

# Histopathological study of endometrium in abnormal uterine bleeding with expression of oestrogen and progesterone receptors

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## ABSTRACT

**Background:** Abnormal uterine bleeding (AUB) is a common gynaecological condition. Studies show that there is altered expression of oestrogen receptors (ER) and progesterone receptors (PR) in the endometrium which may have a role in the aetiology of abnormal bleeding. **Objectives:** To study the histopathological findings of endometrium and expression of oestrogen and progesterone receptors in the endometrium in patients with AUB. **Materials and methods:** A total of 63 endometrial biopsies were examined after obtaining ethical clearance from November 2018 to December 2019. After histopathological examination, all the cases were subjected to immunohistochemistry for ER and PR and were scored by Allred scoring system. **Results:** Majority of the patients were in the age group 41- 50 years and presented with heavy menstrual bleeding. Maximum number of cases were of endometrial hyperplasia (EH) without atypia (63%) followed by proliferative endometrium (21%), disordered proliferative endometrium (11%) and secretory endometrium (5%). Mean Allred score in AUB patients for ER expression in glands and stroma was significantly more than mean score for PR expression (glands –  $7.3 \pm 1.5$  vs  $6.7 \pm 1.8$ ,  $p=0.00$ ; stroma –  $6.2 \pm 1.3$  vs  $5.8 \pm 1.6$ ,  $p=0.00$ ). ER expression in endometrial glands and stroma across various histologic patterns of AUB was higher than PR and this difference was significant in EH without atypia ( $7.2 \pm 1.8$  vs  $6.6 \pm 2$ ,  $p=0.001$ ; stroma –  $6.2 \pm 1.16$  vs  $5.7 \pm 1.8$ ,  $p=0.001$ ). However, in other histological patterns, even though ER expression was higher than PR expression, test of significance could not be applied because of a smaller number of cases. **Conclusion:** Increased expression of ER was observed compared to PR in all histological patterns in patients with AUB. ER expression was significantly more in cases of endometrial hyperplasia without atypia indicating they may have a role in etiopathogenesis of endometrial hyperplasia.

**Keywords:** Abnormal uterine bleeding, endometrium, immunohistochemistry, oestrogen receptor, progesterone receptor, endometrial hyperplasia.

Abnormal uterine bleeding (AUB) is a broad term that describes irregularities in the menstrual cycle involving frequency, regularity, duration, volume of flow and amount of blood loss<sup>1</sup> and is a common cause of gynaecologic referral. It can occur at any age during reproductive period and is a common sign of different uterine disorders ranging from dysfunctional abnormalities (non-organic) to organic lesions such as polyps, hyperplasia with or without atypia, or

carcinoma as well as hormonal and systemic diseases.<sup>2</sup>

The PALM–COEIN system (polyp; adenomyosis; leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction; endometrial; iatrogenic; and not yet classified) aids in classification of women with AUB in a systematic manner which provides reliable information for accurate diagnosis and treatment.<sup>3</sup>

Immunohistochemistry (IHC) shows the presence of

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steroid receptors in human endometrium indicating that these cells respond to steroid hormones oestrogen and progesterone. In cases of abnormal uterine bleeding, there is altered expression of these receptors which may have a role in aetiology. Using specific monoclonal antibodies, receptor expression can be estimated at the cellular level and thus, their exact distribution, localization and intensity in glandular and stromal cells can be studied.<sup>4</sup>

Till date, AUB remains one of the enigmatic diseases. Its aetiology and pattern of expression of these hormonal receptors in the endometrium of AUB cases is still not well understood. We undertook this study to understand the role of these hormonal receptors in the etiopathogenesis of AUB which could have important implications in its management and further implications in management.

**Materials and methods**

This was an observational descriptive study of 63 patients with abnormal uterine bleeding. The study population included the patients diagnosed to have abnormal uterine bleeding who were admitted to Yenepoya Medical College and Hospital. The study was started after obtaining permission from the Institutional Ethics Committee (IEC).

Sample size: Sixty three cases (Sample size calculated as per SPSS version 23 with level of significance 5%, power (1-Beta) = 80%, effect size d= 0.18, with confidence interval of 95%).

Sampling method: Convenience sampling.

Inclusion criteria: The inclusion criteria of the study included patients with abnormal uterine bleeding ranging in the age group 18 to 50 years.

