An Immunohistochemical Study of Estrogen and Progesterone Receptors in Endometrium of Women with Dysfunctional Uterine Bleeding

A. Kavitha[1] and J. Thanka[1*]

[1]Department of Pathology, Sree Balaji Medical College and Hospital Affiliated to Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Abnormal Uterine Bleeding (AUB) is a common complaint that affects large numbers of women from puberty to menopause. It negatively affects health and quality of life of women affected. AUB also has an economic impact for both women and society Abnormal uterine bleeding in premenopausal women is one of the most frequent problems in gynecological practice. Although some of the cases may be due to an organic cause, over 50% of the patients undergoing hysterectomy for menorrhagia have dysfunctional uterine bleeding (DUB). To analyze the percentage and intensity of estrogen receptors (ER) and progesterone receptors (PR) in endometrium of patients with DUB. This study suggests that estrogen and progesterone receptors have an important role in etiopathogenesis of dysfunctional uterine bleeding and alteration in the morphology of endometrium such as development of endometrial hyperplasia. Women in the reproductive age who are complaining of abnormal uterine bleeding, usually have an increase in ER alpha and PR expression in their endometrium.

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*Corresponding author: E-mail: thanka.path@bharathuniv.ac.in;

1. INTRODUCTION

Abnormal Uterine Bleeding (AUB) is a common complaint that affects large numbers of women from puberty to menopause. It negatively affects the health and quality of life of women affected. AUB also has an economic impact for both women and society.

Estrogen and progesterone receptors belong to the nuclear steroid receptor superfamily the effect of these steroid hormones are thought to be mediated through these receptors. The ER and PR IHC expression and distribution pattern may play an important role in endometrial function and pathogenesis 1-4. The study of these receptors -distribution in the endometrial glands could open the gate for medical treatment of cases of AUB and avoid unnecessary surgical intervention. The cause of the bleeding may be due to potentiation of the hormonal action through change in their receptor levels 5. Also there is positive correlation between the endometrial angiogenesis and menstrual disorders. The alternation in blood vessel morphology and density also plays a significant role. Hence the present study was conducted to analyze endometrial estrogen and progesterone receptor expression and blood vessel density in cases of abnormal uterine bleeding.

Abnormal uterine bleeding (AUB) is defined as change in frequency, duration and amount of menstrual bleeding. AUB without any associated organic cause is referred to as "Dysfunctional Uterine Bleeding (DUB)". 1 DUB is fundamentally a diagnosis of exclusion which can be made only with histopathological examination 1, 2 after excluding detectable anatomical abnormality or evidence of systemic diseases. 3 It is one of the most common gynecological problems which accounts for one third of gynecologic out - patient department. It is common in early and late reproductive age group, more than 50% of the patients who were treated by hysterectomy for menorrhagia had DUB. 4 DUB can be seen in both ovulatory and anovulatory cycles. DUB with ovulatory cycles has increased bleeding with regular menstrual cycles. Anovulatory cycles have irregular, prolonged and excessive bleeding. Unopposed estrogen stimulation of endometrium irrespective of serum estrogen levels is the mechanism behind DUB, which causes endometrial hyperplasia which is a known risk factor for endometrial carcinoma. 5 DUB have significant effect on the health status and the quality of life of women, causing severe anemia and infertility due to anovulation. 6 Thus, timely management is very essential [1-5].

Histological examination of endometrial biopsy sample is the investigation of choice to confirm DUB. 7 Various histological patterns seen in DUB. Most of which are effectively treated by hormonal therapy. Endometrial ablation and hysterectomy are done only in severe cases. 8 Advent of receptor modulating drugs and detection of precise location of steroid receptors in endometrium, through which the ovarian hormones act, has reformed medical management of DUB patients [6]. 9 The understanding of steroid receptors in endometrium is extremely important because hormone receptors have role in the etiopathogenesis of DUB. 7, 9 Immunohistochemistry helps to localize receptors (estrogen and progesterone) in tissue sections. 10

Many studies have been done on cyclical variation of estrogen and progesterone receptors in normal endometrium, but very few studies have been done on the pattern of expression of these hormonal receptors in the endometrium of DUB patients. So, this study was undertaken with the aim to know about the expression of estrogen (ER) and progesterone receptors (PR) in endometrium of patients with dysfunctional uterine bleeding to establish the role of hormonal receptors in the etiopathogenesis of DUB, categorizing type of DUB and deciding on management of DUB [7,8].

