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# A STUDY OF HISTOPATHOLOGICAL SPECTRUM OF ENDOMETRIAL LESIONS IN ABNORMAL UTERINE BLEEDING WITH ANALYSIS OF EXPRESSION PATTERN OF ER, PR AND KI-67.

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ABSTRACT

**Introduction:** Abnormal uterine bleeding (AUB) is defined as bleeding that does not correspond to the normal pattern of menstrual cycle. It is one of the most frequently encountered gynaecological problem. A spectrum of histologic changes are noted in the endometrium in AUB which vary according to the age of the patient and includes functional pattern or organic lesions. Histopathologic study of endometrial tissue is a major diagnostic tool in patients presenting with AUB.

**Aim:** To study the various morphologic pattern of endometrial lesions in patients with abnormal uterine bleeding and to analyse the Estrogen receptor(ER), Progersterone receptor(PR) and Ki-67 expression in samples showing hyperplasia and carcinoma.

**Method:** This is a prospective study of 70 endometrial specimens from patients diagnosed clinically as having AUB. The study was done in the department of Pathology JNIMS from January 2017 to June 2019. The samples underwent routine processing and were stained with Hematoxylin and eosin. Immunohistochemistry for ER,PR,KI-67 were done on samples showing hyperplasia or carcinoma.

**Result:** Out of the 70 cases studied, maximum number of cases were seen in the age group of 41-50 years. Age of the patients ranged from 18 to 72 years. In premenopausal women the most common histopathologic pattern was proliferative endometrium (32.7%). In post menopausal women atrophic endometrium was the most frequent pattern(27.7%). Endometrial hyperplasia was noted in 12 cases (17.1%) and there were 3 cases(4.3%) of endometrial carcinoma. ER and PR were positive in all cases of endometrial hyperplasia and 2 cases of endometrial carcinoma. High Ki-67 expression was noted in all the 3 cases of endometrial carcinoma.

**Conclusion:** Histopathologic evaluation of endometrial samples is necessary for deciding the appropriate therapeutic strategy. A wide spectrum of lesions from proliferative endometrium to carcinomas were noted in these patients.

## Introduction

Abnormal uterine bleeding (AUB) is one of the most common and significant presenting complaint in the gynaecology clinic[1,2,3]. It causes considerable morbidity and affects the patient significantly.[3] AUB is responsible for as many as one third outpatient visit to the gynaecology clinic[2]. Abnormal uterine bleeding is generally used to describe any pattern of bleeding from the genital tract that deviates from the normal menstrual cycle with regards to duration, amount, frequency or cyclicity[1,3]. The common presenting complaints are menorrhagia, polymenorrhoea, metrorrhagia and intermenstrual bleeding[1,3].The causes of AUB are varied and include functional causes like normal cyclical endometrium, atrophic endometrium, weakly proliferative endometrium and disordered proliferative endometrium or organic lesions like fibroids, polyps, chronic endometritis, hyperplasia, carcinomas and pregnancy related bleeding. It can also be a result of systemic diseases such as endocrine disorders, disorders of hemostasis or drugs[1,4]. Histopathologic examination is the gold standard procedure for assessment of abnormal uterine bleeding.[1,2,4].

Estrogen (ER) and progesterone (PR) receptors are present in endometrial stroma as well as endometrial glands. They belong to the nuclear steroid receptor superfamily. Detection of ER and PR on tissues can be used as an adjunct to histopathologic examination of AUB. They offer the advantage of tissue localisation and helps in analysing tissue distribution and intensity in stromal and glandular cells[5]. Study of ER and PR expression may be useful in offering evidence based treatment to the patients and also avoid surgical procedures.[5] Loss of ER and PR are also noted in cases of endometrial carcinoma with decreased expression as lesion progressed from endometrial hyperplasia to endometrial carcinoma[6].

Ki-67 is a proliferation marker which is an independent prognostic marker in endometrial carcinomas and its positivity is shown to increase as the severity of endometrial lesion progresses from endometrial hyperplasia to endometrial carcinoma.[6]

The aim of our study was to identify the various histomorphologic patterns of endometrium in patients of AUB and to analyse the expression pattern of ER, PR and Ki-67 on samples exhibiting endometrial hyperplasia and carcinomas. Moreover there are few such studies from the northeastern part of India

## MATERIALS AND METHODS

This study is a prospective analysis of 70 cases of endometrial specimen in patients clinically diagnosed as suffering from abnormal uterine bleeding over a period of 2 years and 6 months from January 2017 to June 2019 in the department of pathology JNIMS, Imphal, India.

