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Original Research Article

A Study on Correlation of ER, PR Status and Serum Estradiol Levels with Histopathology Type among Women with Dysfunctional Uterine Bleeding

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Abstract

Introduction: Dysfunctional uterine bleeding is a clinical term used to describe bleeding not attributable to an underlying organic pathologic condition. Receptor studies in DUB cases show that ER and PR levels in DUB patients were significantly higher. Circulating blood levels of estrogen and progesterone have implications on DUB.A few studies have shown that DUB has high estradiol levels. There is Based on this aim of our study is to evaluate the histopathological features of endometrium in correlation with Serum Estradiol levels and with special emphasis on ER, PR receptors and morphometry in the diagnosis of menorrhagia.

Materials and Methods: This study is a prospective study of the histomorphological profile of endometrium in dysfunctional uterine bleeding in patients in age group of 20-75 years. A total of 60 cases were enrolled during our study period. Endometrial curettage from patients of other age groups were excluded. Immunohistochemically scoring for ER and PR receptors were done with Quick score. Also, intensity scoring was done. The above two scores, score for proportion and the score for intensity are summated to a total maximum score of 8. Score of more than 2 is considered positive.

Results: The common histopathologic diagnosis was proliferative phase in 45 %. ER mad PR were positive in all type of HPE findings in our study with different ranges. The levels of ER and PR expression was significantly raised in DUB patients compared to normal patients.Serum estradiol levels too were positively correlated.

Conclusion: The study of ER and PR expression helps in diagnosing endometrial hyperplasia without atypia, endometrial hyperplasia with atypia and endometrial carcinoma. Measurement of serum estradiol levels in DUB is important because it is markedly increased in case of endometrial hyperplasia and endometrial carcinoma compared to normal proliferative and secretory endometrium.

Keywords: Dysfunctional uterine bleeding, ER, PR, Serum estradiol.

Introduction

Dysfunctional uterine bleeding is a clinical term used to describe bleeding not attributable to an underlying organic pathologic condition. About 10% cases of Dysfunctional Uterine Bleeding are ovulatory and 90%are anovulatory. During an anovulatory cycle, the corpus luteum does not form and hence the normal cyclical secretion of progesterone does not occur. Estrogen will stimulate the endometrium unopposed.

Without progesterone, the endometrium continues to proliferate, Will eventually outgrow its blood supply and sloughs and sheds irregularly for a long time. When this process occurs repeatedly, the endometrium can become hyperplastic, sometimes with atypical or cancerous cells. Throughout the perimenopausal transition there is a significant incidence of DUB due to anovulation.[1]

A better definition is phase before the onset of menopause during which the regular menstrual cycle of a woman transitions to irregular cycles.[2] Perimenopausal bleeding refers to the symptoms of excessive, prolonged acyclic bleeding regardless of the cause.[3] The main reason for checking endometrial histology of the patients in perimenopausal age is to exclude hyperplasia's and carcinomas in the age.[4]

Anovulatory DUB can result secondary to PCOS and hypothyroidism. An acute bleeding episode is best controlled with high-dose estrogen. Treatments may consist of hormonal therapy with estrogen and progestin or cyclical progestin alone.

Surgery may be needed if hormone therapy fails. Surgical options include dilation and curettage (D&C), laser or electro cauterization, endometrial ablation, or hysterectomy. Circulating blood levels of estrogen and progesterone have implications on DUB.A few studies have shown that DUB has high estradiol levels.

The estrogen receptor (ER) and progesterone receptor (PR) expression and distribution pattern might play an important role in normal endometrial function. Receptor studies in DUB cases show that ER and PR levels in DUB patients were significantly higher. Morphometric analysis included gland shape, lumen/gland relative volume, and gland: stroma ratio and number of capillaries/mm2 stroma. Based on this aim of our study is to evaluate the histopathological features of endometrium in correlation with Serum Estradiol levels and with special emphasis on ER, PR receptors and morphometry in the diagnosis of menorrhagia.

Materials and Methods

This study is a prospective study of the histomorphological profile of endometrium in dysfunctional uterine bleeding conducted in the Department of Pathology in a tertiary care teaching hospital for a period of two years in patients in age group of 20-75 years. Same day blood samples for estradiol levels were taken from the respective patients. A total of 60 cases were enrolled during our study period. Endometrial curettage from patients of other age groups were excluded.

The endometrial curettage samples were fixed in 10% formalin, histopathological slides were prepared and stained with Haematoxylin and Eosin stain. All cases of hyperplasia including simple hyperplasia without atypia, complex hyperplasia without atypia, complex atypical hyperplasia and carcinoma were selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for ER and PR status.

