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Histomorphological Profile and Immunohistochemical Analysis of Endometrium in Perimenopausal Bleeding: A 2 Year Study in a Tertiary Care Center

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Abstract: *Perimenopausal bleeding is one of the common conditions for which patients seek advice in the gynecology outpatient department. Histopathological characteristics of endometrial biopsy material for patients with perimenopausal bleeding, by light microscopy remains the gold standard for diagnosis of endometrial pathology. A total of 200 endometrial curetting samples received during a 2 year period in Department of Pathology, Tirunelveli Medical College, Tamilnadu, India were assessed for the histopathological pattern and Estrogen and Progesterone receptor status on those samples showing endometrial hyperplasias. The different patterns reported were proliferative endometrium (49.50%), 48 cases (24%) of hyperplasia in which simple hyperplasia without atypia was higher 42 cases (87.50%). Simple hyperplasia without atypia showed a mean percentage of positive cells, 75% for ER and PR whereas complex atypical hyperplasia showed the mean percentage of positive cells 60% for ER and 70% for PR. Thus, for every morphological pattern of the endometrium analyzed in this study, the average values for PR were higher compared with average values of ER for similar lesions. Estrogen and Progesterone receptors study allows specific determination of the ER, PR receptor content of the cell and hence can be an accurate prediction marker of patient response to endocrine therapy.*

Keywords*: Endometrial hyperplasias, Endometrium, Estrogen and Progesterone receptors, Perimenopausal bleeding.*

I. Introduction

Perimenopausal bleeding is one of the common conditions for which patients seek advice in the gynecological outpatient department. The prevalence increases with age, peaking just prior to menopause. Because most cases are associated with anovulatory menstrual cycles, perimenopausal women are particularly vulnerable. Throughout the perimenopausal transition, there is a significant incidence of DUB due to anovulation (1). Perimenopause is the period 2-8 years preceding the menopause and 1 year after final menses (WHO). However a better definition is the phase preceding the onset of menopause, occurring around the age of 40-50 years (beginning at age 47.5, lasting for 4 years) during which the regular menstrual cycle of a woman transitions to a pattern of irregular cycles (2).

Perimenopausal bleeding refers to the symptoms of excessive, unexpected, prolonged, or acyclic bleeding, regardless of the diagnosis or cause (3). Endometrial biopsy is relatively simple, accurate and inexpensive. The only disadvantage of the endometrial biopsy is that, it is an invasive procedure. The main reason for obtaining endometrial histology in perimenopausal patients with bleeding is to exclude the presence of endometrial hyperplasia or carcinoma of endometrium (4). According to WHO, hyperplasias are classified into simple and complex based on architectural features and typical or atypical based on cytological features (5). Both typical hyperplasia and atypical hyperplasia may regress spontaneously over months or a few years. However, atypical hyperplasia is a precancerous condition that may progress to malignancy and best treated by surgery with hysterectomy. Hyperplasia without atypia regresses spontaneously after D&C or progestin treatment. In patients with atypical hyperplasia, if conserving the uterus is considered, a trial of hormonal treatment may be given. Analysis of steroid hormone receptors plays an important role in patients with perimenopausal bleeding to predict the response to hormonal therapy (4). This study analyses the histomorphological pattern of the endometrium in perimenopausal bleeding and evaluates the expression of ER and PR in endometrial hyperplasias by immunohistochemical method.

II. Materials and Methods

This study is a prospective study conducted in the Department of Pathology, Tirunelveli Medical College and Hospital, Tamilnadu,India, over a period of 2 years. The endometrial curettage specimens, that are clinically diagnosed as DUB/AUB and in perimenopausal age group of 40-50 years were included in the study. Endometrial curettage from patients of other age groups and postmenopausal bleeding cases were excluded from the study. The endometrial curettage samples were fixed in 10% formalin and histopathological slides were prepared and stained with Hematoxylin and Eosin stain. All cases of hyperplasias including simple hyperplasia without atypia, complex hyperplasia without atypia, complex atypical hyperplasia were selected and their representative formalin fixed, paraffin embedded tissue samples were subjected to immunohistochemistry for ER and PR status using a supersensitive polymer HRP system based on non biotin polymeric technology. Immunohistochemical scoring for ER and PR receptors were done with a Quick score (6). The score for proportion and intensity are summated to a total maximum score of 8. Score of more than 2 is considered positive.

