuclear staining of glands (1+,2+) and stroma (0, 1+,2+); C): IHC 100x: PR weak and distinct nuclear staining of stroma (0, 1+,2)

The mean endometrial thickness in cases was 10.5mm and in control was 6.25mm.

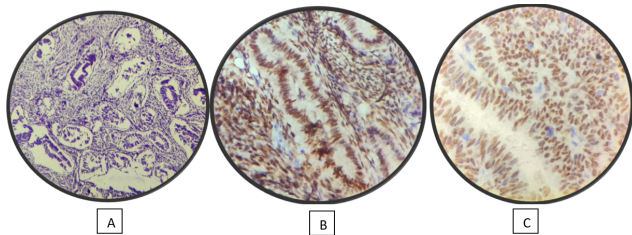
**4. Discussion**

Estrogen and progesterone exert their effect by acting on a specific nuclear receptor proteins such as estrogen receptor

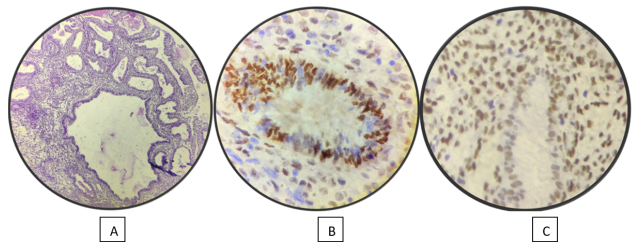
(ER) and progesterone receptor (PR). Histomorphological examination and IHC of steroid receptors in the endometrium is a key factor in the management of abnormal uterine bleeding. The presence of appropriate receptors in target organs determines how tissues respond to steroid hormones.



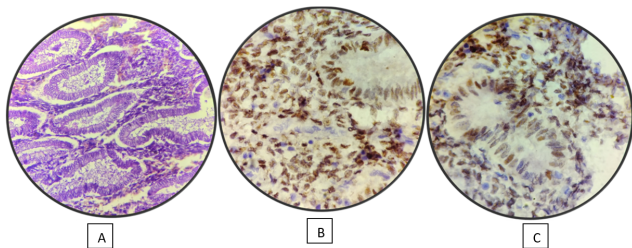
**Figure 3:** **A):** H&E 10X: Early secretory phase; **B):** IHC 100x: ER Strong nuclear staining of glands (3+) and weak and distinct nuclear staining of stroma (0 to 3+); **C):** IHC 100x: PR Strong nuclear staining of stroma (3+) and strong nuclear staining of glands (0, 1+, 2+)



**Figure 4:** **A):** H&E 10X: Late secretory phase; **B):** IHC 100x: ER Strong nuclear staining of glands (3+) and weak and distinct nuclear staining of stroma (1+, 2+); **C):** IHC PR 100x Strong nuclear staining of stroma (3+) and weak to distinct nuclear staining of glands (0, 1+, 2+)



**Figure 5:** **A):** H & E 10x: Endometrial hyperplasia without atypia; **B):** IHC 100X: ER Strong nuclear staining of glands (3+) and weak nuclear staining of stroma (0, 1+); **C):** IHC 100X: PR distinct and Strong nuclear staining (+2, +3) of glands and stroma



**Figure 6:** **A):** H&E 10x endometrial hyperplasia with atypia; **B):** IHC 100x: ER Strong nuclear staining of glands (3+) and weak and distinct nuclear staining of stroma (1+, 2+, 3+); **C):** IHC PR 100x Strong nuclear staining of stroma (3+) and distinct nuclear staining of glands (0, 1+, 2+)

The study of estrogen, progesterone and other hormone receptors in the endometrium have seen increasing interest over the past 25 years. Various techniques can be used to investigate these receptors. Biochemical techniques and tissue homogenization take longer, are more difficult, and are less reliable.<sup>5</sup> Immunohistochemistry enables imaging of receptors in individual cells and analyzing their distribution. Most research has focused on how steroid receptors are expressed in the healthy endometrium. The literature on immunohistochemical evaluation of hormone receptors in patients with abnormal uterine bleeding is extremely rare.

The use of specific monoclonal antibodies against estrogen and progesterone receptors, this study used immunohistochemistry to examine hormonal expressions in endometrium with abnormal uterine bleeding.

The mean age of AUB patients in this study (43.1 years) was almost comparable to this observed in studies by Chakraborty et al,<sup>3</sup> Gleeson et al,<sup>6</sup> Shanthala et al,<sup>1</sup> Priya et al.<sup>2</sup> The mean duration of bleeding in this study was 8.4 days, which was comparable to that of Chakraborty et al. (7.5 days) and Shanthala et al (8.36 days).

The expression of glandular ER was more compared to that of ER and PR in the stroma was more in this study compared to PR glands, which was comparable to studies conducted by Chakraborty et al<sup>3</sup> and Shanthala et al.<sup>1</sup> Also, the expression of ER and PR was higher in all cases than in the control group.

The expression of ERG and ERS was higher in the proliferative phase than in the phase secretory phase and the study was statistically significant (P value <0.05). These results are comparable to the study done by Shanthala et al.<sup>1</sup>

PRG and PRS expression higher in early proliferative phase than others phase and both studies were statistically significant (P value <0.05). These results are comparable to the study done by Shanthala et al.<sup>1</sup>

Barr F et al found that anemia (Hb <12 gm/dl) was seen in 40% of adolescent patients with menorrhagia.<sup>7</sup> In this study, Hb levels in patients with AUB were less than 12 g/dL in 39 (78%) cases. Because excessive AUB can be one of the causes of anemia.

