



Research Article

Histomorphological Patterns of Endometrium in Abnormal Uterine Bleeding and Ki-67 Expression

Authors

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Abstract

Background: Abnormal uterine bleeding (AUB) is a common condition affecting the women of reproductive age that has significant social and economic impact.

In developing countries like India, AUB affects about 5 to 15% of women in reproductive age group and it is increased in older women. Accurate diagnosis of the causative factor of AUB in this age group is of utmost importance so that appropriate management can be established.

Primary Objective:

To evaluate different clinical presentations of AUB in females of the age group of 40-60 years and to study the histomorphological pattern of endometrium in these cases.

Secondary Objective:

To observe Ki- 67 expression in endometrial glands in these conditions.

Keywords: Abnormal uterine bleeding, organic and inorganic causes, endometrium, Ki- 67.

Introduction

Abnormal uterine bleeding is the most common and frequent presenting complaint in Gynaecology Outpatient Department in all age groups, especially in perimenopausal women¹. The endometrium which lines the uterine cavity is one of the most dynamic tissues in the human body. It is characterized by cyclic processes of cell proliferation, differentiation and cell death in response to sex steroids elaborated in the ovary². Abnormal uterine bleeding with different menstrual

patterns has significant effect on women's health, quality of life and ultimately social assimilation suggesting that need of proper evaluation and initiation of treatment should be given a privileged priority¹. In the peri menopausal age variation in normal cyclical pattern may be due to physiological hormonal changes or pathological changes.

Ultrasonography is an appropriate relevant radiological diagnostic tool to identify structural abnormalities in uterus and adnexae. Hysteroscopy guided endometrial biopsy is the preferred

diagnostic technique to detect endometrial pathology, but due to lack of affordability, endometrial biopsy obtained by Dilatation and Curettage (D&C) in developing countries. Accurate diagnosis of the causative factor of abnormal uterine bleeding (AUB) in this age group is of utmost importance so that appropriate management can be established¹.

Perimenopause is defined as the “period around the onset of menopause that is often marked by various physical signs such as hot flushes and menstrual irregularities”. Otherwise perimenopause is also defined as “the coming off from the reproductive years”³. One third of the patient visits to the gynaecologist are for Abnormal Uterine Bleeding and it accounts for more than 70% of all gynaecological consults and in the peri menopausal and postmenopausal years.

A thorough evaluation of patients is important for two main reasons;

- 1) To exclude serious pathology such as carcinoma or complex atypical hyperplasia and
- 2) To identify the cause of bleeding so that proper therapy can be embarked upon.³

Materials and Methods

Type of Study: Observational, Descriptive study

Period of Study: November 2017 TO May 2019

Study Setting: Department of Pathology, Govt. Medical College, Kottayam

Study Population: First 94 cases of endometrial curetting presenting with AUB in the age group 40–60 years which are received in the Department of Pathology, Government Medical College, Kottayam during the study period (November 2017 to May 2019).

Sample size: $N=4pq/d^2$

p = proportion in previous study

q = 100-p

d = allowable error

Proportion p (from previous study was taken based on the clinical prevalence –“Study of Histopathological Pattern of Endometrium in

Abnormal Uterine Bleeding in the Age Group 40-60 Years” by Junu et.al in 2014⁴) was 51.42%.

So, $p=51.42, q=100-51.42 =48.58$.

Taking allowable error $d=10.284$

Sample size, $N=94$

Research Hypothesis

The histomorphological patterns of endometrium in abnormal uterine bleeding are similar in the age group of 40 to 60 years.

Sampling Method: Continuous sampling

Inclusion Criteria

AUB is defined as bleeding from the uterine corpus that is abnormal in volume, regularity and /or timing and has been present for the past 6 months.

- 1) Patients with isolated endometrial causes of AUB of 40 to 60 years of age group

Exclusion Criteria

- 1) Those with lesions of myometrium, vagina and cervix (obtained from the details on the USG report)
- 2) Insufficient tissue samples (blood clots, mucin only- no sufficient endometrial tissue)

Study Tools

- 1) Clinical proforma
- 2) Reagents for Haematoxylin and Eosin staining
- 3) Reagents for doing Ki-67 antibody IHC staining.