III. Results

Among a total of 6583 gynecology histopathological specimens received during the study period, the average incidence of DUB cases were 387 cases (5.88%) with a maximum incidence (67%) of perimenopasual bleeding in the age group of 40-45years. Among the 200 perimenopausal bleeding cases, proliferative endometrium and its variants constitute 99 cases (49.50%), secretory endometrium and its variants 12 cases (6%), hyperplasias, 48 cases (24%), and other cases constitute 20.50% (41cases). (TABLE 1) Chi-square statistical test has been applied to find out the association between the type of proliferative endometrium and age of the person. The significant p-value infers that disordered proliferative endometrium and proliferative endometrium with cystic change are more common among the women in the age group of 46-50 years compared to the women in the age group of 40 to 45 years. Similarly for the women in 40-45 years age group, proliferative endometrium and proliferative endometrium with disintegration is common

In the total of 12 cases of secretory endometrium and its variants, there were 3 cases (24.99%) of early secretory endometrium, 2 cases (16.66%) of mid secretory endometrium, 1 case (8.33%) of late secretory endometrium, 5 cases (41.65%) of deficient secretory endometrium and 1 case (8.33%) of deficient secretory endometrium with stromal hemorrhage. Among the 48 cases of hyperplasia, in typical hyperplasia, simple hyperplasia without atypia constitute 42 cases (87.50%), complex hyperplasia without atypia constitute 4 cases (8.33%), and among atypical hyperplasias, complex hyperplasia with atypia was 2 cases (4.16%) (TABLE 2).

Chi-square with Yate's correction test has been applied to find out the association between the hyperplasias in total and those without atypia (typical). The significant p-value indicates that most of the endometrium without atypia (typical) were with simple hyperplasia. Among the total 42 cases of simple hyperplasia without atypia, the following patterns were observed.They were, simple hyperplasia without atypia 29 cases (69.05%), simple hyperplasia with disintegration 2 cases (4.76%) simple hyperplasia with polyp 2 cases (4.76%), simple hyperplasia with chronic endometritis 2 cases (4.76%), simple hyperplasia with cystic change 6 cases (14.28%) and simple hyperplasia with squamous metaplasia 1 case (2.38%). In this study, 35 cases (83.3%) of hyperplasias were between the age group of 40-45 years and 7 cases (16.66%) in the age group of 46-50 years. Chi-square statistical test has been applied to find out if there is any association between the type of simple hyperplasia and age of the women. The non-significant p-value infers that the type of simple hyperplasia has no association with the age of the women. Between the age group of 40-45 & 46-50 years, there were 3 cases each of complex hyperplasia without atypia and complex hyperplasia with atypia.The other patterns of endometrium were disintegrating endometrium 19 cases(9.5%), irregular shedding 16 cases(8%) and endometrial adenocarcinoma 1 case (0.5%).The other associated organic lesions were 37 cases (18.5%) of fibroid uterus with predominant endometrial pattern simple hyperplasia, 12 cases, (32.43%) and 5cases (2.5%) of endometrial polyp.

Simple hyperplasia without atypia showed moderate staining intensity of ER(Fig.1) in glands and in the stroma with a mean percentage of positive cells 75% in both the glands and stroma, and moderate staining intensity of PR(Fig.2) in glands and in the stroma with a mean percentage of positive cells 75%.Complex hyperplasia without atypia (Fig.3) showed moderate staining intensity of ER(Fig.4) (mean % of positive cells 75%) and PR(Fig.5) (mean % of positive cells 85%) in both glands and stroma. Complex atypical hyperplasia (Fig.6) showed moderate staining intensity of ER (Fig.7) in glands, weak staining in stromal cells (mean% of positive cells, 60%) and moderate intensity levels of PR (Fig.8) in both glands and stroma (mean % of positive cells 70%).

The percentage of cells stained per 1000 cells counted on 40X power field and the intensity of reaction were analyzed and immunohistochemical scoring for estrogen and progesterone receptors were done with Quick score, which showed Estrogen and Progesterone receptor positivity in all cases of hyperplasias in glandular cells and stromal cells .

IV. Discussion

The average incidence of DUB cases in our institution was 5.88% Similar studies done in other study centers such as Kamla Nehru Memorial Hospital Allahabad, and AIIMS New Delhi showed an incidence of 8.32% and 5.3% respectively. In the current study, the incidence of perimenopausal bleeding was 51.68%. The current study also showed higher percentage, 67% (134 cases) in the age group of 40-45 years. Among the various patterns of endometrium observed, the number of cases of proliferative endometrium and its variants was higher (49.50%) in the present study in comparison to other studies of Layla et al (7) and Shazia et al (8).