Exclusion criteria: The exclusion criteria of the study included inadequate specimens, post-menopausal and hysterectomy cases.

The collection of cases was done from November 2018 to December 2019. After taking consent from the patients, the specimens were received in the department of pathology in 10% neutral buffered formalin. The gross findings were documented according to the standard protocol. The specimens were processed, embedded and 4 micron thick sections were cut, which were stained with haematoxylin and eosin and the observations were recorded. All cases were subjected to immunohistochemistry for oestrogen and progesterone receptors, using Flex monoclonal mouse antihuman ER Code EPI protein and Flex monoclonal

mouse anti-human PR Code PgR 636 from DAKO, and universal secondary kit from DAKO which used DAKO Real Envision as the detection system. Control specimen chosen for ER PR was from a case of ductal carcinoma, breast which was ER and PR positive. IHC findings were recorded and categorization was done according to standard protocol as given below.

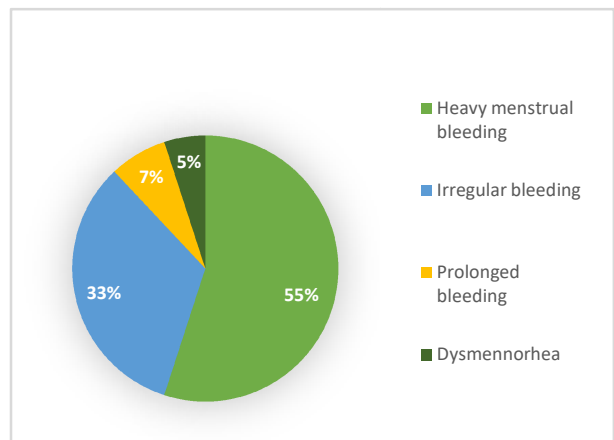
Scoring of IHC sections: Scoring was done by Allred scoring system by calculating proportion score (PS) and the intensity score (IS) as shown in table 1.

**Table 1: Allred scoring system for estrogen and progesterone receptor evaluation**<sup>5,6</sup>

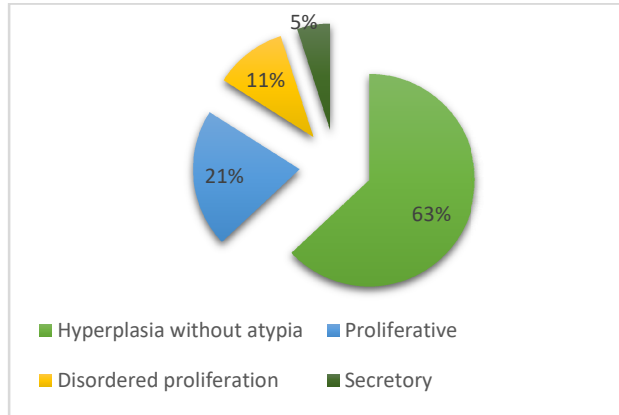
Proportion score (PS)	Positive cells (%)	Intensity Score (IS)	Intensity of staining
Negative	0		
1	>1%	0	None
2	1-10%	1	Weak
3	11-33%	2	Intermediate
4	34-66 %	3	Strong
5	>67 %		
<b>Total score = Sum of proportional score and Intensity score (PS + IS)</b>			<b>Interpretation</b>
0-2			Negative
2-8			Positive

**Results**

Age range in our study was between 24 years to 50 years. Mean age was 41.6 ± 5.9 years. Out of 63 cases, maximum number of cases belonged to the age range between 40-49 years. Heavy menstrual bleeding previously called as menorrhagia was the most common symptom seen in 35 cases in our study followed by irregular bleeding in 20 cases, prolonged bleeding in 5 cases and dysmenorrhea in 3 cases depicted in figure 1.