Study Procedure

Endometrial curettage tissue samples received in the department of pathology for evaluation are collected. Relevant clinical data is taken from the request forms attached along with the specimens. All the specimens will be fixed in 10% formalin, processed and embedded in paraffin, and 3-4 micron thick sections to be made. Sections to be stained with Haematoxylin and Eosin stain (H&E). Immunohistochemistry to be performed on all slides using Ki-67 as primary antibody by Standard Operating Procedure. The percentage of immunopositive nuclei in glandular epithelium to be evaluated.

Table 1

Grading of Ki-67	intensity of staining
0	Absent
1+	Weak
2+	Moderate
3+	Strong
4+	Very strong

Brown staining of the nucleus is considered as positive for Ki-67. Proportion of stained cells in the endometrial glands are evaluated manually counting 10 consecutive high power fields and assigning to them one of the following categories;

Table 2 Proportion scoring for Ki-67

score	Positive cells
0	<5%
1	5-25%
2	26-50%
3	51-75%
4	76-100%

Mean value of proportion score is calculated for each histomorphological pattern of endometrium =

Total of proportion score

Number of cases

Weighted score = intensity score X proportion score

Ki 67 usually stains cells with strong intensity, so intensity score is kept as grade 4. So for Ki -67, 4 is kept constant and Ki-67 weighted score is based on proportion.

Mean score = total weighted score/sample size.⁵

Data Management and Analysis

The data was entered in Microsoft excel and further statistical analysis was done using SPSS software (version 24).

The statistical methods used were;

1. Mean, frequency and proportion for

- 1) Age
- 2) Parity
- 3) Menstrual symptoms
- 4) Histopathology of various endometrial lesions studied
- 5) Ki 67 scoring in various endometrial lesions studied.

2. Strength of association between Ki 67 scoring and cyclical endometrium as well as endometrial hyperplasia and carcinoma was calculated using Fisher's exact test (p value).

Consent

This study was conducted on endometrial curettage samples received in Department of Pathology. Hence informed consent was present.

Results

The present study was conducted on specimens of endometrial curettings of 94 patients who presented with AUB in the age group of 40-60 years, received in the Department of pathology, Government Medical College, Kottayam during the study period. Among the various age groups divided among the study group, with an interval of 5 years, maximum peak was observed in the age group of 40-45 years. Mean age calculated was 49 years. The maximum number of cases belonged to Para 2(62%) followed by belonged to Para 3(18%). The main presenting symptom was menorrhagia (43.6%) followed by postmenopausal bleeding (34.1%).

In the current study, organic morphological changes were observed in 39.4% of the endometrial biopsies and rest were non organic type endometrium (60.6%). The most common pattern was disordered proliferative endometrium followed by endometrial carcinoma, while the least common were atrophic endometrium and endometritis respectively.

Among the various non-organic histomorphological patterns of endometrium, the most common pattern was found to be disordered proliferative endometrium (28%) followed by proliferative endometrium (16%).

Among the organic conditions of AUB, the most common pattern was endometrial carcinoma (20%), followed by endometrial hyperplasia (11%).

Among the total 10 cases of endometrial hyperplasia, 6 cases belonged to endometrial hyperplasia without atypia while 4 cases belonged to endometrial hyperplasia with atypia.

High Ki 67 mean score was observed in endometrial carcinoma, endometrial hyperplasia and disordered proliferative endometrium. The Ki 67 mean score

value was 3.2 in proliferative endometrium and 2.3 in secretory endometrium. The associations of the mean values of Ki 67 proportion score with proliferative and secretory endometrium was calculated and it showed that it was found statistically significant ($p < 0.001$)

The associations of mean values of the Ki 67 proportion score with benign and neoplastic lesions of AUB was calculated and it was found to be statistically significant ($p < 0.001$).

