International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

A Study of Histopathological Patterns of Endometrium and Hormonal Status in Perimenopausal Patients with Abnormal Uterine Bleeding

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Abstract: ***Background**: The endometrium is a dynamic hormonally sensitive and responsive tissue which constantly and rhythmically undergoes changes in the active reproductive life. Abnormal uterine bleeding defined as a bleeding pattern that differs in frequency, duration and amount, that observed during a normal menstrual cycle or after menopause. It affects 10 - 30% of reproductive age group and up to 50% of peri menopausal women. The most common presentations are menorrhagia, metrorrhagia, polymenorrhea, intermenstrual bleeding, peri and post - menopausal bleeding. Hormonal imbalance mainly estrogen and progesterone level are the main factor involved in the pathogenesis of abnormal uterine bleeding. This alteration is better studied by a combination of histopathological and hormonal profile and immunohistochemical evaluation of the endometrium. **Aim and objectives**: To study the histopathological patterns of endometrial biopsies and curettage, hysterectomy specimen and hormonal status [estradiol (E2) and progesterone (PRG)] in perimenopausal patients with abnormal uterine bleeding. **Material and methods**: The study was prospective and includes 72 cases as sample size from August 2022 to February 2024 (18 months) in histopathology and special laboratory department of pathology, Gajra Raja Medical college Gwalior, MP. **Observation and results**: Total N=72 selected cases of AUB patient of perimenopausal age group were included in the study. Overall mean age 44.79 + 4.63 years. The most common age group in the study was 41–45 years (34.73) followed by ~ 40years (30.56%). The most common abnormal uterine bleeding pattern was menorrhagia during the study observation. The following types of histological patterns seen in the endometrium: Proliferative phase [PP] (33.3%), Secretory phase [SP] (16.7%), disordered Proliferative phase (5.6%), pill endometrium (13.9%), Endometrial hyperplasia (EH) without atypia (20.8%), endometrial hyperplasia (EH) with atypia (2.8%), endometrial carcinoma (4.2%), and senile atrophic endometrium (1.4%). The frequency of estrogen level and percentage is categorized into three groups: low (5.6%), normal (22.2%), high (72.2%) and AUC value was 0.722 and p - value 0.0003. Sensitivity and specificity of estrogen value was respectively 82.93% and 61.298%. In this study, progesterone level was categorized into three groups: low (6.9%), normal (54.2%), and high (38.9%). The progesterone level of AUC value was 0.504. Sensitivity and specificity of progesterone value was respectively 75.61% and 45.16%. **Conclusion**: In all patients with AUB, histopathological examination is mandatory. It is the most important diagnostic tool in the evaluation of AUB because it shows a wide range of patterns, from normal endometrium to malignancy. As a result, it assists the physician in planning therapy for successful AUB management and reduces the need for unnecessary hysterectomy. We concluded that the histopathological correlation with the clinical symptoms of AUB was good, and we classified the cases of AUB under FIGO classification (PALM - COEIN). We did find a cut - off value as well as sensitivity and specificity for the serum hormonal status of progesterone and oestrogen in all the histological patterns in people with AUB. The workup for these patients should include an assessment of oestrogen and progesterone level in serum in abnormal uterine bleeding, allowing one to determine the type of therapeutic management.***

Keywords: Abnormal uterine bleeding (AUB); menorrhagia, metrorrhagia, polymenorrhea, intermenstrual bleeding, perimenopausal; post - menopausal; estrogen; progesterone; hysterectomy

1. Introduction

The endometrium is a dynamic hormonally sensitive and responsive tissue which constantly and rhythmically undergoes changes in the active reproductive life[1]. Abnormal uterine bleeding defined as a bleeding pattern that differs in frequency, duration and amount, that observed during a normal menstrual cycle or after menopause[1, 2].

Abnormal uterine bleeding is one of the commonest complaints leading to endometrial sampling by endometrial curettage. It affects 10 - 30% of reproductive age group and up to 50% of peri menopausal women[3]. The most common presentations are menorrhagia, metrorrhagia, polymenorrhea, intermenstrual bleeding, peri and post - menopausal bleeding[4]. Nearly 30% of all gynaecological outpatient attendants are for AUB.