Endometrial tissue were obtained from endometrial biopsy or curettings as well as hysterectomy specimens and fixed in 10% formalin, paraffin embedded and stained with hematoxylin and eosin for microscopic examination.

Cases showing features of endometrial hyperplasia and carcinoma were subjected to immunohistochemistry stain for ER,PR and Ki-67 using avidin-biotin complex peroxidise method and microwave antigen retrieval. Evaluation of ER and PR was done according to the method described by Carcangui et al[7] which is based on the percentage of stained cells and the intensity of nuclear stain. The percentage of positive cells are graded as 1(0%-25% of nuclei stained),2(26% -75%nuclei stained) and 3(>76% of nuclei stained).The staining intensity was scored as 1(absent or weak), 2(strong) and 3(very strong). The sum of both parameters gave the immunohistochemical score. Category I

corresponded to score of 2, category II to a score of 3 or 4 and category III to 5 or 6.

Ki-67 immunoreactivity was calculated according to the percentage of positive cell nuclei. The nuclear staining for Ki-67 was graded by counting Ki-67 labelling index(KI-67 LI). Ki-67 LI was recorded as % of positively stained tumour nuclei in 1000 tumour cells in the hotspot of the tumour. Tumour cells with Ki-67 LI of 5% or more was considered positive. Appropriate controls were used for the immunostaining.

Patients were divided into premenopausal (18-50 years) and post menopausal (>50years) groups along with categorisation in to different age groups.

Inclusion criteria: All patients diagnosed clinically as having abnormal uterine bleeding and who underwent endometrial curettage/biopsy or hysterectomy between January 2017 to June 2019 .

Exclusion criteria: patients with bleeding due to cervical pathology, vaginal pathology, bleeding disorders and pregnancy related complications such as abortions, gestational trophoblastic disease or ectopic pregnancy.

Datas collected were entered in MS Excel spreadsheet 2017 and checked for consistency. Descriptive statistics like mean and proportions were calculated. Ethical clearance was taken from Instituitional ethics committee. All identifiers were removed and strict confidentiality was maintained for all collected data.

## RESULTS

In our study the age of patients ranged from 18 to 74 years with mean age being 48 years. The maximum number of cases were seen in the age group of 41-50years (28/70, 40%) followed by 31-40 years (18/70,25.7%).(Table 1).

**Table1: Age distribution**

| Age (years) | Number of cases out of total 70 cases | Percentage (%) |
|---|---|---|
| <20 | 01 | 1.4% |
| 21-30 | 05 | 7.1% |
| 31-40 | 18 | 25.7% |
| 41-50 | 28 | 40% |
| 51-60 | 12 | 17% |
| >60 | 06 | 8.6% |

Out of 70 patients, 52 cases were premenopausal (74.3%) whereas 18(25.7%) were postmenopausal women. In the 52 cases of AUB in premenopausal women the most common histopathologic finding was proliferative endometrium (32.7%,17/52) followed by endometrial polyp (21.1%, 11/52)). The other diagnoses obtained were hyperplasia (15.4%,8/52), chronic endometritis (13.5%, 7/52), secretory endometrium (7.7,4/52), disordered proliferative endometrium (7.7%,4/52) and endometrial carcinoma (1.9%,1/52).

In the postmenopausal group(18 cases), the most common histopathlogic finding was atrophic endometrium (27.8%,5/18) closely followed by both hyperplastic endometrium (22.2%,4/52) and endometrial polyp (22.2%,4/52).The remaining cases were comprised of endometrial carcinoma (11.1%,2/18),chronic endometritis (11.1%,2/18) and disordered proliferative endometrium (5.5%,1/18).

Overall, proliferative phase endometrium (24.3%,) was the most common histopathologic finding followed by endometrial polyp (21.4%) and endometrial hyperplasia (17.1%). Amongst the 12 cases of hyperplasia there were 9(75%) cases were of simple hyperplasia without atypia, 1case of simple hyperplasia with atypia (8.3%) and 2 cases of complex hyperplasia without atypia(16.7%). We did not encounter any case of complex atypical hyperplasia. Endometrial hyperplasia was most commonly seen in the age group of 41-50 years.(Table 2)

**Table 2: Overall distribution of endometrial pattern on histopathological examination**

| Pattern | No of cases | Percentage (%) |
|---|---|---|
| Proliferative phase | 17 | 24.3% |
| Secretory phase | 04 | 5.7% |
| Hyperplasia | 12 | 17.1% |
| Atrophic endometrium | 05 | 7.1% |
| Disordered proliferation | 05 | 7.1% |
| Chronic endometritis | 09 | 12.9% |
| Endometrial polyp | 15 | 21.4% |
| Endometrial carcinoma | 03 | 4.3% |

We encountered 3(4.3%) cases of endometrial carcinoma (Fig1) in total and it was seen more commonly in the postmenopausal age group (2 cases).