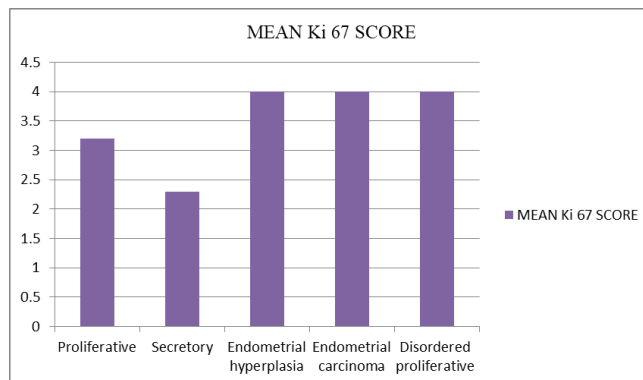


Figure 3: Mean Ki67 score in various endometrial lesions of AUB

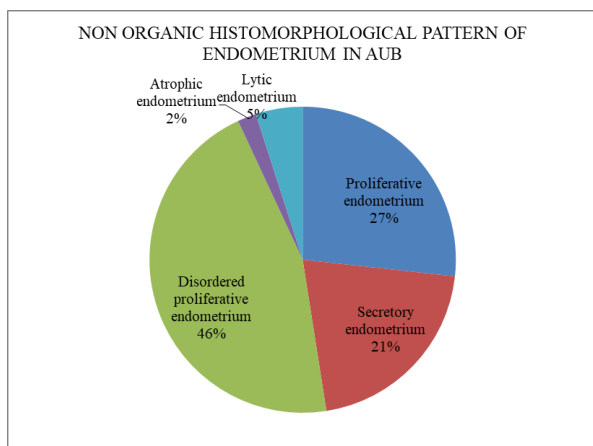


Figure 1: Distribution of different non organic histomorphological pattern of endometrium in AUB among the study group

Disordered proliferative endometrium

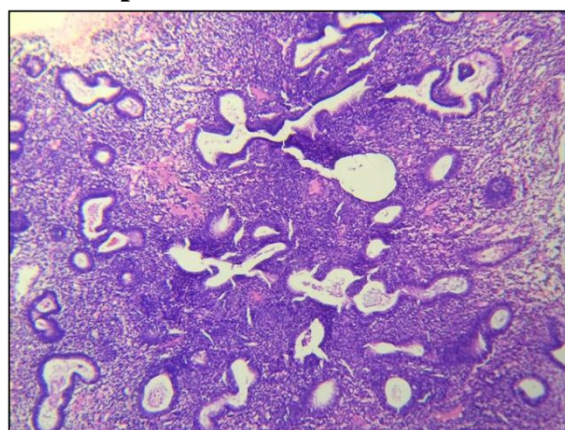


Figure 4: Histopathology (H&E stain, 10X)

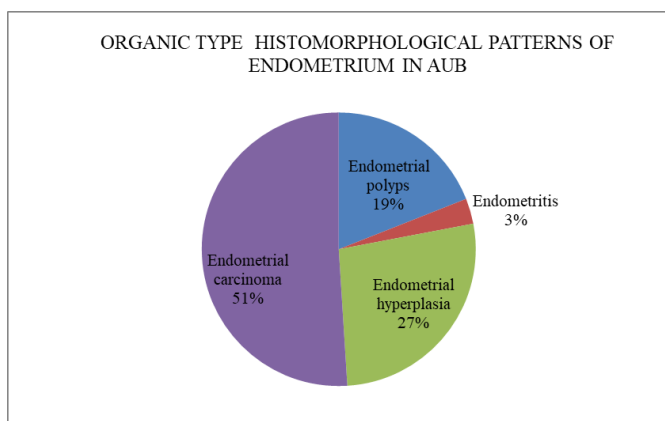


Figure 2: Distribution of different organic type histomorphological pattern of endometrium in AUB among the study group

