Original Article

Immunohistochemical Analysis of Estrogen and Progesterone Receptor Expression in Endometrial Biopsies using Allred scoring System for Abnormal Uterine Bleeding in Perimenopausal Women

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Abstract

Background: Abnormal uterine bleeding (AUB) is a prevalent issue among menopausal women. Earlier histopathology alone has been utilized for diagnosis. Now with advent of immunohistochemistry markers such as estrogen, progesterone receptors, these markers in conjunction with histopathology can enlighten on AUB pathomechanism guiding for early diagnosis and treatment. To evaluate knowledge of estrogen, progesterone receptors in endometrium of perimenopausal women experiencing irregular uterine bleeding. **Material and Methods:** To evaluate utility of Allred Score (AS) for hormone receptor assessment while examining histology of AUB in perimenopausal women. Cross-sectional study conducted for 1.5 years. Ninety endometrial biopsy specimens from women who were clinically diagnosed with AUB were taken. Histochemical (H) score and AS were computed utilizing Fischer's exact test etc. p value <0.05 was considered as substantially statistical significant. **Results:** Commonest finding was disordered proliferative phase endometrium (DPPE) with/without glandular and stromal breakdown, observed in 37.7% cases (n=34/90). Mean (SD) Allred score (AS) was significantly higher in benign entities as compared to malignant entities for both ER and PR. Mean AS-ER was 7.19 (SD 2.92) in benign conditions compared to 4.00 (SD 2.00) in malignant conditions (p = 0.001), mean AS-PR score was 7.10 (SD 0.75) for benign conditions compared to 4.17 (SD 1.17) for malignant conditions (p = 0.001). Similar trends were noted for H-scores. **Conclusion:** Study concludes that in perimenopausal women diagnosed with AUB; Allred score, H-score for hormone receptor is useful. As compared to Allred score, H-score showed better prognostic value. Further researches are needed to authenticate these findings.

Keywords: Endometrium, Endometrial hyperplasia, Immunohistochemistry, Endometrial carcinoma.

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Introduction

Abnormal uterine bleeding (AUB) is commonest menstrual irregularity in reproductive age, characterized by heavy bleeding for a prolonged time, accompanied by irregular cycles.[1] AUB etiological factors are endometrial polyp, hyperplasia, malignancy, disturbance in hypothalamic-pituitary ovarian axis.[2] AUB indicate changes in hormonal environment that increases risk of malignancy by leading to Endometrial Hyperplasia [EH].[3] Histopathology is considered in diagnostic evaluation of AUB regardless of woman's age, menstrual cycle duration, exogenous hormone use.[4] Estrogen and Progesterone are steroidal hormones that affect endometrium by interacting with nuclear receptors estrogen (ER), progesterone (PR) receptors respectively and are identified with Immunohistochemistry.[5,6] This Study aims to summarize histopathological pattern & ER/PR expression with immunohistochemistry using Allred score in perimenopausal women with AUB & compare receptor expressions in different AUB patterns. To best of our knowledge, studies from India on Immunohistochemistry using Allred score (AS) in endometrial biopsy specimens are limited so the study was undertaken.

Materials and Methods

Study design and setting: Observational cross-sectional study was carried out at Pathology department of a teaching hospital providing tertiary care in Uttar Pradesh, India, from December 2020 to June 2022. Research was approved by Institutional Ethics Committee with IEC No. TMU/IEC/20-21/096.

Sample size: As it was a time bound study no formal sample size calculation was done, nevertheless 90 number of cases were evaluated for the study.

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Inclusion criteria

Study included perimenopausal women with age ranging from 40-50 years who had an endometrial biopsy sample with clinical diagnosis of AUB.