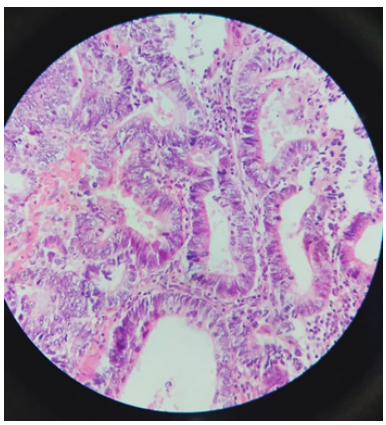

**Fig 1:Photomicrograph showing Endometrial carcinoma (H & E, 100X)**

ER and PR were positive in all cases of endometrial hyperplasia and 2 cases of endometrial carcinoma-endometrioid type (well differentiated stage 1B) (Fig 2)

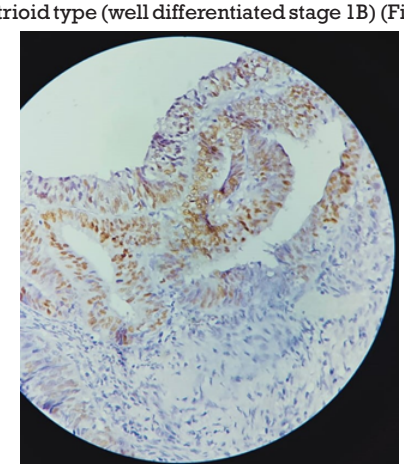

**Fig 2: Photomicrograph showing PR positivity in endometrial carcinoma, 400X)**

ER and PR were negative in one case of endometrioid carcinoma (stage III C1)

All cases of simple hyperplasia without atypia show a low (<5%) Ki -67 expression

Intermediate Ki-67 expression (16-30%) was seen in a case of simple hyperplasia with atypia and all cases of complex hyperplasia without atypia.

High Ki-67 expression (Fig 3) were seen in all the three cases of carcinoma (Table 3).

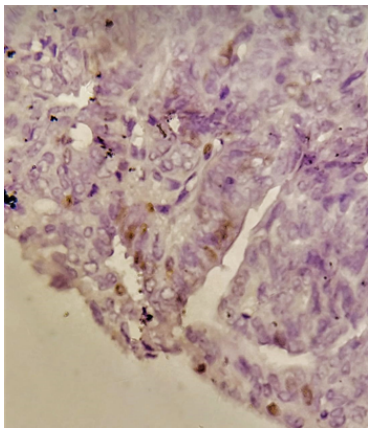

**Fig 3: Photomicrograph showing Ki-67 positivity in endometrial carcinoma,400X)**

**Table 3: Expression patterns of ER, PR and Ki-67.**

| Lesion type | No of cases | ER+/ PR+ | ER- /PR- | Ki-67+ |
|---|---|---|---|---|
| Simple hyperplasia without atypia | 9 | 9 | - | Low |
| Simple Hyperplasia with atypia | 1 | 1 | - | Intermediate (16-30%) |
| Complex hyperplasia without atypia | 2 | 2 | - | Intermediate (16-30%) |
| Endometrial carcinoma | 3 | 2 | 1 | High (>30%) |

## DISCUSSION

Abnormal uterine bleeding(AUB) is a frequently encountered problem in the gynaecology department.AUB refers to any divergence from the normal menstrual cycle pattern. It can be a result of functional causes or organic lesions.AUB can present with menorrhagia, metrorrhagia, polymennorhea, peri and post menopausal bleeding[1,2,4]. Histopathologic study of endometrial tissue is an important diagnostic tool. Immunohistochemistery for ER,PR and KI-67 can be a useful adjunct to understand biologic behaviour and planning of individual treatment strategies[6].

Abnormal uterine bleeding without structural pathology is seen in reproductive women of all ages but is more common in adolescent and perimenopausal women.[8] In the perimenopausal years anovulatory cycles are very frequent which leads to changes in the endometrium which in turn results in irregular bleeding[9]. Perimenopause is defined by World Health Organisation (WHO) as the 2 to 8 years preceding menopause and the 1 year after the final menses.[10]

In our study the maximum number of cases (40%)were from the perimenopausal age group(41-50years) which is compatible with the studies carried out by Bolde SA et al, Vaidya S et al, Doraiswami S et al and Jairajpuri ZS et al[11,12,13,14].

