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Original Research Article

Histopathological evaluation of abnormal uterine bleeding with immunohistochemical study of significant estrogen receptor expression in endometrium of women with different age groups

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ABSTRACT

Background: Abnormal uterine bleeding is symptom, not disease. Bleeding per vaginum is labelled to be pathological when there is constant irregularity in pattern and flow of bleeding, altered duration or increased amount of menstrual blood. Histopathological examination of endometrium biopsies remains a gold standard diagnostic tool.
Methods: A prospective study was done on 150 patients presenting with AUB over the period January 2022 to December 2022 in the department of pathology, Hind institute of medical sciences, Sitapur, Uttar Pradesh, India. The sections were stained with hematoxylin and eosin and special stain estrogen receptor was used.
Results: Majority of the patients were seen in the perimenopausal age group and most common complaint was menorrhagia in 73 patients (48%). The commonest pathology observed in our study was proliferative phase endometrium in 48 patients (32%). Most of the endometrial carcinoma (4%) were presented after the age of 50 years. The association between histopathological patterns and estrogen receptor category were found to be statistically significant as the $p<0.05$.
Conclusions: Disease burden on a global scale is witnessed by women affected by AUB thus having a detrimental impact. Accurate diagnosis of AUB in women over the age of 35yrs is important to rule out preneoplastic lesions and malignancies so that surgical interventions can be avoided by initiating proper medical management. Comparing the hyperplastic and neoplastic endometrium to that of the proliferative phase, ER expression was lower. Endometrial carcinoma with advanced stage often lacks the expression of the receptors.

Keywords: Abnormal uterine bleeding, Endometrium, Estrogen receptor, Hysterectomy, Carcinoma

INTRODUCTION

Abnormal uterine bleeding is a symptom, not a disease which results in diverse structural and functional etiologies. Mostly the women of reproductive age group present with one of the most common gynaecological problems. AUB may be accompanied by pain, discomfort and loss of productivity which may lead to a significant impact on their physical, social and emotional aspects, directly affecting their quality of life and can result in various systemic medical issues like drugs, endocrine disorders or it can be even related with anovulation, fibroids, adenomyosis or neoplasia.[1]Prevalence of AUB varies in each country. According to national health portal in India, the prevalence of AUB is around 17.9%.[2] Bleeding per vaginum is labelled to be pathological when there is constant irregularity in the pattern and flow of bleeding, altered duration (usually >7 days and irregular) or increased amount of menstrual blood (>80 mL/menses).[3] The endometrium is a dynamic remodeling tissue that responds to circulating blood levels of estrogen and progesterone. Upon being reacted by various stimuli, it may be physiological as well as pathological. Only ultrasonography can be a challenging aspect for

diagnosing endometrial abnormalities, so histopathological examination of endometrium biopsies remains a gold standard diagnostic tool.[4] An invasive surgical intervention, hysterectomy remains the only guaranteed 'no bleed' option to prevent AUB for a patient, but various less invasive procedures like medical therapy and endometrial ablation are also available.[5] Estrogen regulates the human reproductive system's growth, development, and physiology. This kind of receptors are found along the glandular cells as well as in the endometrial stromal cells. The existence of estrogen receptors is usually into important forms named as: Estrogen receptor - alpha and Estrogen receptor-beta.[6] Hormone therapy induces a variety of histologic changes in the endometrium, so it is essentially important to rule out the precancerous and cancerous conditions before starting any hormone replacement therapy. Some studies have found that hormonal exposure may lead to mutational changes of genes (KRAS and PTEN) influencing patterns of endometrial carcinoma.[7,8] Younger women usually present with dysfunctional uterine bleeding and pregnancy related issues whereas atrophy and organic lesions are more frequently noticed in older women.[9] The 60% of the population among women in South-east, Eastern Mediterranean and Africa are anemic due to dietary deficiencies, AUB and multiple pregnancies.[10] Immunohistochemistry offers exact tissue localization of receptors at cellular and sub cellular levels and also helps in assessing their distribution.[11] The hormonal imbalance inside the body of a woman is one of the important factors that is related to the pathogenesis of the dysfunctional bleeding of the uterus. The present study is going to focus on the alterations that can also be studied in a better manner through the combination of immunohistochemical evaluation along with the histological evaluation of endometrium.

METHODS

A prospective study was done on 150 patients presenting with AUB over the period April 2021 to March 2022 in the department of pathology, Hind institute of medical sciences, Sitapur, Uttar Pradesh, India.

