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Estrogen and progesterone receptor expression and blood vessel density in abnormal uterine bleeding

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Abstract
Aim: The aim of present study was to analyse endometrial estrogen and progesterone receptor expression and blood vessel density in cases of abnormal uterine bleeding.
Material and method: The present study was conducted in the department of Pathology at SMS Medical College, Jaipur among specimens received from 266 patients presenting with abnormal uterine bleeding in reproductive age group (18-45 years) on endometrial biopsies and hysterectomy specimens. The intensity and distribution of ER, PR was evaluated using a semi-quantitative method Allred score. Also density of blood vessels /10 HPF was evaluated.
Results: Neoplastic cases were found among 9.4% (25) of the subjects whereas non-neoplastic lesions were present in 90.6% (241) of the subjects. Mean Allred scoring of estrogen and progesterone receptor in glandular component was highest among endometrial carcinoma. Mean Allred scoring of estrogen receptor in stromal component was highest among Hyperplasia without atypia (5.13±1.58). Mean progesterone receptor in stroma component was highest among Hyperplasia without atypia followed by disordered maturation and endometrial carcinoma.
Conclusion: The results of the present study showed that ER and PR expression are useful investigations. There expression varies in various causes of abnormal bleeding. Future studies with clinical trials could be done to try medical treatment of AUB.

Keywords: Abnormal uterine bleeding, Endometrial Carcinoma Estrogen receptor, immunohistochemistry, hyperplasia, Progestrone receptor

Introduction

Abnormal uterine bleeding (AUB) is the commonest presenting symptom in gynaecology. It occurs in 9-14% of women between menarche and menopause [1, 2], significantly impacting quality of life and imposing financial burden [3].

Estrogen and progesterone receptors belong to the nuclear steroid receptor superfamily the effect of these steroid hormones are thought to be mediated through these receptors. The ER and PR IHC expression and distribution pattern may play an important role in endometrial function and pathogenesis [4]. The study of these receptors distribution in the endometrial glands could open the gate for medical treatment of cases of AUB and avoid unnecessary surgical intervention. The cause of the bleeding may be due to potentiation of the hormonal action through change in their receptor levels [5]. Also there is positive correlation between the endometrial angiogenesis and menstrual disorders. The alternation in blood vessel morphology and density also plays a significant role. Hence the present study was conducted to analyse endometrial estrogen and progesterone receptor expression and blood vessel density in cases of abnormal uterine bleeding.

Materials and Methods

The present prospective cross-sectional hospital based study was conducted in the department of Pathology at SMS Medical College, Jaipur on specimens received from patients with abnormal uterine bleeding presenting with AUB in reproductive age group (18-45 years). The sample size for the present study was 266. The source of data for this study were patients undergoing endometrial biopsy/hysterectomy for complaints of abnormal uterine bleeding presenting in reproductive age group (18-50 years) over a period of 2 years. In the present study IHC was performed among 50 subjects, out of which 25 were neoplastic and 25 were non-neoplastic as control. The subjects were selected according to the following Criteria.

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Inclusion criteria
Hysterectomy and endometrial samples of reproductive and peri-menopausal age groups having abnormal uterine bleeding and patients presenting with menstrual irregularities like, irregular cycles, excessive and prolonged menstrual bleeding.

Exclusion criteria
Inadequate samples, patients with provisional diagnosis of infertility and women on hormone replacement therapy, oral contraceptive pills, intrauterine contraceptive devices or steroidal and non-steroidal anti-inflammatory medications.

Sample handling
Grossing and processing was done on the Hysterectomy specimens and endometrial tissues received. Specimens were fixed in 10% formal saline and then processing was done. Endometrial tissue was fixed as a whole while the hysterectomy specimen was cut and fixed overnight. The tissues were processed routinely to obtain 4-5 µ thick paraffin sections and stained with Haematoxylin and Eosin. Lesions were categorised according to PALM-COIEN.
Sections stained by H & E stain were used for dating of endometrium. Findings were correlated with clinical history to formulate cause of abnormal uterine bleeding. Overall vascularity of endometrium in functional is layer was judged by counting average number of blood vessels in 10 HPFs. Number of vessels was evaluated in all cases on high power using 4µm sections stained with Haematoxylin and Eosin and the photomicrographs were taken at 40x magnification.