According to Chakraborty et al.<sup>3</sup> the AUB group showed an increase in ER and PR during the proliferative phase and decrease during the secretory phase.

These results support the cyclic fluctuation of steroid receptors. In this study Cyclic variation of steroid receptors has also been observed. Moreover, it supports the idea that the estrogen hormone increases the synthesis of both receptors during the proliferative phase, while the progesterone hormone suppresses the synthesis of both estrogen and progesterone receptors in the secretory phase.

A study conducted by Chakraborty et al.<sup>3</sup> discovered that patients with AUB have a Atypical endometrial histology (hyperplasia) occurs at a significantly higher rate. The mean

HSCORE ER and PR decreased in women with atypical hyperplasia.

In contrast to proliferative endometrium and hyperplasia without atypia. When you're in we discovered that endometrial hyperplasia with atypia has higher ER and PR expression than all other endometrial stages in the current study. However, their findings suggest that steroid receptors are not the only cause of hyperplasia. Other factors that can down-regulate receptors in atypical hyperplasias could be to blame for atypia and carcinoma. This mechanism could explain how atypical hyperplasia progresses to endometrial carcinoma, where autonomy releases aberrant cells' receptor dependence.

In this study, patients with abnormal uterine bleeding had higher levels endometrial estrogen and progesterone receptors than the normal group, which may have been due to higher local concentrations of estrogen in the endometrium during late secretion phase. In earlier research, it was found that there are both estrogen and progesterone receptors downregulated in atypical endometrial hyperplasia.<sup>8</sup> But atypical in this study endometrial hyperplasia shows greater expression of ER and PR at all stages.

An interesting finding was the evidence of a significant increase in both ER and PR in glands and endometrial stroma in women with AUB compared to the normal group ( $p < 0.001$ ). This supported a previous study by S. Chakraborty et al.<sup>3</sup> that the increased concentration of estrogen and progesterone receptors may play a role in the etiopathogenesis of abnormal uterine bleeding.<sup>3,9</sup>

This study found greater variability for glandular ERs than for other receptors in endometrial hyperplasia ( $\chi^2 = 22.02$ ,  $p$  and  $t$ ;  $0.001$ ), which was consistent with the results of Thornton et al.<sup>10</sup> This may be due to increased glandular proliferation and increased glandular to stromal ratio in endometrial hyperplasia. The findings of this study contradicted those of Critchley et al. which showed no appreciable difference between normal endometrium and abnormal uterine bleeding on estrogen and progesterone receptors. This may be due to inconsistency in the way patients were selected for the study. Critchley et al excluded patients who had endometrial carcinoma, endometrial hyperplasia without atypia, or discrepancies between endometrial maturation and menstrual date.<sup>8</sup>

Vinanti et al. evaluated ER and PR in 24 cases of abnormal uterine bleeding and 12 cases of infertility in 2020. They came to the conclusion that ER and PR expression in normal endometrium varied cyclically. It was highest during the proliferative phase, followed by the periovulatory phase, and then declined significantly during the late secretory phase. Infertility and abnormal uterine bleeding with endometrial dysfunction were associated with increased ER expression in late secretory endometrium when compared to controls.<sup>11</sup>

Even though endometrial hyperplasia had the highest levels of ER and PR, it was not distinguishable from other typical menstrual histological patterns.

In contrast to those in the AUB group who had normal histological patterns, Chakraborty et al<sup>3</sup> discovered that Hormone therapy is the most successful initial treatment method for doctors today management of AUB.

Hormonal therapy is now the most effective first-line therapy for the medical management of AUB. Endometrial ablation and hysterectomy, both invasive procedures associated with anaesthesia and surgical complications, are used in difficult cases. The majority of patients with endometrial hyperplasia undergo endometrial ablation or hysterectomy because they are less amenable to medical management.<sup>12</sup>

SPRMs (selective progesterone receptor modulators) have both agonistic and antagonistic effects. Their effects are determined by a person's species, tissue type, hormonal status, and drug dose. They are used in a variety of clinical settings, including emergency contraception, long-term estrogen-free contraception, myomas, endometriosis, and tumors. Mifepristone is the most thoroughly researched progesterone antagonist (PA) to date. Low-dose mifepristone has an anti-proliferative effect in premenopausal women.<sup>12,13</sup>

## 5. Conclusion

Endometrial thickness can be measured using transvaginal ultrasound in addition to pelvic ultrasonography. Immunohistochemistry of ER and PR, in addition to histological examination of endometrial samples, has the advantage of allowing tissue localization of these hormone receptors in endometrial aspirate samples and adjuvant investigation in the management of patients with abnormal uterine bleeding. Endometrial hyperplasia is distinguished by histological changes as well as receptor levels. Patients with AUB have a higher prevalence of endometrial hyperplasia, which has significantly higher concentrations of estrogen and progesterone receptors. This study also identifies patients with elevated ER and PR receptor location and concentrations, indicating that they would benefit from targeted therapy and avoid invasive surgical procedures. The search for anti-receptor agents is ongoing and could be fruitful in the future.

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None.

## 7. Conflict of Interest

None.

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