Abnormal uterine bleeding (AUB) is a collective terminology that includes both organic and non - organic causes. An endometrial biopsy is usually done for abnormal uterine bleeding to rule out organic pathology. Age and menstrual history are particularly important, because the etiologies of abnormal uterine bleeding differ according to the age and menstrual status[5]. In women of reproductive age group, pregnancy complications, including abortion are more common, whereas in postmenopausal women atrophy and organic pathologies are common[6].

A diagnosis of abnormal uterine bleeding can only be made after the histopathological examination has ruled out organic causes. Three patterns are commonly seen - The first is called as "estrogen breakthrough bleeding", which occurs in the presence of continuous estrogen production by a "persistent follicle". The proliferated endometrium increases in size so that it outgrows its own blood supply and breakthrough bleeding ensues. The second one is called "estrogen withdrawal bleeding" which is due to "failed follicle", i. e. the follicle produces subnormal estrogen. Both these causes are attributed to anovulation. The third finding is "ovulatory endometrium" due to follicular or luteal phase defects.

The cyclical release of estrogen and progesterone from the ovaries control the normal cyclical physiological changes that occur in the endometrium of women during the reproductive period. The concentration of receptors for these hormones also varies cyclically during the menstrual cycle[7]. Estrogen and progesterone receptors are also expressed in hyperplasias and endometrial cancers, especially type I. These receptor levels can give important information about prognosis and the amenability to hormonal therapy.

Hormonal imbalance is the main factor involved in the pathogenesis of abnormal uterine bleeding. This alteration is better studied by a combination of histopathological and hormonal profile and immunohistochemical evaluation of the endometrium[8].

In order to evaluate endometrial samples, information regarding age, the phase of her menstrual cycle and use of any exogenous hormones along with clinical examination are a pre requisite.

In this study we have attempted to analyse different histopathological patterns of endometrium and hormonal status in abnormal uterine bleeding (AUB) and observe the incidence of various pathologies in different perimenopausal age groups.

Aim and Objectives

Aim:
To study the histopathological patterns of endometrial biopsies and curettage, hysterectomy specimen and hormonal status [estradiol (E2) and progesterone (PRG)] in perimenopausal patients with abnormal uterine bleeding.

Objectives:
1) To determine the histopathological patterns of endometrial biopsies and curettage and hysterectomy specimen in perimenopausal female with Abnormal Uterine Bleeding.
2) To categorise the causes of abnormal uterine bleeding according to age group and pattern of bleeding.
3) To determine the hormonal profile: Serum estrogen and serum progesterone status of non - malignant and malignant endometrium in perimenopausal age group.
4) To compare the result of present study with the previous studies.

2. Material and Methods

The study was prospective and includes 72 cases as sample size from August 2022 to February 2024 (18 months) in histopathology and special laboratory department of pathology, Gajra Raja Medical college Gwalior, MP.

Formula:

$$n = \frac{Z^2\alpha/2 \times PQ}{d^2}$$

$$= \frac{(1.96)^2 \times 25 \times (100 - 25)}{(10)^2} = 72$$

P= 25% proportional to endometrium hyperplasia (Sajitha K et al)

Inclusion Criteria
All the patients having diagnosis of AUB and undergoing procedure like Dilatation and Curettage/ biopsy and hysterectomy would be included.

Exclusion Criteria:
1) Critically ill patients.
2) Patients getting biopsies done from private hospitals.
3) Postmenopausal women.
4) Hysterectomy specimen apart from abnormal uterine bleeding.
5) Endocervical biopsy for primary infertility.
6) Bleeding due to cervical pathology, vaginal pathology, bleeding disorder and pregnancy related complications.