2. METHODOLOGY

This is a prospective, cross - sectional study, was done in the Department of Pathology, Sree Balaji Medical College and Hospital, Chromepet, Chennai, from September 2015 to September 2017. The study material included 50 cases of pipelle sampling / TAH& BSO specimen which was received by the Department of Pathology. Pipelle procedure or Hysterectomy was performed for clinical diagnosis of DUB in the Department of Obstetrics and Gynecology at Sree Balaji Medical College and Hospital, Chennai. Complete history, menstrual history, physical examination findings (general, per

abdomen, per vaginal, per speculum), hormonal status, hematologic values and transvaginal ultrasound findings noted from patients records for exclusion of genital tract abnormality and for measurement of endometrial width.

Inclusion criteria: Women aged between 20 - 50 years.

Patients with history of menstrual irregularities like, irregular cycles, excessive and prolonged me natural bleeding. Clinically confirmed diagnosis of DUB, after ruling out structural abnormalities by physical examination and ultrasound investigation. Women with normal estrogen and progesterone levels.

Exclusion criteria: Women on hormone replacement therapy, oral contraceptive pills, intrauterine contraceptive devices and long- term therapy of aspirin. Women with history thyroid dysfunction (abnormal TSH level) and history bleeding diathesis (abnormal PT level).

Histologic features of endometrial malignancy: The cases were selected based on following inclusion and exclusion criteria. The specimen was received in 10% neutral buffered formalin. After adequate fixation, the gross morphology of the specimens were noted. Endometrial samples were all embedded and representative bits were taken from hysterectomy specimens. After tissue processing, 3 - 5 - micron thick sections were taken and stained with hematoxylin and eosin as detailed in (Annexure II) and are studied microscopically.

Immunohistochemistry was performed on paraffin embedded sections taken on glasss lides pre - coated with poly- L- lysine, using Pathn Situ poly Excel IHC detection systems, which uses micro - polymer based technology wherein the HRP based polymer conjugates with secondary antibody bound to primary antibody which reacts with DAB (3 - 3 diaminobenzidine).

Substrate - chromogen conjugation gives brown color.

Statistical Methods Used for Data Analysis: Data were analyzed using SPSS version 21. The significance of the results were assessed by determining the probability factor "p" value. $P< 0.005$ – Significant, $p> 0.005$ - Not significant. Distribution of histopathologic patterns across the DUB and control groups were compared using Chi square test. Data were normally distributed and changes in ER/ PR expression between DUB and control groups were analyzed using independent samples test. ER/ PR expression across various HP patterns was compared using median test in DUB group.

3. RESULTS

The clinical profile of DUB patients is summarized in Table 1. Majority of patients were more than 35 years, constituting 60% of total DUB patients (Fig. 1). The mean age of patients in DUB group was 41.08 years. The mean duration of menstrual blood flow in DUB group was long (8.04 days), although the mean duration of the cycle (28.86 days) was nearly normal. Upon analyzing parity among DUB patients, a large proportion (84%) of patients was noted to be multiparous of which 66% had 2 children (Fig. 2). The mean endometrial thickness was 6.72 mm. DUB patients were divided according to endometrial thickness (Fig. 3), a significant proportion of patients had endometrial thickness more than 4.5 mm (n= 38, 76%).