**Figure 1: Frequency of type of menstrual disturbance seen in our study**

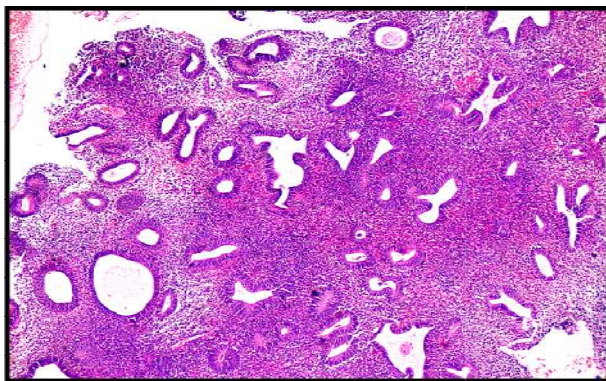


**Figure 2: Various histological patterns in our study**

**Table 2: Final Allred score [Proportion score (PS) + intensity score (IS)] in all AUB cases (n= 61)**

Allred score (PS + IS)	ER expression in gland - No. of cases (%)	ER expression in stroma - No. of cases (%)	PR expression in gland- No. of cases (%)	PR expression in stroma - No. of cases (%)
3	0	0	02(3%)	04(7%)
4	0	0	03(5%)	06(10%)
5	02(3%)	07(12%)	02(3%)	04(7%)
6	01(2%)	24(39%)	8(13%)	21(34%)
7	19(31%)	25(41%)	15(25%)	24(39%)
8	39(64%)	05(8%)	31(51%)	02(3%)

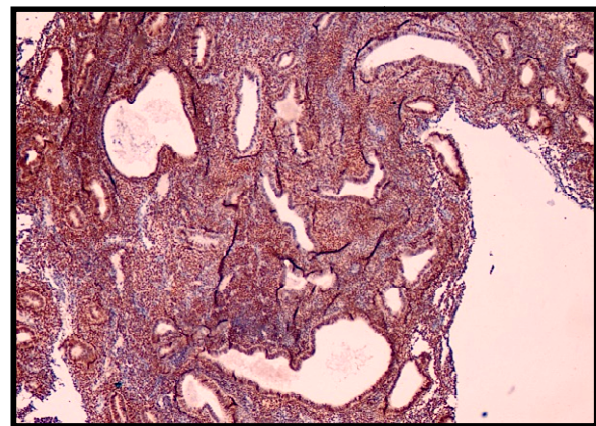
We studied 63 endometrial biopsies and divided them into four histological patterns based on the microscopic features of glands, glandular lining and the stromal changes. The histological patterns identified were proliferative endometrium, secretory endometrium, disordered proliferative endometrium and hyperplasia without atypia. Most common pattern was found out to be hyperplasia without atypia as shown in figure 2 and 3. No cases of atypical endometrial hyperplasia were received in this period.



**Figure 3: Endometrium with hyperplasia without atypia showing variable sized and cystically dilated glands with increased gland to stroma ratio (H&E 4x)**

Proliferative endometrium (n= 13) showed tubular glands lined by pseudo stratified nuclei with regular spacing between the glands. The stromal cells showed oval nuclei and indistinct cytoplasm. Numerous mitotic figures were noted in glands and stroma. Secretory endometrium accounted for minimum number of cases (n= 3). Two out of three cases were of early secretory phase showing tortuous glands with prominent subnuclear vacuolations and luminal secretions in more than 50% of the glands but no stromal change. The remaining one case showed predominantly stromal changes with oedema and predecidualisation. Disordered proliferative endometrium (n= 7) was identified by disorganised proliferative phase glands with focal branching and glandular dilatation but relatively normal ratio of glands to stroma. Maximum number of cases were of

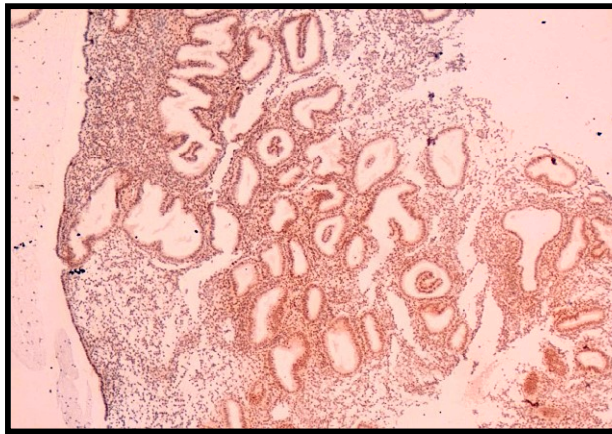
hyperplasia without atypia (n=40) having irregular, variable sized closely packed glands. Glands were cystically dilated, branched and showed outpunching. Few of the glands were having back-to-back arrangement, separated by sparse to abundant stroma, causing increase in gland to stroma ratio. Glandular cell nuclei showed pseudostratification and uniform outlines, lacking cytologic atypia.