Case Selection and Process
This one and half year prospective study would be done in the Department of Pathology, a tertiary care hospital which would include 72 cases of clinically diagnosed Abnormal uterine bleeding of perimenopausal age group and evaluated. Histopathological examination of endometrial biopsies and hysterectomy specimen would be done, followed by clinical correlation hormonal profile. Hormonal profile - Oestrogen and Progesterone levels will be done by Chemiluminescence (CLIA) machine (MAGLUMI - 800) available in department of Pathology. Tissue submitted in histopathological section of pathology department were processed, paraffin embedded and sections stained with Haematoxylin and eosin stain. Then stained section examined under the microscope and histopathological evaluation done.

Simultaneously, the blood sample of same patient collected in plain vial and separated serum will be used for hormonal assay. We took the history of patients along with relevant investigations. Age, sex, BMI, menstrual history and clinic - radiological findings of the patient was recorded carefully.

Reference value of estrogen - Males: <87pg/ml
Female: Follicular phase: 15 - 112pg/ml, Preovulatory phase: 136 - 251pg/ml, Luteal phase: 48 - 172pg/ml, Menopausal and post - menopausal phase: 10 - 66pg/ml, Hormonal contraceptives: 15 - 95pg/ml.

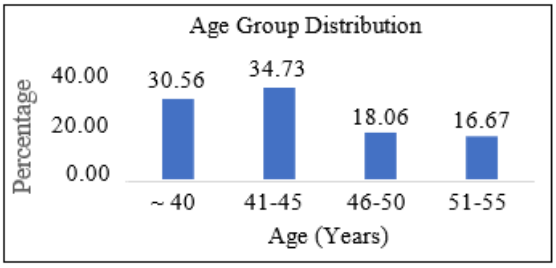
Reference value of progesterone -
Males: 0.23 - 1.50ng/ml (meadian - 0.57 ng/ml)
Females (non - pregnant): Follicular phase: 0.36 - 1.21ng/ml

(median - 0.53ng/ml), Ovulation phase: 0.39 - 22.87ng/ml (median - 1.24ng/ml), Luteal phase: 2.12 - 26.44ng/ml (median - 12.86ng/ml), Post - menopausal: 0 - 0.89ng/ml (median - 0.36ng/ml)
Pregnant females: Early pregnancy 0 - 12th week: 1.17 - 49.9ng/ml (median 24.23ng/ml),
Mid pregnancy 13 - 28th week: 15.4 - 68.9ng/ml (median 37.77ng/ml), Late pregnancy 29 - 40th week 59.8 - >80ng/ml.

3. Observation and Results

Total N=72 selected cases of AUB patient of perimenopausal age group were included in the study. Overall mean age 44.79 + 4.63 years. The most common age group in the study was 40–45 years (65.29%).

In the existing study, the age group among perimenopausal patient with AUB was categorized into four groups as follows: 1) ~ 40 year (30.56%); 2) 41 - 45 years (34.73%); 3) 46 - 50 years (18.06%); and 51 - 55 years (16.67%). The most common age group in the study was 41–45 years (34.73) followed by ~ 40years (30.56%).



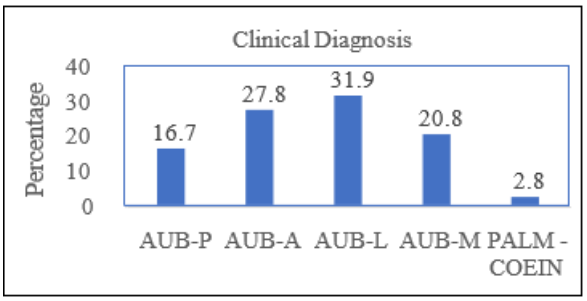
Graph 1: Age group distribution among perimenopausal patient with AUB.

Among 72 patients, the patterns of bleeding shown during the study were as follows: menorrhagia (65.2%), metrorrhagia (16.7%), menometrorrhagia (11.1%), polymenorrhea (5.6%), and oligomenorrhea (1.4%). The most common abnormal uterine bleeding pattern was menorrhagia during the study observation.