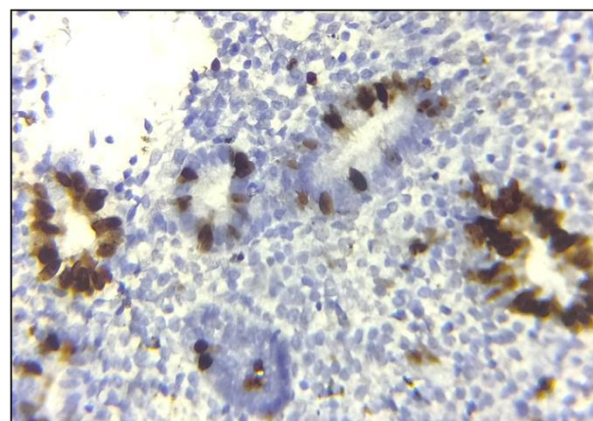


Figure 5: Section showing Ki 67 positivity in nucleus (40X) (score 2)

Endometrial Hyperplasia

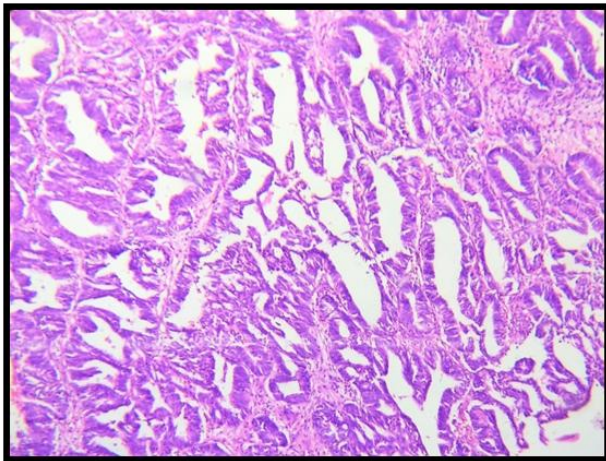


Figure 6: Endometrial hyperplasia with atypia (H&E, 10X)

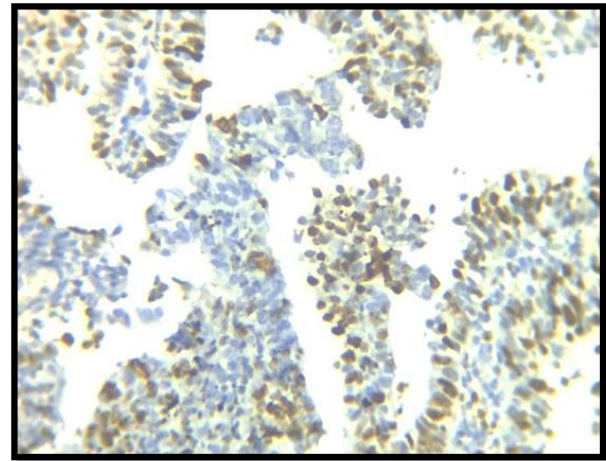


Figure 9: Section showing Ki 67 positivity in nucleus (40X) (score 4)

Proliferative Endometrium

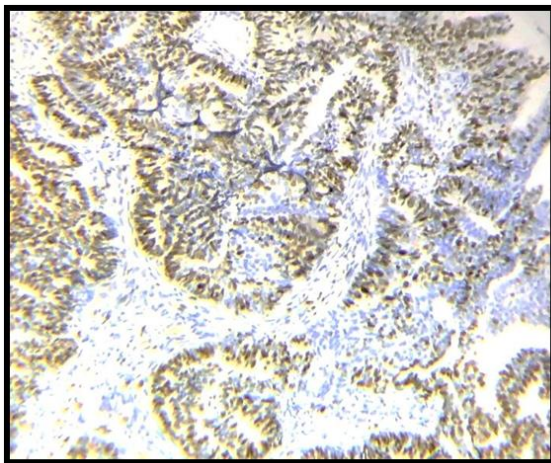


Fig 7: Ki 67 positivity in nucleus (40X) (score 4)