Square test was done to analyze variation in distribution of histopathologic patterns across normal (n= 50) and DUB (n= 50) groups. There was more of secretory histopathologic patterns in normal subjects and significant increase in hyperplasia without atypia (n= 17, 34%) and atypical endometrial hyperplasia (n= 2, 4%) patterns in DUB group (Fig. 6). The Chi - Square ratio was significant (χ^2 = 16.29, p = 0.001).

Table 1. Clinical profile of DUB group

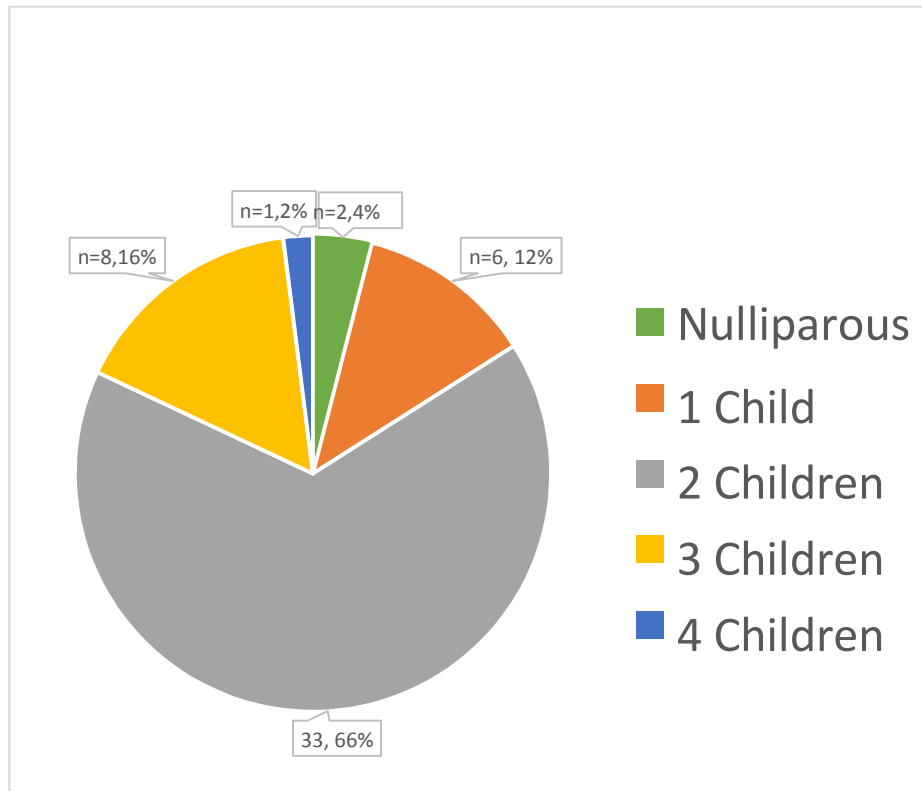| Profile	Mean± SD
Age	41.08 ± 6.67
Parity	2 ± 1
Endometrial thickness	6.72 ± 2.3
Cycle duration	28.86 ± 5.20
Bleeding days	8.04 ± 3.20

Fig. 1. Distribution of DUB patients according to parity

Table 2. Cross tabulation of histopathologic patterns across DUB (n= 50) and normal group (n= 50)

		PE	SE	HWA	A.E.H	Total
Normal	Count	21	29	0	0	50
	Expected count	18	22.5	8.5	1	50
	% within group	4 2.0%	5 8.0%	0%	0%	100%
	% total	21%	29%	0%	0%	50%
DUB	Count	15	16	17	2	50
	Expected count	18	22.5	8.5	1	50
	% within group	30.0%	32.0%	34.0%	4.0%	100%
	% total	15%	16%	17%	2%	50%

PE - Proliferative endometrium SE - Secretory endometrium
HWA - Hyperplasia without atypia
AEH - Atypical endometrial hyperplasia

Table 3. Comparison of changes in distribution of HP Patterns across DUB and control groups using Chi Square test.