Table 1: Pattern of Bleeding among perimenopausal patient with AUB

Patterns of Bleeding		
	Frequency	Percentage
Menorrhagia	47	65.2%
Metrorrhagia	12	16.7%
Menometrorrhagia	8	11.1%
Polymenorrhea	4	5.6%
Oligomenorrhea	1	1.4%
Total	72	100.0%

In the existing study, we observed the following types of clinical diagnoses: AUB - P (16.7%), AUB - A (27.8%), AUB - L (31.9%), AUB - M (20.8%), and COEIN (2.8%). The most common clinical diagnosis was AUB - L (31.9%) observed during the study.



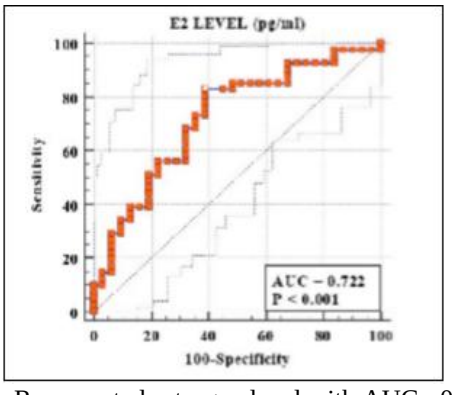
Graph 2: Graphical representation of clinical diagnosis of AUB among perimenopausal women.

Among 72 patients, we observed the following types of radiological findings during the study: leiomyoma (31.9%), adenomyosis (27.2%), polyp (16.7%), hyperplasia (11.1%), malignancy (9.7%), and endometritis (2.8%). During the study observation, the most common radiological finding was leiomyoma (31.9%).

Table 2: Histopathological Patterns of endometrium among perimenopausal patient with AUB

Histological pattern of endometrium		
	Frequency	Percentage
Proliferative phase	24	33.3%
Secretory phase	12	16.7%
Disordered Proliferative phase	4	5.6%
Pill Endometrium	10	13.9%
EH Without Atypia	15	20.8%
EH With Atypia	3	2.8%
Endometrial Carcinoma	3	4.2%
Senile Atrophic Endometrium	1	1.4%
Total	72	100%

We observed the following types of patterns in the endometrium based on histopathological findings: Proliferative phase [PP] (33.3%), Secretory phase [SP] (16.7%), disordered Proliferative phase (5.6%), pill endometrium (13.9%), Endometrial hyperplasia (EH) without atypia (20.8%), endometrial hyperplasia (EH) with atypia (2.8%), endometrial carcinoma (4.2%), and senile atrophic endometrium (1.4%). In our study, 56.9% had organic cause of AUB, whereas 43.1% had functional cause of AUB observed during the histopathological examination. Among 72 patients, the frequency of estrogen level and percentage is categorized into three groups: low (5.6%), normal (22.2%), high (72.2%) and AUC value was 0.722 and p - value 0.0003.



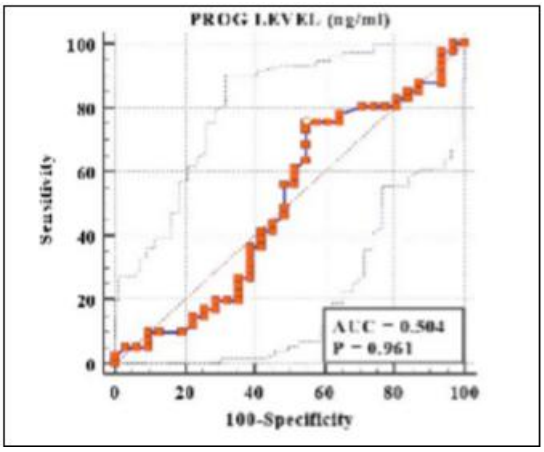
Graph 3: Represented estrogen level with AUC= 0.722 and p - value <0.001.

Sensitivity and specificity of estrogen value was respectively 82.93% and 61.298%. In the present study, >170 pg/ml was a threshold value for estrogen level to decide organic / functional causes of AUB.

Table 3: Sensitivity and specificity of estrogen level

AUC	Associated criterion	Sensitivity	Specificity	P – value
0.722 (0.604 - 0.821)	>170	82.93%	61.29%	0.0003

In this study, progesterone level was categorized into three groups: low (6.9%), normal (54.2%), and high (38.9%).