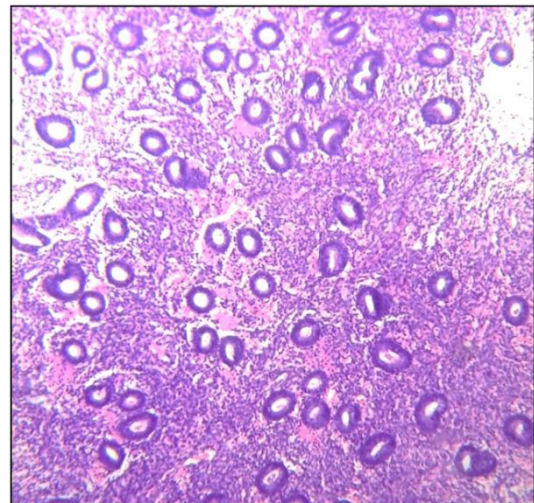


Figure 10: Histopathology (H&E stain, 10X)

Endometrial Carcinoma

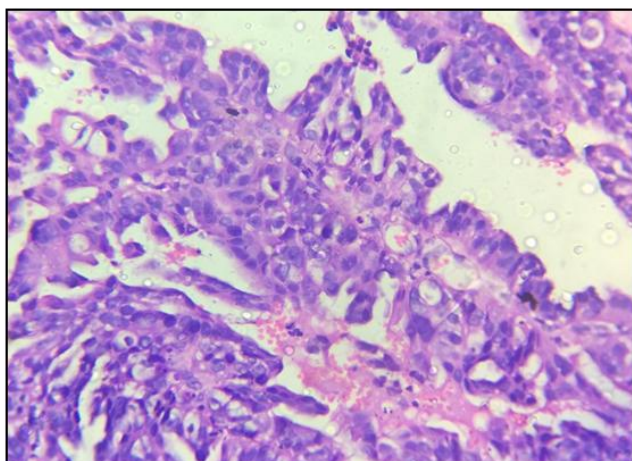


Figure 8: Histopathology (H&E stain, 40X)

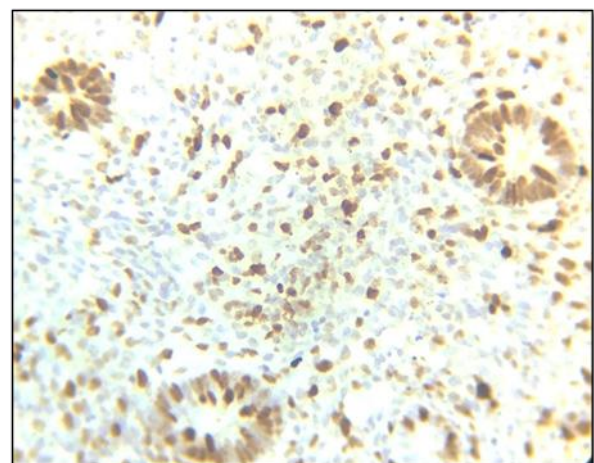


Figure 11: Section showing Ki 67 positivity in nucleus (40X) (score 3)

Secretory Endometrium

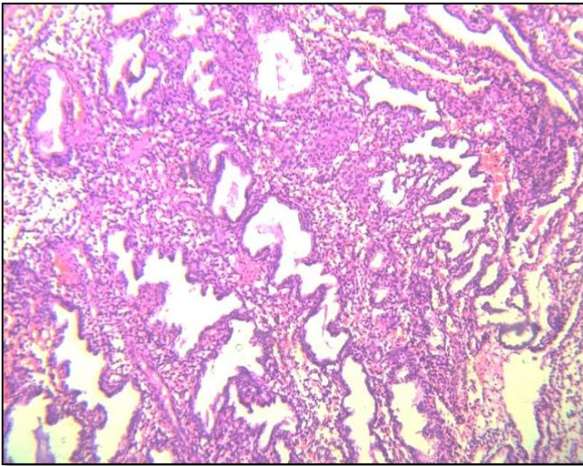


Figure 12: Histopathology (H&E stain, 10X)