Among the cases of secretory endometrium and its variants in the present study,the incidence of early secretory endometrium were 24.99% and the age incidence of secretory endometrium were higher (74.99%)in 40-45 years age group.The incidence of endometrial hyperplasia in the present study was 24% .Of the total 200 cases of perimenoupausal bleeding, the incidence of simple hyperplasia without atypia in the present study were 21% which is in concurrence with the study of Shazia et al (8) (25%) and in the study of Archana Bhosle et al (9) 17.8% .In the present study among the 48 cases of endometrial hyperplasias,simple hyperplasia without atypia was most common,with a total of 42 cases (87.50%) similar to the study of Amera Takreem et al(10), who observed 66.6% of simple hyperplasia without atypia in a study of 100 perimenopausal bleeding patients. In our study, 4 cases were complex hyperplasia without atypia (8.33%) and 2 cases were complex atypical hyperplasia (4.16%). The present study also revealed the maximum number of cases of endometrial hyperplasia in the age group of 40-45 years. Simple hyperplasia with atypia which is extremely unusual (11) were not observed in our study.

Complex hyperplasia without atypia were 2% in the present study similar to the study of Layla et al (7). Complex atypical hyperplasia were 1%, similar to the study of Shazia et al (8)(1%), and the incidence was observed to be increasing with increasing age (2 cases, 46-50 years). The other associated organic lesions in perimenopausal age were fibroid uterus 18.5% and endometrial polyps 2.5% and the predominant pattern of endometrium in perimenopausal women with fibroid uterus was simple hyperplasia 32.43% Lyla et al (7) observed that the incidence of endometrial polyp rises as age increases, has a maximum incidence in the fifth decade of life and declines gradually after menopause (12). The present study showed an increasing incidence of endometrial polyps in older age groups 46-50 years (80%). Our result is comparable to other studies.

Hyperplasia in perimenopausal women is a major cause of abnormal uterine bleeding. The transition from complex hyperplasia to carcinoma of the endometrium was reported to occur at rates of 26.7% and 29% by Wentz et al and Allahbadia G. et al respectively. This shows the importance of detection of hyperplasias in preventing the disease progression to more advanced stages (13). Samhita Chakraborty et al (2005) (14) suggested that increased ER and PR lead to a local unopposed estrogen effect. This up regulates estrogen and progesterone receptor protein and this cycle leads to hyperplasia of the endometrium, if the stimulus persists.

Endometrial hyperplasia, which is a potential precancerous lesion of the endometrium (15) may show altered expression of sex hormone receptors. Hormonal therapy is an effective treatment strategy in the management of patients with perimenopausal bleeding and it supports the role of these receptors in the etiopathogenesis of hyperplasias (14). Nyholm et al (15) reported that ER and PR levels were high in simple and complex hyperplasia without atypia, and low in simple hyperplasia with atypia and complex atypical hyperplasia.

In the study by Daniela et al, (16) of endometrial hyperplasias, 100% of endometrial hyperplasias were positive for stromal and epithelial PR. Daniela et al[85], Samhita Chakraborty et al (14) and Nyholm et al (15)observed that the mean score of ER and PR decreased in cases of hyperplasias with atypia as compared to hyperplasias without atypia. Samhita Chakraborty et al (14) suggested that the sex steroid receptors may not be the only factor responsible for hyperplasias. There may be other factors responsible for changes leading to atypia and carcinoma, that may down regulate the receptors in atypical hyperplasias. In the study of Daniela et al (2012) (16), it was observed that the percentage of positive cells for estrogen receptors were 41.50% for simple hyperplasia, 72.3% for complex hyperplasia, 57% for complex atypical hyperplasia and the percentage of positive cells for progesterone receptors were 43.8% for simple hyperplasia, 78.5% for complex hyperplasia, 75.4% for complex atypical hyperplasia.

In the current study, the highest values of percentage of positive cells for estrogen receptors (ER)were observed in case of simple hyperplasia without atypia 75% and complex hyperplasia without atypia 75%, followed by complex atypical hyperplasia with 60%.Analyzing the PR expression of various types of endometrial hyperplasia, we observed that complex hyperplasia without atypia has the highest mean values of percentage of positive cells for progestrone receptors (85%), followed by simple hyperplasia without atypia (75%) and complex atypical hyperplasia (70%). In the present study,we observed that the mean score of ER and PR decreased in cases of hyperplasias with atypia as compared to hyperplasias without atypia, which is in concurrence with the above mentioned studies of Daniela et al(16), Samhita Chakraborty et al (14) and Nyholm et al(15) .After a comparative analysis it was noted that, of ER and PR expression, progesterone receptors are better expressed than estrogen receptors. Thus, for every morphological pattern of the endometrium analyzed

in this study, the average values for PR were higher compared with average values of ER for similar lesions.