Graph 4: Represented as progesterone level with AUC = 0.504 and p - value 0.961

Table 4: Sensitivity and specificity based on organic/functional causes of AUB

AUC	Associated criterion	Sensitivity	Specificity	P – value
0.504 (0.383 - 0.624)	>2.98	75.61%	45.16%	0.9611

In the present study, progesterone of AUC value was 0.504. Sensitivity and specificity of progesterone value was respectively 75.61% and 45.16%. >2.98 ng/ml was the threshold value for progesterone to decide organic / functional causes of AUB.

Histopathological Images

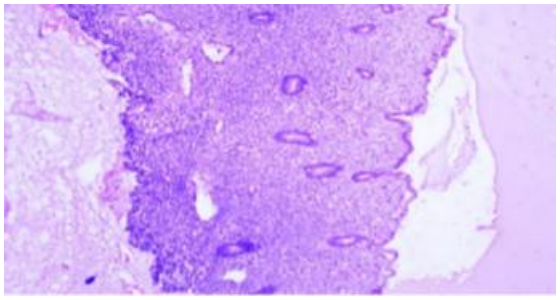


Figure 1: Proliferative endometrium showing small round/ tubular glands in dense stroma, (H&E,100x)

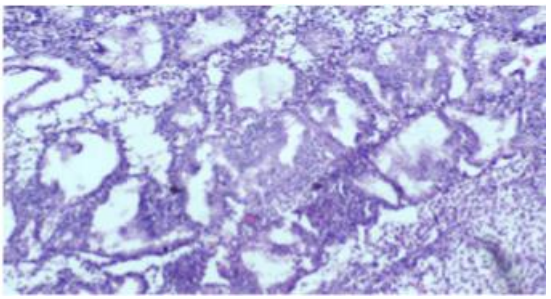


Figure 2: Secretory endometrium showing tortuous glands with dilated lumen containing abundant secretions. (H&E,400x)

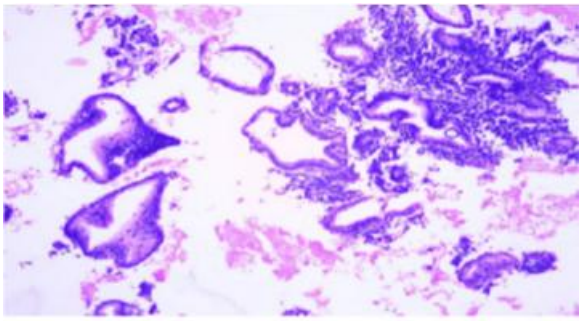


Figure 3: Disordered proliferative endometrium showing varying size and shape of cystically dilated spaces separated by stroma. (H&E, 100x)

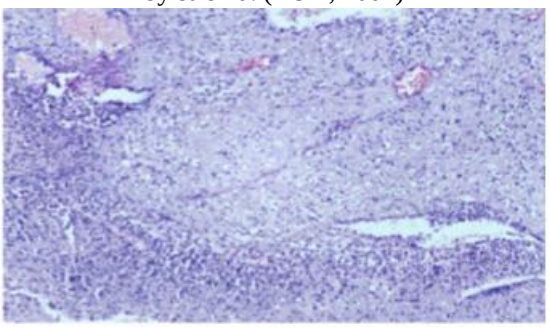


Figure 4: Pill endometrium showing dense pseudodecidualization of endometrial stroma, probably exogenous progesterone effect. (H&E, 100x)

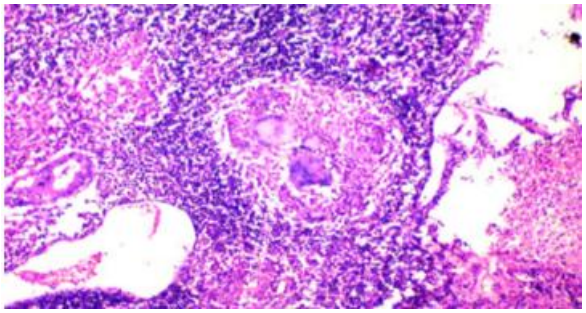


Figure 5: Tubercular endometritis showing endometrial tissue with well-formed granuloma and giant cells, lymphocytic infiltration. (H&E, 100x)