Exclusion criteria

Individuals with insufficient endometrial samples and those with a history of verified postmenopausal bleeding were not included. Laboratory request form was utilized to collect data regarding demographic and clinical information. Biopsy samples received in Histopathology were fixed with formalin and then paraffin-embedded tissue blocks were obtained according to standard laboratory practices. Three sections were prepared, one for histopathology and the other two for immunohistochemistry for estrogen receptor (ER) and progesterone receptor (PR). Intensity of the immunohistochemical reaction and percentage of stained cells per 1000 cells were assessed at 40x magnification utilizing Allred score. Allred score was computed by summing the intensity score (IS) and percentage score (PS). Intensity scoring was based on the average intensity of the positively coloured nuclei, with grade1 considered as weak intensity, Grade 2: moderate intensity, Grade 3: strong intensity. Proportion scoring was performed as follows: 0 meant that none of nuclei were positively stained, 1 meant that less than 1% of the nuclei were positively stained, 2 meant that 1-10% of the nuclei were positively stained, 3 meant that 10-33% of the nuclei were positively stained, 4 meant that 34-66% of the nuclei were positively stained and 5 meant that more than 67% of the nuclei were positively stained. Another score which is used is H. Score, it yields an overall score (0-300) established on the total percentiles of cells stained weakly, moderately and strongly on an ordinal basis. Statistical analysis: As this was descriptive study, no formal sample size calculation was performed. Statistical package for social sciences (SPSS) software for Windows, version 25.0 (publisher: IBM Corp., Armonk, New York, USA, 2017), was used to conduct the statistical analysis. The demographic and clinical data were summoned using descriptive statistics. Utilizing Shapiro-Wilks and Kolmogorov-Smirnov tests, normality of the data's distribution was attempted. The mean (standard deviation [SD]) was used to confer continuous values, whereas percentage represented frequency for categorical variables. Fisher's exact test (in cases where the anticipated observations in a cell were fewer than n=5) and the chi-square test were used to assess the proportional differences between benign and malignant situations for discrete variables. Utilizing independent Student's t-test, mean difference between two groups for continuous variables were attained. Friedman test was used to compare the two values. All analyses were conducted with a significant threshold of $p < 0.05$.

Results

During the study period, 138 endometrial biopsy samples were received, of which 11 samples were classified as inadequate and 37 were from postmenopausal women. Therefore, altogether 90 samples were permitted in this study. Of which most of samples (n=54/90; 60.0 %) belonged to the 40-45 age group, followed by 34.4 % (n=31/90) from the 46-50 age group and 5.6 % (n=5/90) from the over 50 age group (Table 1). The consistent finding in the biopsy samples was disordered proliferative phase endometrium (PPE) and PPE with glandular and stromal breakdown which was found in 37.7% (n=34/90) of cases followed by Endometrial hyperplasia without atypia was diagnosed in 16 cases (17.8%) and least cases of proliferative phase endometrium (PPE) was noted around 4 cases(4.4%) (Table 2). Mean (SD) Allred score (AS) and H-score were significantly higher in benign conditions compared to malignant entities for both ER and PR. (Table 3). Mean AS-ER was 7.19 (SD 2.92) in benign conditions compared to 4.00 (SD 2.00) in malignant conditions ($p = 0.001$). Similarly, Mean AS-PR score was 7.10 (SD 0.75) for benign conditions compared to 4.17 (SD 1.17) for malignant conditions ($p = 0.001$). Similar trends were seen for H-scores (Table 3). The Allred score for ER was highest for PPE (proliferative endometrium) with normal, disrupted or glandular and stromal breakdown [7.64 (SD 0.80)], followed by hyperplasia without atypia [7.50 (SD 0.50)] and endometrial polyps, including hyperplastic polyps [7.00 (SD 0.92)]. Similar results were observed when performing Allred scoring for the progesterone receptor (Table 4). The highest mean (SD) H-score ER was found in proliferative endometrium exhibiting normal, impaired or breakdown of the stroma and glands [200.00 (SD 25.54)], followed by hyperplasia without atypia [190.30 (SD 32.08)] and endometrial polyps, including hyperplastic polyps [166.42 (SD 37.86)]. Similarly, the highest mean (SD) H-score PR was found in proliferative phase endometrium with normal, impaired or combined glandular and stromal breakdown [187.50 (SD 22.29)], followed by hyperplasia without atypia [179.38 (SD 27.26)] and endometrial polyps, including hyperplastic polyps [162.42 (SD 32.37)] (Table5). It should be noted that the two analyses use different scoring systems and units, so a direct comparison of means may not be appropriate. Nevertheless, both analyses support that proliferative phase endometrium with normal, disrupted or combined glandular and stromal breakdown has the highest expression of ER and PR. The information from these analyses may be useful in understanding the underlying biology of different types of endometrial tissue and may have clinical implications for the diagnosis and treatment of endometrial disease.