Inclusion criteria included women presenting with AUB in all age groups over the age of 18 years.

Exclusion criteria excludes patients with bleeding due to cervical pathology, hysterectomy done for obstetric complication, insitu or intrauterine contraceptive devices.

After a detailed gross examination of the specimen, multiple bits were taken from the representative sites, processed and paraffin block was be made. The specimens were processed in automated tissue processor. The 3-4 micrometer thick sections were taken, stained routinely with H and E Stain and special stain like IHC marker: ER was used. Primary antibody used for estrogen receptor was monoclonal mouse anti-estrogen receptor clone ID-5 (sentier). Expression of estrogen receptor was obtained in endometrial lining of glands and stroma. The IRS score was calculated as follows: IRS=SI×PP. Where SI is the optical staining intensity.

Grade 0=no, grade 1=weak, grade 2=moderate, grade 3=strong staining. Where PP is the number of positive stained cells. The PP was estimated by counting approximately 200 cells and it was defined as 0=no staining, 1=<10%, 2=11-50%, 3=51-80%, 4=>81.9%. Where IRS-Immunoreactive score; SI-optical staining intensity; PP-number of positive stained cells

Ethical approval

The study was carried out following the Helsinki declaration for research on human subjects. Approval for the study was obtained from the institutional ethics committee before the commencement of study. Informed consent was obtained from the patients.

Statistical analysis

The data was entered in Microsoft excel and managed using SPSS software version 16. Analysis was done in the form of percentages and proportions and represented in tables and graphs

RESULTS

A total number of 150 endometrial sample obtained were analyzed for histomorphological pattern. The patients were categorised into reproductive (18-40 year), perimenopausal (41-50 years) and menopausal (>50 years) age groups.

Majority of the patients were seen in the perimenopausal age groups. Frequency distribution of cases according to menstrual history, where 73 subjects were found in HMB i.e., 48.7%, which was the most common complaint, 31 subjects were found in IMB i.e. 20.7%, 11 subjects were found in HPMB i.e. 7.3%, 4 subjects were found in FMB i.e. 2.7 %, 21 subjects were found in PMB i.e. 14.0%, and 10 subjects were found in PMB i.e. 6.7% (Table 1).

The association between histopathological patterns and ER category shows 40 cases with strong expression 3+ positivity (Figure 7), 88 cases with intermediate expression 2+ positivity (Figure 8), 16 cases with weak expression 1+ positivity and 6 cases were negative for ER (Figure 9). The association were found statistically significant as the $p<0.05$ (Table 2).

The commonest pathology observed in our study was proliferative phase endometrium (Table 3), (Figure 2) in 48 subjects (32%) followed by secretory phase in 33 patients (22%) (Figure 3), disordered proliferative endometrium in 11 subjects (Figure 4) and endometrial hyperplasia in 18 subjects (12%) (Figure 5). Most of the endometrial carcinoma (4%) were presented after the age of 50 years (Figure 6).

Table 1: Association between the frequency distribution of the cases according to menstrual history and age interval.

Pattern of bleeding	>18-30 year	31-40 year	41-50 year	51-60 year	>60 year	Total	Percentage (%)
Heavy menstrual bleeding (Menorrhagia)	1	29	41	2	-	73	48.7
Intermenstrual bleeding (Metrorrhagia)	1	12	17	1	-	31	20.7
Heavy and prolonged menstrual bleeding (Menometrorrhagia)	2	3	6	-	-	11	7.3
Frequent menstrual bleeding (Polymenorrhagia)	-	2	1	1	-	4	2.7
Post menopausal bleeding	-	-	11	6	4	21	14
Infrequent menstrual bleeding (Oligomenorrhea)	-	5	3	2	-	10	6.7
Total	4	51	79	12	4	150	100

Table 2: Represent the frequency distribution of histopathological patterns with ER categories.