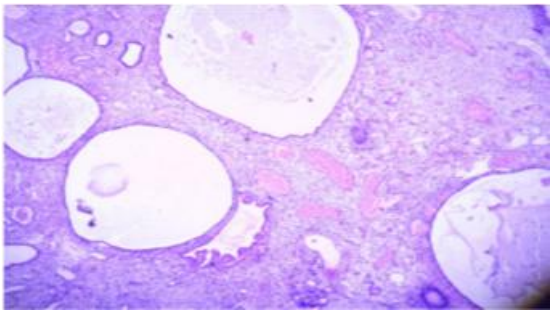


Figure 6: Showing endometrial hyperplasia without atypia, (H&E, 100x)

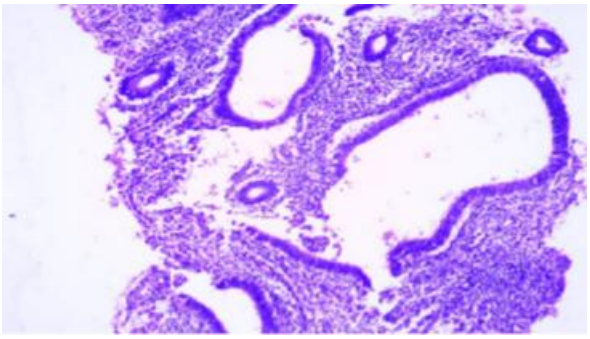


Figure 7: Endometrial hyperplasia with atypia, (H&E, 100x)

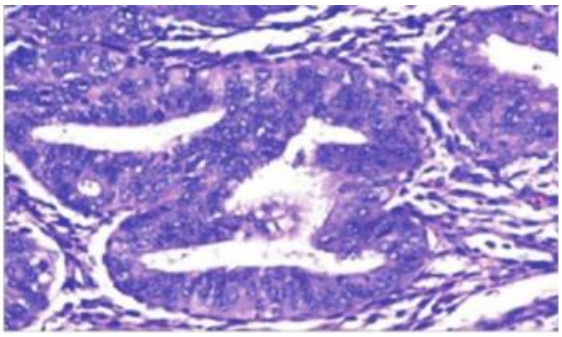


Figure 8: Showing endometrial intraepithelial neoplasia (H&E, 400x)

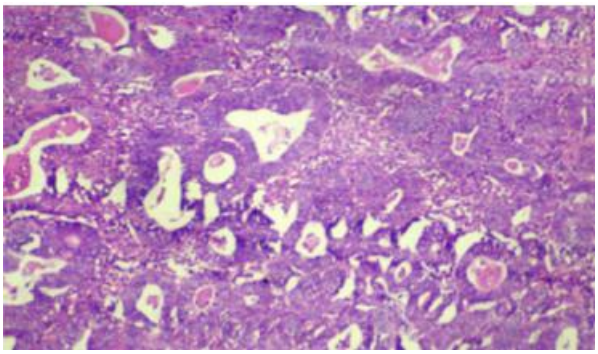


Figure 9: Endometrioid carcinoma (H&E, 100x)

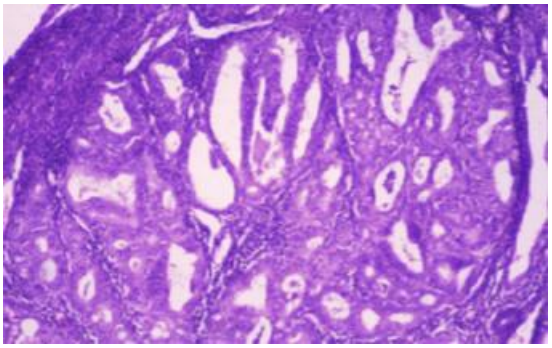


Figure 10: Endometroid Carcinoma (H&E,100x)