Immunohistochemical Evaluation:

Immunohistochemical analysis of ER and PR were done in paraffin embedded tissue

samples for all cases which were diagnosed as dysfunctional uterine bleeding, hyperplasia's, including simple hyperplasia without atypia, complex hyperplasia without atypia, complex atypical hyperplasia and carcinoma using supersensitive polymer HRP system based on non-biotin polymeric technology. 4 µ thick sections from formalin fixed paraffin embedded tissue samples were transferred on to gelatin coated slides. Heat induced antigen retrieval was done. The antigen is bound with mouse monoclonal antibody (Biogenex) against estrogen and progesterone receptors.

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization. The percentage of cells stained per 1000 cells counted on 40X power field and the intensity of reaction were analyzed. Immunohistochemical scoring for ER and PR receptors were done with Quick score[5]. Also intensity scoring was done. The above two scores, score for proportion and the score for intensity are summated to a total maximum score of 8.Score of more than 2 is considered positive.

Results

The study was conducted on 60 patients clinically diagnosed as DUB in the department of Gynecology in a tertiary care teaching hospital. The age group of these patients ranged from 22 years to 72 years with maximum number of cases in the range of 41 to 50 years (41.66%,N=25). Only (3.5%, N=2) were found to be more than 70 years.

Most of the cases were in the perimenopausal age group (38.34%, N= 23) followed by reproductive age group (41.66 %, N =25) and postmenopausal age group (20%, N=12). The common symptom is menorrhagia with 100% of cases.

Dilatation and curettage was performed on 71.66%, N=43 of patients and total abdominal hysterectomy with bilateral salphingo-oophorectomy was done for 28.34%, N= 17 of patients.

The common histopathologic diagnosis was proliferative phase in 45 % (N=27). Out of these 41.6% and 75% did not correlate with LMP respectively. Secretory endometrium was seen in 23.3% (N=14), in the age group of 22-50yrs. 60% of the cases did not correlate with LMP. Hyperplasia without atypia was seen in 11.6 % (N=7), Atrophic endometrium, atypical hyperplasia and endometrial carcinomas constitutes (5% N=3, 3.3% N=2, 3.3% N=2) respectively.

Table 1: Type of lesions

S.no	Histopathological diagnosis	No of cases	Percentage
1	Proliferative phase	27	45%
2	Secretory phase	14	23.3%
3	Hyperplasia without atypia	7	11.6%
4	Disorderly proliferative phase	5	8.3%
5	Endometrial carcinoma	2	3.3%
6	Atypical hyperplasia	2	3.3%
7	Atrophic endometrium	3	5%

Estrogen and progesterone receptor for IHC staining:

Positive staining of both ER and PR was seen in the nuclei of both glands and stroma. The intensity of the cells stained and percentage stained were analyzed and immunohistochemical scoring for ER and PR was done with quick score.

ER and PR showed nuclear positivity .Staining was graded in both epithelial and stromal component including cases of hyperplasia without atypia, atypical hyperplasia and malignancies of endometrium. Vascular smooth muscle cells and endothelial cells were consistently negative in all the cases.

Estrogen and progesterone receptor staining in hyperplasia without atypia:

Hyperplasia without atypia showed moderate staining intensity of ER in glands and stroma with a mean percentage of 60.5% positivity in both endometrial glands and stroma. PR also showed strong staining intensity in both glands and stroma with a mean percentage of 90.4% positivity.

Estrogen and progesterone receptors staining in atypical hyperplasia:

Atypical hyperplasia showed strong staining intensity of ER in glands and stroma with a mean percentage of 100% positivity in both endometrial glands and stroma.PR also showed strong staining intensity in both glands and stroma with a mean percentage of 100%.

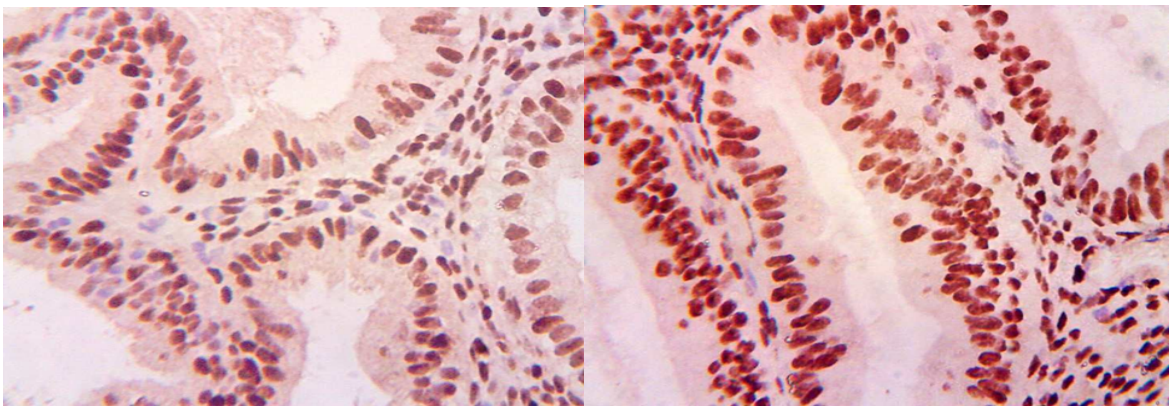

Figure 1: IHC of ER and PR in atypical hyperplasia

Estrogen and progesterone receptors staining in endometrial carcinoma:

Endometrial carcinoma showed weak staining intensity of ER in glands and stroma with a mean percentage of 25% positivity in both endometrial glands and stroma. PR also showed weak staining intensity but more than ER in both glands and stroma with a mean percentage of 37.5%.

Estrogen and progesterone receptors staining in proliferative phase:

Proliferative phase showed moderate staining intensity of ER in glands and stroma with a mean percentage of 45% positivity in both endometrial glands and stroma. PR showed staining intensity more than ER in both glands and stroma with a mean percentage of 65%.

Estrogen and progesterone receptors staining in secretory phase:

Secretory phase showed moderate staining intensity of ER in glands and stroma with a mean percentage of 45% positivity in both endometrial glands and stroma. PR showed staining intensity more than ER in both glands and stroma with a mean percentage of 70%.

Estrogen and progesterone receptors staining in disorderly proliferative phase:

Disorderly proliferative phase showed moderate staining intensity of ER in glands

and stroma with a mean percentage of 70% positivity in both endometrial glands and stroma. PR showed staining intensity more than ER in both glands and stroma with a mean percentage of 80%.

Table 2: Estrogen receptor expression

Type of endometrium	Glandular cells	Stromal cells
Proliferative phase	Positive (45%)	Positive (45%)
Secretory phase	Positive (45%)	Positive (45%)
Disorderly proliferative phase	Positive (70%)	Positive (70%)
Atrophic endometrium	Positive	Positive
Hyperplasia without atypia	Positive (60.5%)	Positive (60.5%)
Atypical hyperplasia	Positive (100%)	Positive (100%)
Endometrial carcinoma	Positive (25%)	Positive (25%)

Table 3: Progesterone receptor expression

Type of endometrium	Glandular cells	Stromal cells
Proliferative phase	Positive (65%)	Positive (65%)
Secretory phase	Positive (70%)	Positive (70%)
Disorderly proliferative phase	Positive (80%)	Positive (80%)
Atrophic endometrium	Positive	Positive
Hyperplasia without atypia	Positive (90.4%)	Positive (90.4%)
Atypical hyperplasia	Positive (100%)	Positive (100%)
Endometrial carcinoma	Positive (37.5%)	Positive (37.5%)

Gland: stroma ratio was very high in endometrial carcinoma, atypical hyperplasia, hyperplasia without atypia. The most common glands seen were round to oval in shape. Simple endometrial hyperplasia, complex endometrial hyperplasia and endometrial carcinoma had branching, back to back glands.

Simple endometrial hyperplasia, complex endometrial hyperplasia and endometrial carcinoma had increased mitosis, vascularity and nuclear stratification.

Table 4: Serum estradiol levels

ESTRADIOL				
		Mean	Std. Deviation	Std. Error
Endometrial carcinoma	2	308.95	3.46	2.45
Hyperplasia without atypia	7	162.00	57.51	21.74
Proliferative phase	27	58.25	39.87	7.81
Secretory phase	14	99.35	49.03	13.10
Disorderly proliferative phase	5	117.29	65.02	29.08
Atypical hyperplasia	2	202.60	28.37	20.06
Atrophic endometrium	3	22.82	33.08	19.10
Total	60	93.06	70.35	9.15

Serum estradiol levels were increased in hyperplasia without atypia and complex hyperplasia with atypia ranging from 111.3pg/ml-206.3pg/ml. Serum estradiol levels were markedly increased in cases of malignancies with values above 300 pg/ml.

Serum estradiol levels in disorderly proliferative endometrium was slightly increased compared to secretory and proliferative phase with a mean value of 117.29 pg/dl

Discussion

Menstrual disorders are common, accounting for almost 3% of all outpatient referrals and over 20% of referrals to gynaecology outpatients' clinics. In this study the maximum age incidence of patients suffering from menorrhagia was between 41-50 years which is similar to the studies done by Pilli GS *et al* [6] and Archana [7] *et al* reported that the common age incidence was between 40-50 years, while Sadia Khan *et al* [8] reported as 45-55 years, Shazia Riaz *et al* [9] as 45-50 years and Layla *et al* [10] more than 52 years.