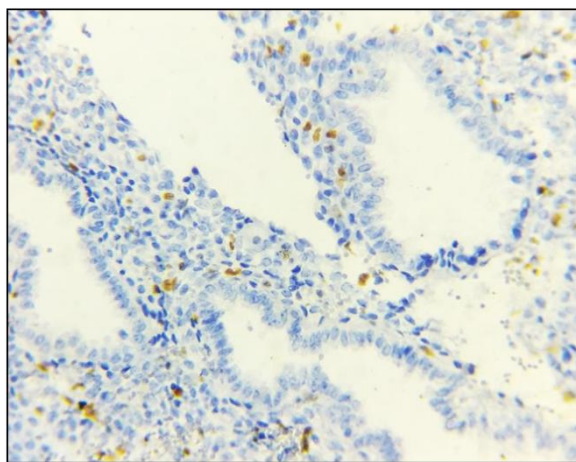


Figure 13: Section showing Ki 67 negativity in nucleus (40X) (score 0)

Discussion

Evaluation of AUB in perimenopausal age is a crucial step, since excessive or prolonged bleeding may cause disruption of women's daily activities, provoke serious medical consequences or exacerbate anemia and in a certain percentage of cases may eventually be life threatening if left untreated.¹

In perimenopausal women, abnormal uterine bleeding may display in different menstrual patterns, these are heavy menstrual bleeding, frequent menstrual cycles, inter-menstrual bleeding, dysmenorrhea and amenorrhea followed by continuous heavy bleeding.

Dilatation and curettage is a useful and cost-effective method of detecting intrauterine pathologies and very few lesions escape detection. It

is commonly used in developing countries with limited resources. The sensitivity of endometrial biopsy for the detection of endometrial pathology and for detection of cancer has been reported to be as high as 96% with 2-6% false negative rates.⁶ However, hysteroscopy guided biopsy is the gold standard both for screening as well as diagnostic purposes and can be performed as outpatient procedure.

The present study was conducted on specimens of endometrial curettings of 94 patients who presented with AUB in the age group of 40-60 years, received in the Department of pathology, Government Medical College, Kottayam during the study period. The reason for selecting this age group is that women of this age group are in their climacteric period and organic causes are common in this age group. Among the various age groups divided among the study group, with an interval of 5 years, maximum peak was observed in the age group of 40-45 years. Mean age calculated was 49 years. As women approach menopause, cycles shorten and often become intermittently anovulatory due to decline in the number of ovarian follicles and fluctuation in the estradiol level.⁷

These findings were comparable with similar studies conducted by Sreelakshmi et al and Talukdar et al. In the current study, organic morphological changes were observed in 39.4% of the endometrial biopsies and rest were non organic type endometrium (60.6%). These findings were comparable with studies conducted by Supriya et al and Junu et al.

The most common pattern among the non organic types of AUB was proliferative endometrium (30.3%) followed by secretory endometrium (27.4%) in the study conducted by Sreelakshmi et al, while it was proliferative endometrium (20.56%) followed by secretory endometrium (16.11%) in the study conducted by Talukdar et al .

Table 3: Comparison of different non organic histomorphological patterns of endometrium in AUB among the study group with similar studies

	Proliferative	Secretory	Disordered proliferative	Lytic	Atrophic
Present study (2017,n=94)	16%(n=15)	13%	28%(n=26)	3%(n=3)	1%(n=1)
Sreelakshmi et al (2017,n=135)	30.3%(n=41)	27.4%	6.6%(n=9)	0.7%(n=1)	3.7%(n=5)
Talukdar et al (2016,n=180)	20.56%(n=37)	16.11%	0	1.67%(n=3)	7.78%(n=14)