Both typical hyperplasia and atypical hyperplasias may regress spontaneously over months or a few years. However, atypical hyperplasia is a precancerous condition that may progress to malignancy and best treated by surgery with hysterectomy. Hyperplasia without atypia regresses spontaneously after D&C or progestin treatment (14,17). In patients with atypical hyperplasia, if conserving the uterus is considered, a trial of hormonal treatment may be given (4,18). Progesterone receptor rich lesions have a better response rate to progestins than lesions which are progesterone receptor poor (19).

Analysis of the steroid hormone receptors plays an important role or may be an indication in perimenopausal bleeding patients to predict the response to hormonal therapy (4,14). Immunohistochemistry explains the response of the patient to hormonal therapy in cases of hyperplasia of endometrium, and suggest that, in these cases, there are many alterations in the cellular DNA, but does not allow the prediction of the cases of atypical hyperplasia which will progress into endometrial carcinoma (16). In our study, the cases of atypical hyperplasia, which are steroid receptor positive, might have responded well if these patients were given hormonal therapy[4].

V. Conclusion

Our study throws a light on many important facts about perimenopausal bleeding. The present study revealed that the incidence of perimenopausal bleeding cases constituted 51.68%. The distribution of cases of perimenopausal bleeding was maximum in the age group of 40-45 years (67%) compared to 46-50 years. The most common histological pattern of endometrium observed was proliferative endometrium and its variants (49.50%). Among hyperplasias, which constituted 24% of the total cases, simple hyperplasia without atypia was higher (87.50%). In the total of 6 cases of complex hyperplasia, 66.67% were complex hyperplasia without atypia and 33.33% complex atypical hyperplasia, and they were equally distributed between the age group of 40-45 & 46-50 years. The other associated organic lesions in perimenopausal age were fibroid uterus 18.5% and endometrial polyps 2.5% and the predominant pattern of endometrium in perimenopausal women with fibroid uterus was simple hyperplasia 32.43%.

Analyzing the ER, PR expression by immunohistochemical method revealed that there is a significant difference in the epithelial and stromal expression of PR between simple hyperplasia and complex hyperplasia. Among ER and PR expression, progesterone receptors were better expressed than estrogen receptors. Thus, for every morphological aspect of the endometrium analyzed in this study, the mean values for PR were higher compared with mean values of ER for similar lesions. The cases of atypical hyperplasia, which were steroid receptor positive, might have responded well if these patients were subjected to hormonal therapy (4,18). The response rate to progestins for the lesions which are progesterone receptor rich are better compared to progesterone receptor poor lesions (19). Analysis of the steroid hormone receptors plays an important role or may be an indication in perimenopausal bleeding patients to predict the response to hormonal therapy.

Immunohistochemical analysis of the Estrogen and Progesterone receptors in hyperplasias of endometrium allows a more specific determination of the cell ER, PR receptor content and hence yields a more accurate prediction of the response of the patient to endocrine therapy. The immunohistochemical studies of ER and PR in endometrial hyperplasia and its significance on the prognosis of the disease and hormone therapy are few in the medical literature compared to receptor studies on breast cancer. Our data will definitely be an important addition to the existing literature. These studies should facilitate the development of rational strategies for the prevention and treatment of grave and lethal endometrial disorders.

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TABLE 1: Distribution of endometrial patterns in perimenopausal bleeding cases

Type of Endometrium	**No. of cases**	**Percentage**
Proliferative endometrium and its variants	99	49.50%
Secretory endometrium and its variants	12	6%
Hyperplasias	48	24%
Others	41	20.50%
Total	200	100%

TABLE 2: Types of hyperplasias

Type of Endometrium	**No. of cases**	**Percentage**
TYPICAL		
Simple Hyperplasia without atypia	42	87.50%
Complex Hyperplasia without atypia	4	8.33%
ATYPICAL		
Simple Hyperplasia with atypia	0	0%
Complex Atypical Hyperplasia	2	4.16%
Total	**48**	**100%**

Chi-square with Yate's correction value =7.45, P<0.05.