In this study the most common pattern seen was proliferative phase (45%N=27). Similar results were given in studies done by Sadia Khan *et al* [8] reported proliferative phase (46%), Shazia Riaz [9] (33%), Archana *et al* [7] (53%).Pilli GS *et al* [6] reported endometrial hyperplasia as most common (44%), Layla *et al*10 reported secretory phase in 24% of cases.

In this study serum estradiol levels were high in simple endometrial hyperplasia and complex endometrial hyperplasia and very high in endometrial carcinoma. The mean serum estradiol level in this study for patients with endometrial carcinoma was 308.95pg/dl (N=2). Similarly for atypical hyperplasia it was 202.60pg/dl (N=2), disorderly proliferative phase it was 117.29 pg/dl (N=5) In our study serum estradiol levels was markedly increased in endometrial hyperplasia and endometrial carcinoma compared to normal proliferative and secretory endometrium with a significant p value of <0.005. Similar studies by Vysotskiĭ MM *et al* [11] reported high estradiol levels were detected in the blood serum of patients with glandular cystic polyps, atypical and glandular hyperplasia.

Lépine J *et al* [12] stated that circulating levels of estradiol levels were significantly higher in endometrial carcinoma compared to normal endometrium. Moen MH [13] *et al* stated that estradiol levels are increased in perimenopausal women with menometrorrhagia. However some studies found normal and decreased estradiol levels in atypical hyperplasia and endometrial carcinoma.

Hong Yul Choi [14] *et al* reported that secretory substance in the epithelial cells of the endometrial glands during the secretory phase and menstrual phase was mainly glycogen and concluded that PAS staining is superior to routine hematoxylin and eosin staining for the detection of epithelial secretory substance. Eddie SM [15] *et al* stated that PAS stain is superior to H & E. However in this study there was no advantage of PAS over H&E.

Spona J [16] *et al* stated that receptor levels were highest in well-differentiated group of endometrial carcinoma. Hu K [17] *et al* reported decreased levels of estrogen receptors in both atypical hyperplasia and adenocarcinoma. Teleman S [18] *et al* reported high level of both ER and PR in simple and complex hyperplasias and a significant decrease in atypical conditions.

Guo Y [19] *et al* reported that ER and PR rates in adenocarcinoma were higher than in the normal histological types. Samhita Chakraborty [20] *et al* reported increased levels of ER and PR levels in DUB cases. Li SF, Shiozawa T stated that it has been shown that a loss of sex steroid receptors is an early event in endometrial carcinogenesis, and endometrial carcinoma generally has a lower level of steroid receptors than does normal endometrium or endometrial hyperplasia[21].

ER-α expression is decreased in EC in both glands and stroma in relation to non-malignant tissue and is further decreased as EC grading is advanced. The expression of ER-α is lower in the stroma than in the glands of EC, indicating that stroma cells are significantly more affected than the epithelial cells.

PR expression of either one or both of the two PR isoforms PR-A or PR-B is also reduced or absent in EC. PR-A shows the exact same pattern of expression as ER-α in the gland and stroma cells, as well as in the different portions of EC specimens. The levels of ER and PR were significantly reduced compared to normal endometrium. In our study the levels were near normal in proliferative, secretory phase and was increased in hyperplasia's and decreased in endometrial carcinomas.

The levels of ER and PR expression was significantly raised in DUB patients compared to normal patients. Both hyperplasia's without atypia and atypical hyperplasia's may regress spontaneously over months or 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. Progesterone receptor rich lesions have a better response rate to progestin than lesions which are progesterone receptor poor.

Analysis of the steroid hormone receptors play an important role or may be an indication in perimenopausal bleeding patients to predict the response to hormonal therapy. 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 of the cellular DNA, but does not allow the prediction of the cases of atypical hyperplasia which will progress into endometrial carcinoma. In our study, the cases of atypical hyperplasia which are steroid receptor strong positive, might have responded well if these patients were given hormonal therapy.

Conclusion

In our study ER and PR levels were very high in simple and complex endometrial hyperplasia and decreased in endometrial carcinoma. It was slightly increased in disordered proliferation and irregular shedding and almost normal expression was seen in secretory and proliferative endometrium.

The study of ER and PR expression helps in diagnosing endometrial hyperplasia without atypia, endometrial hyperplasia with atypia and endometrial carcinoma since ER and PR expression was markedly increased in endometrial hyperplasia without atypia, endometrial hyperplasia with atypia and markedly decreased in case of endometrial carcinoma. The response rate to progestin for the lesions which are progesterone receptor rich are better compared to progesterone receptor poor lesions.

Measurement of serum estradiol levels in DUB is important because it is markedly increased in case of endometrial hyperplasia and endometrial carcinoma compared to normal proliferative and secretory endometrium. Serum estradiol levels were moderately increased in disorderly proliferative endometrium compared to normal proliferative and secretory endometrium.

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