Antibody
Primary antibody used for ER and PR was Monoclonal mouse Anti-Estrogen Receptor clone ID5 (Sentier) and Monoclonal mouse Anti-Progesterone Receptor clone PR88 (Sentier) respectively. Expression of ER and PR receptors was observed in endometrial lining and stroma [4].

Scoring criteria
The intensity and distribution of ER and PR was evaluated using a semi-quantitative method - Allred score. In Allred system of scoring, score 0-5 was given to cells depending on the proportion of cells which were stained (proportion score [PS]) and score 0 -3 depending on the intensity of staining (intensity score [IS]). Allred score is (PS + IS = AS) [6].

Statistical analysis
Data collected was tabulated in an excel sheet. The mean and standard deviation of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). Statistical difference between was determined using chi square test and the level of significance was $p < 0.05$.

Results
In the present study, endometrial biopsy and hysterectomy specimen were obtained from 76.3% and 23.7% of the total specimen (266) respectively.
Among the neoplastic cases, Hyperplasia without atypia, Atypical Hyperplasia and endometrial carcinoma was reported among 80%, 12% and 8% of cases respectively.

Disordered maturation, Secretory/Progesterone effect, Proliferative/Oestrogen effect, Non-secretory endometrium, Polyp, Adenomyosis, Leiomyoma and Endometritis were found among 44.81%, 22.41%, 13.28%, 4.98%, 4.56%, 5.39%, 3.32% and 1.24% of the non-neoplastic cases respectively (Table 1).
0-9 blood vessels / 10HPF was revealed among 14.1% of the non-neoplastic cases as compared to 4% of neoplastic cases. 40-49 blood vessels/10 HPF was found among 84% and 17.4% of the neoplastic and non-neoplastic cases respectively (Table 2).
In the present study, average blood vessel density was highest among Hyperplasia without atypia (42.60±3.21) followed by Polyp (38.80±3.78), Endometrial Carcinoma (38±0) and Atypical Hyperplasia (36.71±4.78). The least average blood vessel density was found among disordered maturation (20.17±3.81) followed by different phases of endometrium in context of leiomyoma (22.36±4.16) and non-secretory endometrium (24.51±4.98) as shown in Table 3.
In the present study, mean Allred scoring of estrogen and progesterone receptor in glandular component was highest among Endometrial Carcinoma (7±0, 7.67±2.92) followed by Hyperplasia without atypia (6.33±0.58, 7.67±2.92) respectively. Mean Allred scoring of estrogen and progesterone receptor in stromal component was highest among Hyperplasia without atypia i.e. 5.13±1.58, 6.90±2.92 respectively as shown in Table 4

Table 1: Diagnostic spectrum of cases with AUB (PALM-COEIN)

Spectrum	N	%
Neoplastic cases (9.3%)		
Hyperplasia without atypia	20	80
Atypical hyperplasia	3	12
Endometrial Carcinoma	2	8
Total	25	100
Non-Neoplastic cases (90.7%)		
Disordered maturation	108	44.81
Secretory phase/ Progesterone effect	54	22.41
Proliferative phase/ Oestrogen effect	32	13.28
Non-secretory endometrium	12	4.98
Polyp	08	3.32
Adenomyosis	13	5.39
Leiomyoma	11	4.56
Endometritis	3	1.24
Total	241	100

Table 2: Blood vessels density in Neoplastic and Non Neoplastic categories

Blood vessels/10HPF	Neoplastic		Non-neoplastic	
	n (25)	%	n (241)	%
0-09	1	4	34	14.11
10-19	1	4	98	40.66
20-29	0	0	1	0.41
30-39	1	4	61	25.31
40-49	21	84	42	17.43
40-59	1	4	2	0.83
60-69	0	0	2	0.83
80-90	0	0	1	0.41
Chi square	10.32			
p value	<0.01*			

*: statistically significant

Table 3: Average number of blood vessels in different diagnostic categories/10 HPF

Spectrum	Cases	Average/10HPF
Hyperplasia without atypia	20	43
Atypical hyperplasia	3	37
Endometrial Carcinoma	2	38
Disordered maturation	108	20
Secretory/ Progesterone effect	54	31
Proliferative/ Oestrogen phase	32	34
Non-secretory endometrium	12	25
Polyp	08	39
Adenomyosis	13	33
Leiomyoma	11	22
Endometritis	03	30