**Figure 4: IHC positive for ER in hyperplasia without atypia – strong nuclear staining in glands and stroma (4x)**

The expression of ER and PR in our study was done by Allred scoring system. Out of 63 cases, 61 cases were

studied for IHC as 2 cases were inadequately stained. Immunohistochemical study showed ER and PR expression in the nuclei of cells in endometrial glands and stroma (figure 4 and 5).



**Figure 5: IHC positive for PR in hyperplasia without atypia - Intermediate nuclear staining in glands and stroma (4x)**

**Table 3: Expression of oestrogen and progesterone receptors in endometrial glands and stroma (n=61)**

Categories	Mean Allred score for ER expression	Mean Allred score for PR expression
Glands	7.3±1.5	6.7±1.8
Stroma	6.2±1.3	5.8±1.6

P value = 0.00 by paired student t test

**Table 4: Expression of oestrogen receptors and progesterone receptors in endometrial glands in various histological patterns of AUB in our study (n= 61)**

Categories	ER expression in gland	PR expression in gland
Proliferative phase	7.46± 0.5	7.08± 1.1
Secretory phase	7.6±0.5	6.6 ±0.5
Disordered proliferative endometrium	7.2± 1.1	7.1± 1.0
Hyperplasia without atypia	7.2 ±1.8	6.6 ± 2

\* p value = 0.001 in simple hyperplasia without atypia by paired student t test

**Table 5: Expression of oestrogen receptors and progesterone receptors in endometrial stroma in various histological patterns of AUB (n= 61)**

Categories	ER expression in stroma	PR expression in stroma
Proliferative phase	6.46±0.8	6.31±1.2
Secretory phase	7 ±0	6.6± 0.5
Disordered proliferative endometrium	5.7±0.4	5.7± 1.2
Hyperplasia without atypia	6.2±1.6	5.7±1.8

\*p value = 0.001 in hyperplasia without atypia by paired student t test

The percentage of positively stained cells and intensity of each case was noted each in both glands and stroma. Maximum Allred score of 8 was found in oestrogen receptors expression (64%) in glands followed by

progesterone receptors expression (51%) in glands as shown in table 2.

Analysis of expression of ER and PR in all cases of AUB (n=61) revealed that mean Allred score in AUB patients for ER expression in glands and stroma was significantly more than mean score for PR expression (7.3±1.5 vs 6.7 ± 1.8, p=0.00; stroma – 6.2±1.3 vs 5.8±1.6, p=0.00) (table 3).

Analysis of ER and PR expression in endometrium across various histologic patterns of AUB group revealed that in cases of hyperplasia without atypia, expression of oestrogen receptors in endometrial glands (p value = 0.001) and stroma (p value = 0.001) was found to be significantly higher than expression of progesterone receptors by paired student t test (table 4 and 5).

In other histological patterns, even though ER expression is higher than PR expression, test of significance could not be applied because of less number of cases.

### Discussion

Variations from normal menstrual cycle including alteration in its regularity, frequency of menstrual bleeding, duration of flow and amount of blood loss account for AUB.<sup>7</sup> It negatively affects health and quality of life and in turn is a burden for the society.<sup>8</sup> International Federation of Gynaecology and Obstetrics (FIGO) gave PALM-COEN classification as the aetiology of AUB in non-gravid women in reproductive age groups.<sup>9</sup>

Proliferation of endometrial glands and stroma is mainly regulated by steroid hormones oestrogen and progesterone. These hormones act through nuclear steroid receptors called as oestrogen and progesterone receptors. Change in these receptor expressions can regulate the intensity of the action of hormones either by potentiation or suppression and may be the cause of abnormal bleeding. Therefore, the study of these

receptors in the endometrial glands and stroma could open the gate for medical treatment of cases of AUB by avoiding unnecessary surgeries.

The present study evaluated 63 cases of endo-

metrial biopsies and degree of expression of ER and PR in both endometrium glands and stroma of patients presenting with AUB by Allred scoring, which is a well-established and validated method.