	Value	DF	P value (2 tailed)
Pearson chi square	16.292	3	0.001
Likelihood ratio	18.601	3	0.001
Number of valid cases	100		

Changes in ER/ PR expression in normal and DUB groups

Comparison of ER/ PR expression between DUB and normal groups showed variable expression of the receptors Table 3. In DUB group, the mean receptor scores are, ER in glands (180.72 ± 39.1), ER in stroma (175.98 ± 44.4). PR in glands (164.06 ± 41.5) and PR in stroma (165.56

± 40.1) were almost equal. Among normal group, the maximum score was that of PR in gland (107.52 ± 18.7) and least for ER in stroma (99.30 ± 19.17). Overall, the mean concentrations of PR in both glands and stroma were more than that of ER in g lands and stroma in normal group, But in DUB group the mean concentration of ER in glands and stroma were more than that of PR (Fig. 7). An Independent samples t test was performed to compare the ER and PR expression in DUB and normal groups. There was a significant increase in ER/ PR receptor expression (all $p < 0.001$) in both endometrial glands and stroma in DUB group compared to normal group.

In proliferative phase, the maximum receptor concentrations were those of progesterone in stroma (mean= 143.31 ± 46.42) and estrogen in gland (mean= 143.03 ± 52.92), followed by PR in glands (mean= 142.00 ± 49.87). The concentration of ER in stroma were the least (mean= 140.14 ± 52.09), but not much difference in staining pattern noted. In secretory phase, PRG and PRS were comparable 122.51 ± 35.43, 120.96 ± 39.96 respectively and ERS were the least 112.31 ± 34.02.

In hyperplasia without atypia, the mean concentration of ER in stroma was significantly higher (197.89 ± 37.03) which was com parable to ER in gland (197.50 ± 44.82). Also, it was more than mean ERG and ERS concentration in all other histologic patterns.

In atypical hyperplasia ERS have maximum mean of 121.50 ± 14.85 and PRS have least 111.50 ± 0.707 expression. A non- parametric median test to compare ER/ PR expression across various HP pattern in DUB group.
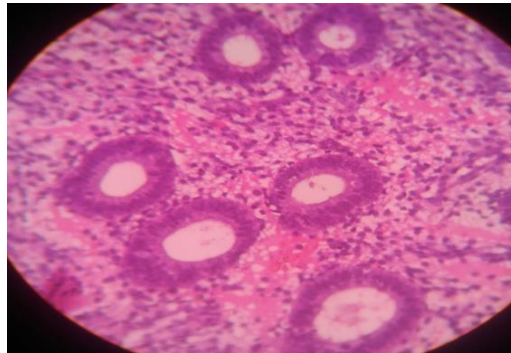
CASES

Proliferative Endometrium

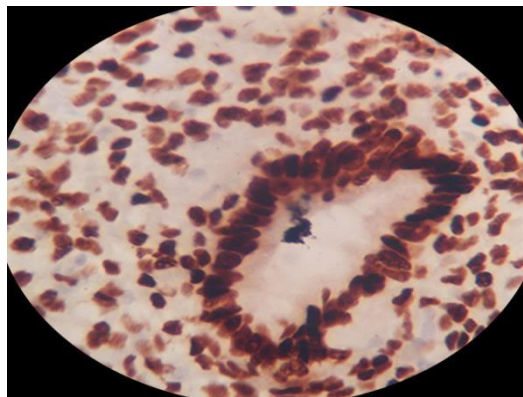**Fig. 2. Proliferative Phase (H & E, 100x mag.)**