Table 4: Mean Allred scoring of estrogen and progesterone receptor expression in glandular and stromal component of different categories

Spectrum	N	ER expression Mean Allred score/8		PR expression Mean Allred score/8	
		Glandular Component	Stromal Component	Glandular Component	Stromal Component
Hyperplasia without atypia	20	6.3	5.1	7.7	6.9
Atypical hyperplasia	3	5.7	3.3	4.3	3.7
Endometrial Carcinoma	2	7.0	2.0	8.0	4.0
Disordered maturation	10	5.5	3.3	7.0	4.5
Secretory/ Progesterone effect	6	4.0	4.0	1.0	1.0
Proliferative/ Estrogen effect	4	4.1	4.1	6.0	2.0
Non-secretory endometrium	1	0	0	4.0	2.0
Polyp	1	6.3	4.7	6.0	4.0
Adenomyosis	1	6.0	2.0	6.0	2.0
Leiomyoma	1	4.8	4.8	6.5	2.0
Endometritis	1	4.3	4.3	4.3	0

Discussion

The present study was conducted to evaluate endometrial estrogen and progesterone receptor expression and blood vessel density in cases of abnormal uterine bleeding. In the present study, maximum AUB cases were in the age group of 31-40 years (45.96%). The mean age of the study subjects was 39.1±6.3 years (range 22-50 years). These results were in accordance with the study done by Singh P *et al.* [7] who revealed that cases presenting with AUB were of 36-45 years of age which accounted for 74.73%. Mean age of presentation was 37.9 years. Mostafa AM *et al.* [8] reported that mean age of the females of the control group was 34.81 years (range 27-40).

In the present study, among the neoplastic cases, Hyperplasia without atypia, Atypical Hyperplasia and endometrial carcinoma was reported among 80%, 12% and 8% of the subjects respectively. These results were in accordance with the study done by Baghel P *et al.* [9]. She reported simple hyperplasia without atypia among 12 cases (5.6%), whereas with atypia was seen only in 3 cases (1.40%). The most common patterns in the present study were disordered maturation followed by Secretory/Progesterone effect and Proliferative/Oestrogen phase. These results in accordance with the study done by Tiwari A *et al.* [10] who found disordered proliferative endometrium was most common (12%) other than normal endometrial patterns.

In the present study, mean blood vessels were highest among Hyperplasia without atypia (42.60±3.21) followed by polyp (38.80±3.78), The least mean blood vessels was found among disordered maturation (20.17±3.81) followed by endometrium in case of leiomyoma (22.36±4.16). Makhija D *et al.* [11] in their study revealed that the endometrial blood vessels showed characteristic changes in various phases of menstrual cycle. They were concentrated more in basal layer in the proliferative phase and were distributed more in functional layer in the secretory phase. The average blood vessels per HPF were 4.471 ± 0.095 in complex hyperplasia and 6.38 ± 0.78 in pill endometrium. Mean blood vessels per HPF were 3.9 ± 0.16 in proliferative and secretory phase and 3.73 ± 0.38 in Non-secretory cases.

In the present study, overall it was observed that ER and PR expression were higher in neoplastic as compared to non-neoplastic cases. Similar results were reported by Armando A *et al.* [12] in their study. High expression in the glandular epithelia indicates a higher sensitivity of these structures to steroid hormones, which may be responsible for the development of benign polyps in the presence of low serum estrogen levels, while malignant polyps appear to be developed by a different etiology.

In our study, there was a significant increase in ER and PR expression in the endometrial glands in patients with AUB. Patients with AUB had a significant higher endometrial thickness and ER and PR levels in the endometrium. Therefore examination of estrogen and progesterone receptor expression is more important than the biochemical examination of the hormone itself because it gives an idea about the actual effects of these hormones. Therefore, it is a useful investigation on which clinical trials could be done to try medical treatment of AUB in reproductive age group.

Conclusion

The results of the present study concluded that endometrial ER and PR expression in endometrium helped in establishing its trend in and providing insight in the pathogenesis of abnormal uterine bleeding. The blood vessel density showed the possibility of quantifying microscopically the morphologic alterations that occur in blood vessels in pathological process and predict angiogenesis in AUB. However morphometric evaluation will aid to better diagnosis and treatment in cases of AUB. Therefore ER, PR expression and blood vessel density are

useful investigations on which clinical trials could be done to try medical treatment of AUB in reproductive age group.

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