AUB affects various age groups. In the present study, the age ranged between 24 to 50 years and mean age was 41.6 years which is comparable with Upadhyaya et al<sup>10</sup> (mean age - 43.15 years) and Dasgupta et al<sup>11</sup> (mean age 46.2 years). Most common age range was between 41-50 years. This could be explained as AUB is common in perimenopausal age group<sup>12-14</sup> and it was similar to other studies<sup>15-19</sup> while Sadia Khan et al<sup>20</sup> reported the age groups as 45-55 years, Shazia Riaz et al<sup>21</sup> as 45-50 years and Layla et al<sup>22</sup> as more than 52 years.

Heavy menstrual bleeding was the most common symptom seen in 35 cases (55%) in our study which was in accordance with other studies<sup>18, 19</sup>. It was followed by irregular bleeding in 20 cases (33%), prolonged bleeding in 5 cases (7%) and dysmenorrhea in 3 cases (5%).

The most common histological pattern in the present study was found to be hyperplasia without atypia (63%) having closely packed, variable sized and cystically dilated glands showing branching and outpunching. Few of the glands were having back-to-back arrangement, separated by sparse to abundant stroma, causing increase in gland to stroma ratio. Glandular cell nuclei showed pseudo-stratification and uniform outlines, lacking cytologic atypia. Hyperplasia without atypia was also the most common histological pattern in studies by Pilai et al<sup>15</sup> and Rajesh Patil et al<sup>24</sup>, which could be attributed to the fact that most of the patients were perimenopausal having anovulatory cycles. This is in contrast to studies by Shagufta et al<sup>25</sup> and Saraswathi et al<sup>26</sup> showing only 4.9% cases and 6.1% cases of endometrial hyperplasia respectively. The next common histological finding was proliferative endometrium (21%) followed by disordered proliferative endometrium (11%) and secretory endometrium (5%).

In our study, high expression of oestrogen receptors was noted in the endometrium on overall assessment of AUB cases. Mean ER expression in glands was more than mean PR expression in glands which is in accordance with studies by Mostafa et al<sup>27</sup>, Marsden et al<sup>28</sup> and Jiang W et al<sup>29</sup> implying oestrogen receptors are overexpressed in AUB which leads to excessive glandular proliferation. On the contrary, Merla et al<sup>30</sup> observed higher average value of PR than ER for every type of endometrium.

It was also observed that glandular ER and PR were significantly more than stromal ER and PR which is in concordance with other studies<sup>29, 31</sup>. However, Mostafa et al<sup>27</sup>, on studying 30 patients and 30 controls, observed greater expression of progesterone receptors in stroma than

in glands and greater expression of oestrogen receptors in glands than in stroma.

In cases with hyperplasia without atypia, preponderance of oestrogen receptors was noted in glands and stroma as compared to expression of progesterone receptors indicating oestrogen has an important role in etiopathogenesis of endometrial hyperplasia. Studies by Pradyot Singh et al<sup>32</sup>, Masjeed et al<sup>33</sup> and Chakraborty et al<sup>34</sup> reported high level of ER and PR in cases with hyperplasia without atypia, which is in concordance with our study except Sathpathy et al<sup>7</sup>, who reported decreased expression of receptors in hyperplasia without atypia.

In proliferative endometrium, secretory endometrium and disordered proliferative endometrium, expression of ER was found to be more than expression of PR both in glands and stroma. Since the number of cases were less, the statistical significance could not be calculated.

The strength of this study is that significant number of cases with hyperplasia without atypia could be evaluated as per recent WHO classification whereas limiting factor was low number of cases of proliferative, secretory and disordered proliferative endometrium.

#### **Conclusion**

In our study, we found increased expression of oestrogen receptors compared to progesterone receptors in all the histological patterns in patients with AUB. It was also found that ER expression was more in cases of endometrial hyperplasia without atypia than in normal endometrium. This finding was similar to most of the studies indicating their role in etiopathogenesis of endometrial hyperplasia without atypia and could also help to predict the response to hormone therapy in management of endometrial disorders with abnormal bleeding. Assessment of oestrogen and progesterone receptors in abnormal uterine bleeding should be included in armamentarium of workup of these patients, so one can decide the type of medical management depending on expression of oestrogen and progesterone receptors.

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**Conflict of interest:** None. **Disclaimer:** Nil.



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