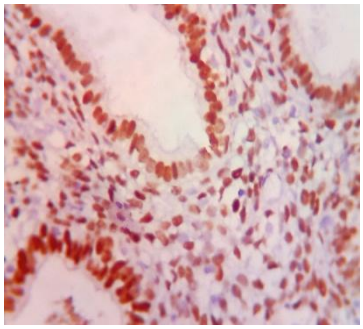

Figure 1: Simple Hyperplasia without atypia (ER,400X)

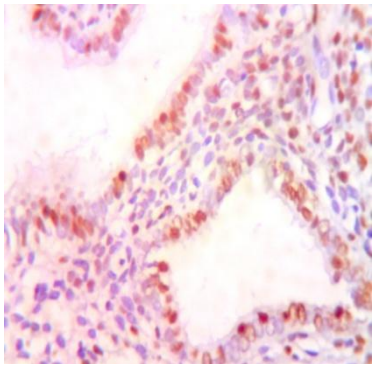

Figure 2: Simple Hyperplasia without atypia (PR,400X)

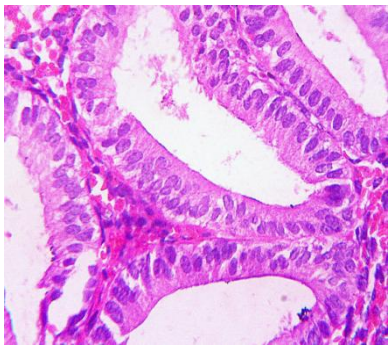

Figure 3 : Complex Hyperplasia without atypia (H&E,400X)

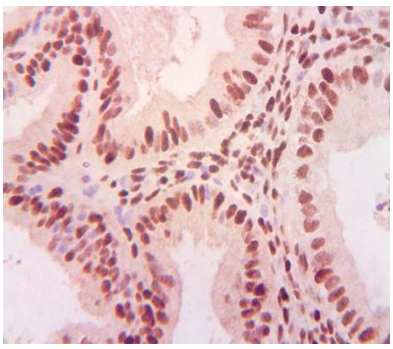

Figure 4: Complex Hyperplasia without atypia (ER,400X)

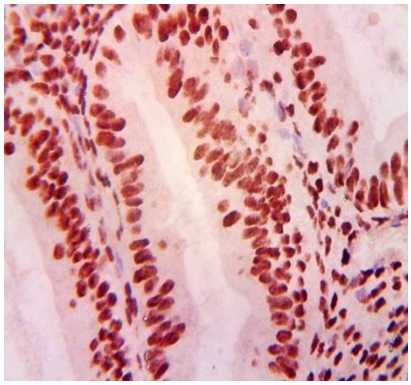

Figure - 5: Complex Hyperplasia without atypia (PR,400X)

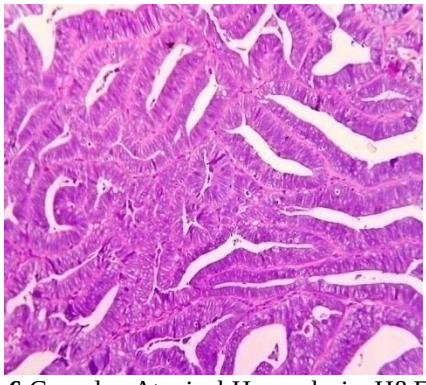

Figure - 6:Complex Atypical Hyperplasia. H&E (100X)

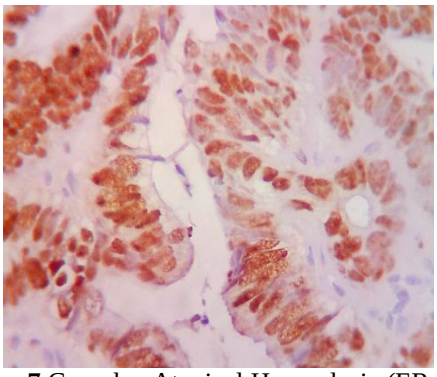

Figure - 7:Complex Atypical Hyperplasia (ER,400X)

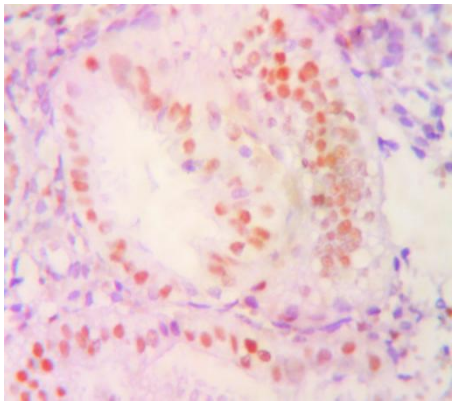

Figure – 8 : Complex Atypical Hyperplasia (PR,400X)