Variables	ER category				P value
	0- (Negative)	1+ Positive (Weak expression)	2+ Positive (Intermediate expression)	3+ Positive (Strong expression)	
Proliferative phase	0 (0)	0 (0)	18 (20.5)	30 (75)	0.001
Secretory phase	0 (0)	1 (6.3)	24 (38.6)	8 (20)	
Disordered proliferative phase	0 (0)	1 (6.3)	8 (9)	2 (5)	
Atrophic endometrium	0 (0)	4 (25)	9 (10.2)	0 (0)	
Chronic endometritis	0 (0)	0 (0)	9 (10.2)	0 (0)	
Endometrial polyp	0 (0)	3 (18.8)	5 (5.7)	0 (0)	
Endometrial hyperplasia with atypia	1 (16.7)	1 (6.3)	4 (4.5)	0 (0)	
Endometrial hyperplasia without atypia	0 (0)	4 (25)	8 (9.1)	0 (0)	
Menstrual phase	4 (66.7)	0 (0)	0 (0)	0 (0)	
Endometrial adenocarcinoma grade 1	0 (0)	1 (6.3)	2 (2.3)	0 (0)	
Endometrial adenocarcinoma grade 2	0 (0)	0 (0)	1 (1.1)	0 (0)	
Endometrial adenocarcinoma grade 3	0 (0)	1 (6.3)	0 (0)	0 (0)	
Serous endometrial carcinoma	1 (16.7)	0 (0)	0 (0)	0 (0)	
Total	6 (100)	16 (100)	88 (100)	40 (100)	

Table 3: Association between histopathologial pattern and number of patients.

Histopathological pattern	Number of patients	Percentage (%)
Proliferative phase	48	32
Secretory phase	33	22
Menstrual phase	4	2
Endometrial hyperplasia (with and without atypia)	18	12
Disordered proliferative endometrium	11	7
Atrophic endometrium	13	8
Chronic endometritis	9	6
Endometrial polyp	8	5
Endometrial carcinoma	6	4
Total	150	100

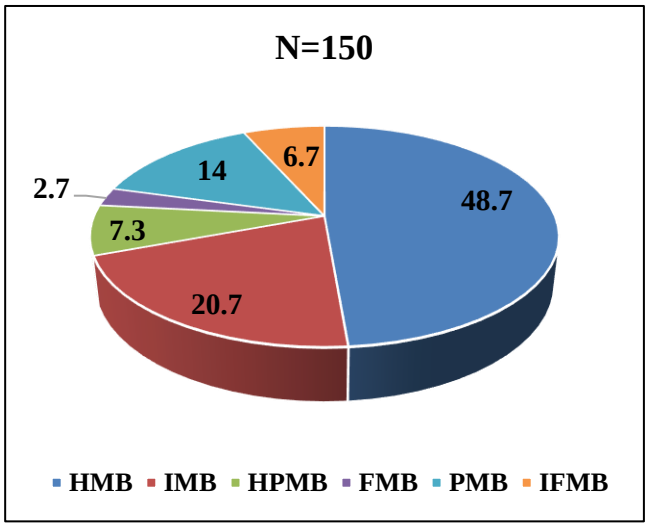

Figure 1: Frequency distribution of the cases according to menstrual history.

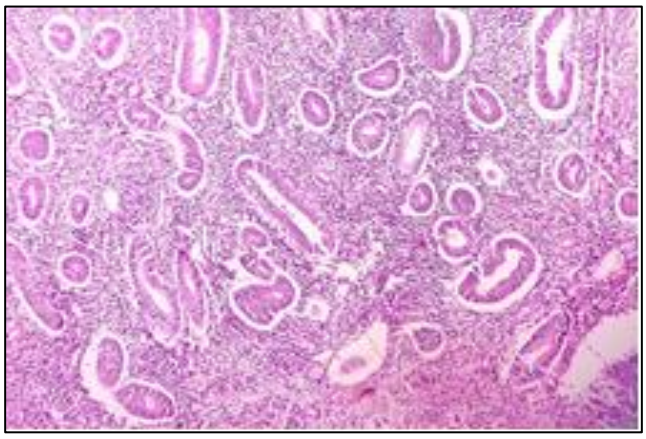

Figure 2: Proliferative phase endometrium.

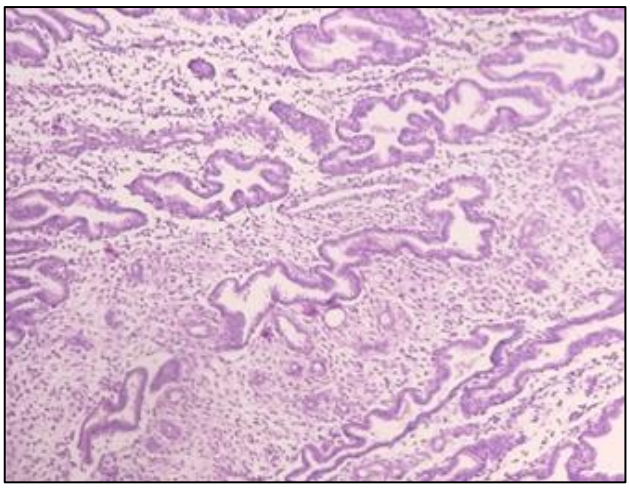

Figure 3: Secretory phase endometrium.