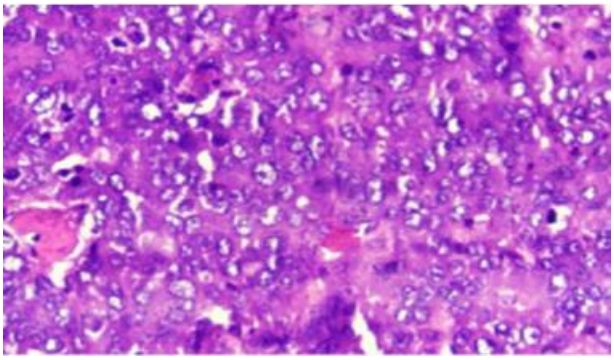


Figure 11: High grade endometrioid carcinoma showing high degree of nuclear pleomorphism, hyperchromatic nuclei with prominent nucleoli and frequent mitoses (H&E,400x)

4. Discussion

The human reproductive system has captivated people for ages due to its distinctiveness and susceptibility to a broad range of both benign and malignant disorders. Abnormal uterine bleeding is a common issue that is frequently reported in gynaecologic practice. Abnormal uterine bleeding refers to any bleeding from the vaginal tract that deviates from the typical standards in terms of volume, length, regularity or frequency. Reports indicate that the perimenopausal, premenopausal, and postmenopausal age groups are the ones most affected. The researchers have found that the sensitivity for detecting histopathologic abnormalities in endometrial biopsy samples is 96% [11 - 14].

The Pathology and Obstetrics & Gynaecology department at GRMC, Gwalior examined 72 women with AUB as part of the study. We obtained detailed history, clinical examination and routine investigations. The team offered USG as an initial non - invasive investigation. The team conducted further investigations using endometrial curettage, either through D and C or Pipelle's biopsy. We carried out additional interventions based on the endometrial histopathology. We compared various parameters of our study with those of different authors.

1) Age group

In the present study, overall mean age was 44.79 ± 4.63 years. Most common age group was 41 - 45 years (34.73%). Mishra J. et al. also observed that overall mean age was 44.03 years. She also observed that the 41–45 - year - old age group was more common in the study [15]. Mune SB. et al. also found that the study's overall mean age was 44.2 years [16]. Studies by Sadbhawana R. et al.[17], Malathi BG et al.[18],

Sharma R. et al.[19], and Behera B. et al.[20] corroborated our findings. The reason behind this may be due to the fact that these patients are in their climacteric period. As women approach menopause, cycles shorten and often become intermittently anovulatory due to a reduction in the number of graffian follicles and the estradiol level [21].

2) Pattern of Bleeding

In our study, 65.2% had menorrhagia, 16.7% had Metrorrhagia, 11.1% had Menometrorrhagia, 5.6% had Polymenorrhagia and 1.4% had Oligomenorrhea observed during the study. According to Mune SB et al. study, menorrhagia pattern (47.6%) was the most common pattern of bleeding[16]. Malathi BG. Et al.1[8] and Sharma P. et al.[22] study also having supportive findings with our study results. Abid M. et al. study was different from our study findings. He observed that polymenorrhagia was more common findings during his study[23].

3) FIGO classification (PALM - COEIN)

In the present study, 31.9% had AUB - L, 27.8% had AUB - A, 20.8% had AUB - M, 16.7% had AUB - P and 2.8 % had COIEN type of clinical diagnosis observed during our study. According to Mishra J. et al. study, 73.2% had AUB - L, 13.4% had AUB - A, 12.2% AUB - M and 0% COIEN type of clinical diagnosis during her study[15].

4) Functional/Organic Cause of AUB on the basis of Histopathological Examination

In the present study, 43.1% had functional whereas 56.9% had organic cause observed during our study. Sadbhawana R. et al. study was different from our study findings. She observed functional group was higher than organic[17]. Incidence of functional cause of AUB in our study was lower than Mune SB. et al.[16], Ara & Roohi [24] 62.1%, Muzaffar et al [25] 61%, Abdullah LS [26] 61.5%.