Clusters of cases in this age group could be due to decreased ovarian function resulting in low estrogen level which can no longer mantain the endometrial growth. Further a lesser number of patients were seen in patients above 60 years (8.6%) which may be due to early diagnosis and treatment.

In our analysis the overall predominant histologic pattern noted was proliferative endometrium(24.3%). Our finding was in tandem with the studies of Bolde SA et al[11](22.8%), Khare A et al[15](26.8%) and Saera Afghan et al[16](34.6%). This was followed by endometrial polyp (21.4%) and endometrial hyperplasia (17.1%)in our study. Bolde SA et al[11] found endometrial hyperplasia as the second most common pattern (19.40%).

This study found that the occurrence of endometrial hyperplasia was highest in the perimenopausal group (41-50years) constituting 58.33%. Similarly, Abdullah LS et al[2], Sajeetha KR et al[17] and Bhatta S et al[18] found endometrial hyperplasia as the leading cause of AUB in this age group.

In our study, proliferative endometrium was the major cause of AUB in the perimenopausal age group(41-50years) accounting for 47% of cases. Our study is comparable to the findings of Salvi Anuradha et al[19](53%) and Damle Rajshree P et al[20](34%). However Bhatta S[18] et al reported a lower value of 29.16%. Proliferative endometrium was also a predominant finding in 31-40 years (35.2%) in our study which is comparable to the study done by Doraiswami S et al[13] who reported 33.3%.

In the present study atrophic endometrium(27.8%) was the major finding in the postmenopausal age group followed by hyperplastic endometrium(22.2%). Our findings are compatible with the studies conducted by Salvi Anuradha et al[19] (atrophic endometrium-30.7% and hyperplasia-26.1%), Damle Rajshri P et al[20] (atrophic endometrium-25.8%, hyperplasia-19.4%). Sclerotic degeneration of the vessel wall or local abnormal hemostatic mechanism is considered as the major mechanism of bleeding due to atrophic endometrium.

We found 3 cases of endometrial carcinoma in our study (4.3%) where one case was in noted in the 41-50 age group and 2 cases were in the postmenopausal age group. The findings are comparable to those of Doraiswami Set al[13](4.4%) and Khare A et al(3.7%.) Prajabati Rujuta et al[21] however reported only 0.9% cases in this age group . The different observations may be explained by the more patient representation in the peri and post menopausal age groups and also consideration of only endometrial cause in our study.

Estrogen receptor(ER) and Progersterone receptor(PR) expression by the endometrium decreases as the lesion progresses from endometrial hyperplasia to endometrial carcinoma. In the present study, ER and PR positivity were seen in all cases of endometrial hyperplasia (100%) and 2 cases (66.7%) of endometrial carcinoma. These findings were in tandem with the findings of Orejuela FJ et al[22]. Bozdogan O et al[23] observed estrogen positivity in 96.1% of hyperplasia and 86.3% of carcinomas whereas all cases of hyperplasia (100%) and 90.9% of carcinomas were positive for progesterone receptors. Stoin SC et al[24] observed decreased ER and PR expression in advanced stage cancers compared to well differentiated and early stage cancer.

Ki-67 a well established cell proliferation marker has been used widely in analysing various endometrial lesions associated with AUB. In the present study, high Ki-67 labelling index was seen in all cases of endometrial carcinoma whereas negative or lo Ki-67 labelling index was observed in all cases of simple endometrial hyperplasia without atypia. Moderate Ki-67LI was seen in cases of complex hyperplasia .CR Shevra et al[25] also found high Ki-67 labelling index in 100% of endometrial carcinoma and in 77.65% of endometrial hyperplasias. Zidaan AA[26] et al observed increased Ki-67 positivity in endometrial carcinoma compared to endometrial hyperplasia.

Our study is limited by a small sample size. Study involving a larger sample size may provide better insight into the the different endometrial pathology in patients clinically presenting with AUB.

## CONCLUSION

Abnormal uterine bleeding is a common gynaecological problem with significant patient morbidity and has a variety of causes according to the age. Histopathologic evaluation of endometrial samples is an important diagnostic tool in AUB. Our study revealed that proliferative endometrium was the

most common endometrial pattern seen in AUB followed by endometrial polyps.

Incorporation of ER, PR and Ki-67 immunostains in histopathological examination of endometrium in AUB patients will help in understanding its biological behaviour which in turn could help in individual treatment strategies and prognostication.

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