[Table 1] Majority of research Population belonged to 40-45 years (60%) followed by 46-50 years (34.4%) and above 50 years is 5.6%

[Table 2] As per histopathological diagnosis least frequency of cases was noted in Proliferative phase endometrium (PPE) around 4.4% (4) and maximum frequency of cases were noted in Disordered Proliferative endometrium (DPP) around 24.4%(22) cases.

[Table 3] The mean Allred score and H-score for ER(estrogen receptor), PR (Progesterone receptor) is higher for Benign lesions as compared to Malignant lesions with substantial statistical significance (p value 0.001)

[Table 4] The mean Allred score for ER (estrogen receptor) was higher 7.64 as compared to Allred score for PR (Progesterone receptor). Maximum Mean Allred score (7.64) for ER (estrogen

receptor) was noted in Proliferative phase endometrium followed by hyperplasia without atypia (Mean allred score 7.50) and least mean allred score for ER was noted in Endometrial carcinoma (3.40)

[Table 5] The mean H score for ER (estrogen receptor) was higher 200.00 as compared to H score for PR (Progesterone receptor). Maximum Mean H score (200.00) for ER (estrogen receptor) was noted in Proliferative phase endometrium followed by hyperplasia without atypia (Mean H score 190.30) and least mean H-score for ER was noted in Endometrial carcinoma (72.0).

Table 1: Distribution of Study Population according to age

Age groups	Frequency	Percent
40-45 years	54	60.0%
46-50 years	31	34.4%
Above 50 years	5	5.6%

Table 2: Histopathological Diagnoses – Frequency and proportions

Diagnosis	Frequency	Percent
Proliferative Phase Endometrium (PPE)	4	4.4%
PPE with glandular and stromal breakdown	12	13.3%
Disordered Proliferative Endometrium (DPP)	22	24.4%
Secretory phase endometrium	10	11.1%
Chronic Endometritis	6	6.7%
Endometrial polyp (including Hyperplastic polyp)	7	7.7%
Endometrial Hyperplasia (EH) without atypia	16	17.8%
Endometrial hyperplasia (EH) with atypia	8	8.9%
Endometroid Adenocarcinoma	5	5.5%
Total	90	100%

Table 3: Percentage Score, Intensity score, Allred score and H score – estrogen and Progesterone Receptors

Score	Benign		Malignant		p-value
	Mean	Standard Deviation	Mean	Standard Deviation	
Estrogen Receptor (ER)					
Percentage Score (PS)	4.65	0.48	2.67	1.03	0.001
Intensity score (IS)	2.54	0.50	1.33	1.03	0.001
Allred Score (AS = PS+IS)	7.19	0.72	4.00	2.00	0.001
H score	184.46	37.54	86.67	42.39	0.001
Progesterone Receptor (PR)					
Percentage Score (PS)	4.65	0.50	2.83	0.75	0.001
Intensity score (IS)	2.43	0.52	1.33	0.52	0.001
Allred Score (AS = PS+IS)	7.10	0.75	4.17	1.17	0.001
H score	171.07	34.13	88.33	48.24	0.001

Table 4: Diagnoses-wise distribution of Allred score

S No.	Diagnosis	Allred Score - estrogen receptor (AS-ER)		Allred Score - progesterone receptor (AS-PR)	
		Mean	SD	Mean	SD
1a 1b 1c	Proliferative phase endometrium Proliferative phase endometrium with Glandular and stromal breakdown Disordered Proliferative Endometrium	7.64*	0.80	7.34#	0.56
2	Secretory phase	6.70	0.46	6.90	0.53
3	Chronic endometritis	6.00	0.00	5.83	0.37
4	Endometrial polyp (including hyperplastic)	7.00	0.92	6.4	0.72
5	Hyperplasia without atypia	7.50	0.50	7.38	0.78
6	Hyperplasia with atypia	6.75	0.66	7.13	0.59
7	Endometrial carcinoma	3.40	1.36	3.80	0.74