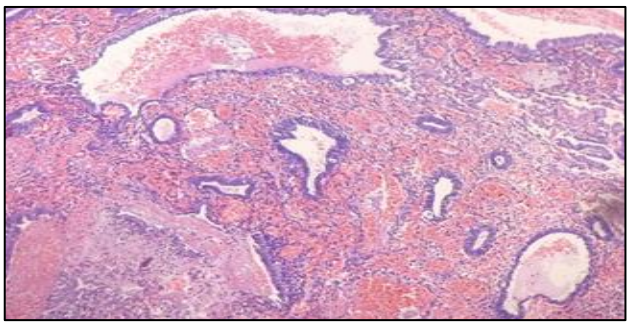

Figure 4: Disordered proliferative phase.

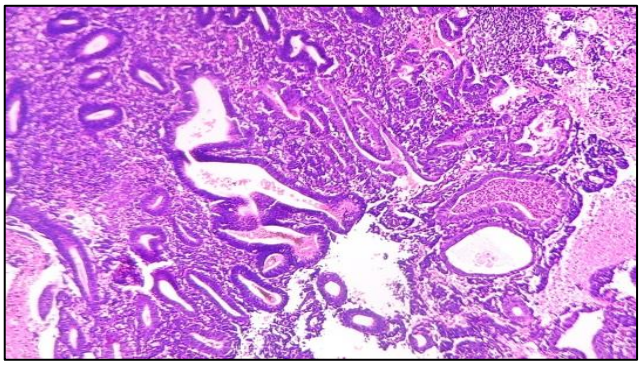

Figure 5: Endometrial hyperplasia without atypia.

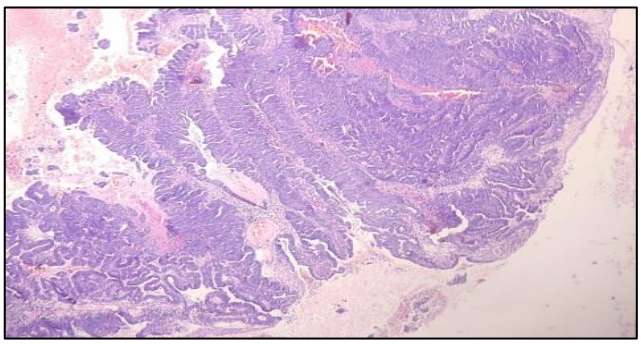

Figure 6: Endometrial adenocarcinoma.

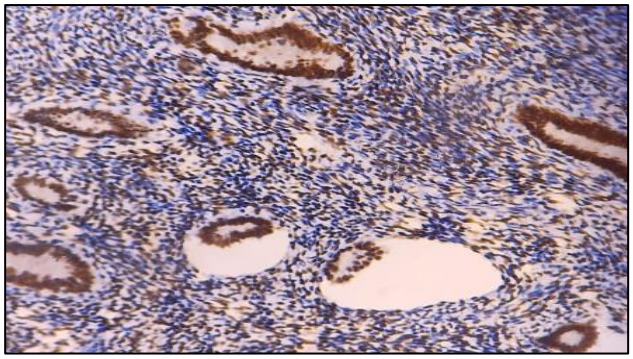

Figure 7: Proliferative endometrium with very strong ER expression.

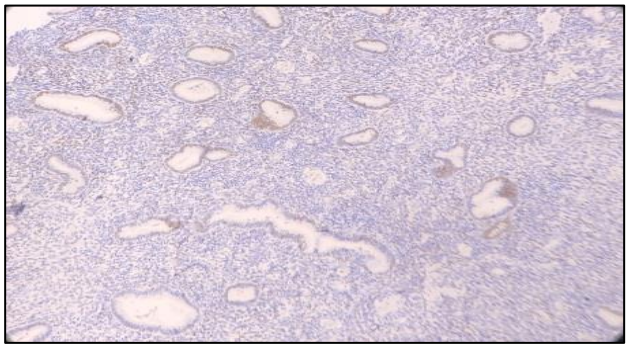

Figure 8: Endometrial hyperplasia without atypia with intermediate expression.