5) Histological Pattern of Endometrium in AUB based on Age Group

In the present study, 33.33% more common proliferative phase type of histopathological pattern was observed.38.8% was observed most common pattern in Sharma R. et al. study[9], Mishra J. et al.[15], Sarika et al.[17], and Anuradha Salvi et al. studies were concordance with our study results [28]. Histological pattern was not significantly associated with age group. Mune SB. Et al. study was different from our study findings which show 27.8% [16].

6) Estrogen Hormone Level

Overall mean estrogen level was 274.59 ± 199.13 pg/ml. In the present study, the estrogen level was categorized into three groups: low (5.6%), normal (22.2%), and high (72.2%). Age group distribution, pattern of bleeding and histological patterns was not significantly associated with level of oestrogen. In the present study, we predicted estrogen level based on functional /organic causes AUB and HPE. >170pg/ml of estrogen was a threshold value to convert organic cause from functional cause of AUB. Sensitivity and specificity of this threshold value was respectively 82.93% and 61.29%. AUB - L of mean estrogen value was higher than compared to AUB - P, AUB - A, AUB - M and AUB - E. Among bleeding patterns, mean estrogen value was observed as high in polymenorrhea.

7) Progesterone Level

Overall mean progesterone level was 12.03 ± 13.14 pg/ml. In the present study, the progesterone level was categorized into three groups: low (6.9%), normal (54.2%), and high (38.9%). Age group distribution and pattern of bleeding was not significantly associated with level of progesterone. Histopathology of pattern was significantly associated with progesterone level. In the present study, we were predicted progesterone level based on functional /organic causes of AUB and HPE. AUB - E of mean progesterone value was higher than compared to AUB - P, AUB - A, AUB - M and AUB - L. Among bleeding patterns, mean progesterone value was observed as high in menometrorrhagia.

Many demographic factors can influence endometrial hormonal status and bleeding patterns like women's age other than perimenopausal group, large geographic area with large sample, ethnicity, extreme of hormones fluctuation are beyond our study.

In this study we cannot suggests that perimenopausal women with abnormal uterine bleeding may have an increased risk of cardiovascular, osteoporotic events, cancer metastasis and other long - term consequences. Even extreme elevation of hormones like estrogen and progesterone cannot exclude carcinoma endometrium. We cannot categorize endometrial carcinoma on a basis of molecular biomarker. We are not included advanced imaging studies like MRI.

5. Future Research Directions

1) Molecular biomarkers for risk stratification.
2) Imaging advancements like MRI for early and better detection.
3) Personalized risk assessment and prevention strategies.
4) Investigating the role of progesterone and oestrogen in endometrial cancer prevention.

6. Conclusion

In all patients with AUB, histopathological examination is mandatory. It is the most important diagnostic tool in the evaluation of AUB because it shows a wide range of patterns, from normal endometrium to malignancy. As a result, it assists the physician in planning therapy for successful AUB management and reduces the need for unnecessary hysterectomy. We concluded that the histopathological correlation with the clinical symptoms of AUB was good, and we classified the cases of AUB under FIGO classification (PALM - COEIN).

Leiomyoma, the most common aetiology contributing to AUB in the perimenopausal age group, was identified. Thus, we found that the FIGO (PALM - COEIN) classification is a useful system clinically and histopathologically, as both are complementary to each other, and allocating a proper category helps in optimizing the patient's treatment. Clinically, distinguishing between adenomyosis and leiomyoma can be challenging due to their striking similarities in signs and symptoms. Therefore, histopathological analysis helps differentiate the two. This emphasizes the importance of histopathological examination as a complementary diagnostic tool in classifying AUB's

structural causes.

In the present study, we did find a cut - off value as well as sensitivity and specificity for the serum hormonal status of progesterone and oestrogen in all the histological patterns in people with AUB. The workup for these patients should include an assessment of oestrogen and progesterone level in serum in abnormal uterine bleeding, allowing one to determine the type of therapeutic management.

Conflict of interest and funding
In this study there is no conflict of interest and not funded.

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