The AUB in the proliferative phase may be due to anovulatory cycle⁸. The exact cause of bleeding in atrophic endometrium is not known, it is thought to be due to anatomic vascular variation or defective local hemostatic mechanism.¹ Disordered proliferative pattern of the endometrium is somewhat difficult to define; it refers to a proliferative phase endometrium due to persistent oestrogen stimulation. It occupies the lower end of spectrum which passes through the hyperplasia to endometrial carcinoma on the other end. Early diagnosis of the lesion will prevent further disease progression.¹ It denotes an endometrial appearance that is hyperplastic but without an increase in endometrial volume⁷. Disordered proliferative

patterns resemble a simple hyperplasia, but the process is focal rather than diffuse. In the present study, among the organic conditions of AUB, the most common pattern was endometrial carcinoma (20%), followed by endometrial hyperplasia (11%). In the study conducted by Sreelakshmi et al, the most common pattern observed among the organic types of AUB was endometrial hyperplasia (19.9%) followed by equal number of all other organic conditions of AUB, while both Junu et al and Talukdar et al reported that, the most common pattern of organic type AUB, was endometrial hyperplasia followed by endometrial polyps in their studies.

Table 4: Comparison of different organic histomorphological patterns of endometrium with similar studies

	Age range	Endometrial carcinoma	Endometrial hyperplasia	Endometrial polyp	Endometritis
Present study (2017,n=94)	40-60 years	20%(n=19)	11%(n=10)	7.4%(n=7)	1.06%(n=1)
Sreelakshmi et al (2017,n=135)	45-55 years	0.7%(n=1)	19.9%(n=27)	0.7%(n=1)	0.7%(n=1)
Junu et al (2014,n=500)	40-60 years	2.63%(n=3)	82.45%(n=94)	9.65%(n=11)	5.26%(n=6)
Talukdar et al (2016,n=180)	40-55 years	1.11%(n=2)	46.11%(n=82)	2.22%(n=4)	2.22%(n=4)

The disparity between these studies may be due to the difference in the age group selected. Endometrial hyperplasia is a common histopathological finding in perimenopausal women often causing symptoms of irregular or prolonged bleeding. Heavy bleeding is secondary to sustained level of oestrogens. The overgrowth not only affects glands and stroma but there is also abnormal vascularization. Endometrial hyperplasia is one of the risk factor for the development of endometrial

carcinoma, early diagnosis and initiation of treatment is very important. So, perimenopausal women with heavy or irregular menstrual bleeding are often advised to have an endometrial sample taken to exclude endometrial disease.⁷ Identification of endometrial hyperplasia is important because they are thought to be precursors of endometrial carcinoma.

High Ki 67 mean score was observed in endometrial carcinoma, endometrial hyperplasia and disordered

proliferative endometrium. The Ki 67 mean score value was 3.2 in proliferative endometrium and 2.3 in secretory endometrium .These findings were comparable with study conducted by Arjunan et al.

Table 5: Comparison of Mean Ki67 score expression in cases of AUB with similar studies

	Mean Ki67 expression		
	Proliferative phase	Secretory phase	Endometrial hyperplasia
Present study(2017,n=94)	Score 3.2	Score 2.3	Score 4
Arjunan et al(2016,n=50)	Score 3	Score 1	Score 5.2

Conclusions

The conclusions are

- The most common clinical type of AUB was menorrhagic type of AUB (n=41, 43.6%), in which disordered proliferative endometrium was the commonest histomorphological pattern.
- In the study group, the most frequent histomorphological pattern of endometrium was disordered proliferative endometrium (n=26, 28%) followed by endometrial carcinoma (n=19, 20.2%)
- High mean Ki 67 score was seen in organic and non-organichistomorphological patterns of endometrium. Highest score was 4, which was observed in endometrial hyperplasia, disordered proliferative endometrium and endometrial carcinoma.

Acknowledgement

I express my sincere gratitude to Kerala University of Health Sciences (KUHS) for giving me the opportunity to do this thesis by accepting my topic of research. This helped me to improve my research skills.

I express my sincere gratitude to DrSankar S. and Dr. Priya P.V who gave me valuable suggestions and guidance during my research work. I also express my gratitude to all my colleagues and technical staff who helped me to complete my research work.

Funding: No funding sources

Conflict of Interest: None Declared

Ethical Approval: The study was approved by the Institutional Ethics Committee Review Board, Government Medical College, Kottayam, Kerala, India.

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