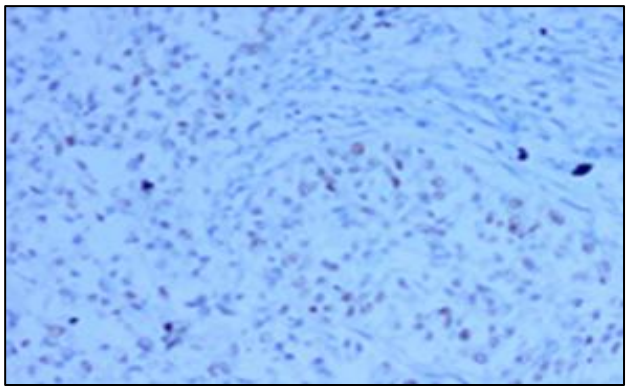

Figure 9: Poorly differentiated (Grade 3) endometrioid carcinoma with weak ER expression.

DISCUSSION

Endometrium is a dynamic hormonally sensitive and responsive tissue and it constantly and rhythmically undergoes changes in the actual reproductive life. It is the mirror of hormonal status and is highly influenced by endogenous hormones and with advanced age the normal physiological functional capacity of endometrium gets altered.[12] Abnormal uterine bleeding was more common among the perimenopausal age group as compared to postmenopausal age in the present study which was in concordance with the results of the study by Samal et al, Muzaffar et al and Singh et al.[1,7,12] Endometrial sampling could be effectively used as the first diagnostic step in AUB. It is a simple, cost-effective and appropriate method that provides accurate diagnostic yield. The highest incidence of AUB was seen in 41 to 50 years age group. According to WHO, perimenopausal is defined as 2 to 8 years preceding menopause and 1 year after the final menses.[13] Various patterns in AUB is the result of shortening of the cycle of the women with increasing age and leading to decline in the number of ovarian follicles and fluctuations in estradiol level resulting in anovulation.[14] In our study Proliferative phase was the most common pattern which correlates with the studies made by Samal et at, Nandkishore et al and Baral et al, whereas secretory phase was predominant finding in Vaidya et al and Singh et al.[1,4,12,16] Endometrial hyperplasia was most common pattern observed in the studies by Sagitha et al (25%) and Riaz et al (26%) but in our study, it's incidence is lower (12%) compared favorably with Dangal et al (10.7%).[6,17,18] Risk of endometrial carcinoma advances with age, in our study incidence is 4%. It was in concordance with study by Mirza et al (5%) and Doraiswami et al (4.4%).[19,21] Carcinoma cases was higher than in studies by Nandkishore et al (1%) and Abdullah et al (1.8%).[4,20] Estrogen receptor status plays prognostic and predictive roles in endometrial cancer. The ovaries regularly manufacture estrogens. The proliferative phase exhibited a greater level of ER expression than the secretory phase. Comparing the hyperplastic and neoplastic endometrium to that of the proliferative phase, ER expression was lower which found similarity in the studies by Ilie et al and Mylonas et al.[22,23] Patients with lack of ER receptor expression in endometrial cancer are typically more aggressive tumours and have a lower chance of survival so the estrogen receptor positivity expression is positively associated with the prognosis of endometrial carcinoma. Endometrial carcinoma with advanced stage often lacks the expression of the receptors.

Limitations

Few of the limitations in the study was due to inadequate samples received from the clinical department because of improper sampling is also a source of discomfort for the patient as the sampling has to be repeated and the intensity of ER expression is subjective to assess, especially between strong (2+) and very strong (3+). Thereby recommending that hysteroscopy should be used in AUB in perimenopausal age group. It should be the part of basic investigations of AUB in perimenopausal age group more so in cases where no pathology was found in sonography. Added advantage of hysteroscopy is the simultaneous treatment of endometrial lesions.

CONCLUSION

Disease burden on a global scale is witnessed by women affected by AUB thus having a detrimental impact. Accurate diagnosis of the causative factor of AUB in any age group, especially in women over the age of 35yrs is

of importance to rule out preneoplastic lesions and malignancies. Immunohistochemistry reveals that ER expression decreases progressively as we go from the proliferative pattern through hyperplasias to adenocarcinoma. The proportion of ER positive patients in each group was investigated in this qualitative investigation. Surgical interventions can be avoided by initiating proper medical management.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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