*Individual means: 1a – 7.00; 1b – 7.75; and 1c – 7.45

Individual Means: 1a – 5.83 1b – 6.92; and 1c – 7.59

Table 5: Diagnosis wise distribution of H-score

S No.	Diagnosis	H Score -estrogen receptor (ER)		H Score -progesterone receptor (PR)	
		Mean	SD	Mean	SD
1a 1b 1c	Proliferative phase endometrium Proliferative phase endometrium with Glandular and stromal breakdown Disordered Proliferative Endometrium	200.00*	25.54	187.50#	22.29
2	Secretory phase	163.00	19.51	161.50	16.28

3	Chronic endometritis	120.00	15.27	109.17	16.43
4	Endometrial polyp (including hyperplastic)	166.42	37.86	162.42	32.37
5	Hyperplasia without atypia	190.30	32.08	179.38	27.26
6	Hyperplasia with atypia	150.65	37.95	143.75	39.27
7	Endometrial carcinoma	72.0	22.49	64.50	17.60

* Individual means: 1a – 170.00; 1b – 209.50; and 1c – 204.70
# Individual Means: 1a – 185.00 1b – 184.17; and 1c – 190.00

Discussion

Current research demonstrated that proliferative phase endometrium, whether exhibiting signs of breakdown or not, was the predominant histopathologic finding accounting for 42.2% of cases. Endometrial adenocarcinoma enlisted cases were 5.5% of cases (n=5/90). Above clinical findings were in accordance with studies done by Roopmala et al. and Tiwari et al,[7,8] as in the study done by Roopmala demonstrated prominent findings as proliferative phase endometrium in 31% of cases, and adenocarcinoma in 2.87% cases and study done by Tiwari et al. depicted similar findings as proliferative phase endometrium in 30% of cases, and adenocarcinoma in 4% of cases but findings were not consistent with study done by Sajita et al. as they documented endometrial hyperplasia as the most frequent histopathologic finding in 25% of patients.[9] Discrepancies among these findings may be attributed to referral bias and variations in patient demographics across the institutions where the studies were conducted.

Present research depicted significant increase in mean AS (Allred Score) and H scores for ER/PR expression in benign conditions as compared to malignant ones. Additionally present study demonstrated higher ER/PR expression in endometrial hyperplasia without atypia as compared to hyperplasia with atypia, consistent with findings observed by Bhattacharya et al.[10] These findings underscore the importance of diminished ER/PR expression as a prognostic indicator in endometrial disease, similar findings were observed by Shanthalal et al.[11] Furthermore present research demonstrated that proliferative endometrium exhibited significantly higher levels of ER/PR expression compared to secretory endometrium, in focus with findings detected by Mylonas I et al.[12] Current investigation represents one of the few studies to explore immunohistochemical expression utilizing Allred score in endometrial biopsy samples within the Indian context. By employing this method, this study contributes to comprehensive immunohistochemical characterization of endometrial lesions, augmenting existing data within this geographical region. Current study highlights utility of ER/PR expressivity levels as prognostic indicators in distinguishing benign from malignant endometrial conditions. Having these scoring methods of Allred score and H-score for ER / PR have been identified as valuable tools for this purpose, with higher scores correlating with benign disease. These findings underscore the importance of immunohistochemical testing for ER /PR in endometrial samples to inform prognosis and guide treatment decisions, particularly regarding hormonal therapy. Nevertheless, this research is subjected to restriction as sample size is modest in number and all specimens were obtained from one research area. Consequently, generalized findings to broader Indian populace may be constrained and referral bias could have influenced results in current study. To mitigate these constraints and validate our observations, we advocate for future large-scale, multicenter investigations that encompass diverse clinical outcomes.

Conclusion

Immunohistochemical evaluation of ER and PR using Allred and H-Scores is a valuable adjunct in assessing abnormal uterine bleeding in perimenopausal women. These scoring systems can guide prognosis and therapeutic decisions, especially regarding hormonal therapy. Future multicentric studies with molecular integration are required to validate and broaden their clinical applicability.

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Conflicts of interest

There are no conflicts of interest.

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