Fig. 3. Proliferative Phase (ER, 400x mag.). Endometrium with glands showing 4+ nuclear staining and stromal nuclei showing variable intensity (1 to 3+)
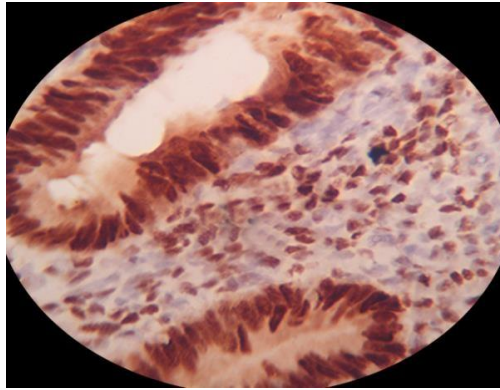
Fig. 4. Proliferative Phase (PR 400x mag). Endometrium showing strong nuclear staining of glands (3+) and weak nuclear staining of stroma (0 to 3+)
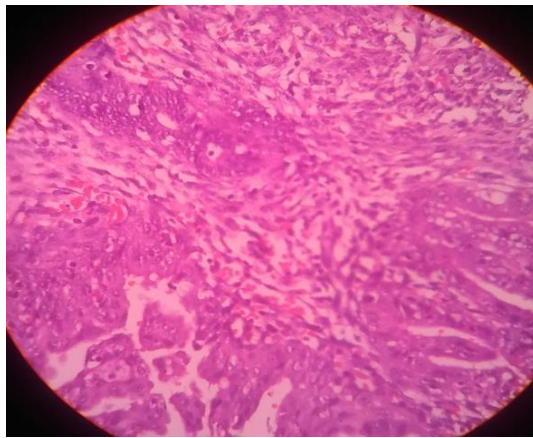

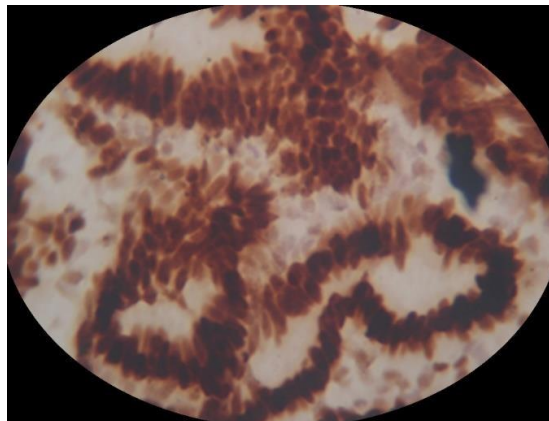**Fig. 5. Hyperplasia without atypia (H&E, 100x mag.)**

Fig. 6. Endometrial hyperplasia without atypia (ER, 400x mag.). Endometrium showing strong nuclear (3+ to 4+) staining of both glands and stroma seen
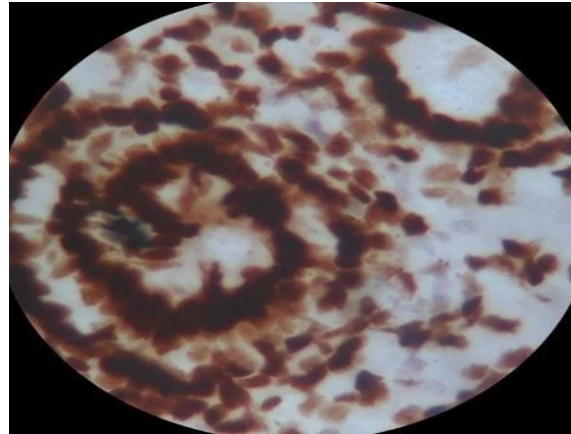
Fig. 7. Hyperplasia without atypia (PR, 400x mag.). Endometrium showing strong nuclear (4+) staining of glands and (3+) staining of stroma

Table 4. Comparison of mean values of ER and PR expression in endometrial glands and stroma in DUB group and control group using Independent samples *t* test

Group	ERG	ERS	PRG	PRS
Normal n= 50 Mean ± SD	100.08 ± 18.2	99.30 ± 19.17	107.52 ± 18.7	105.74 ± 16.9
DUB n= 50 Mean ± SD	180.72 ± 45.2*	175.98 ± 44.4*	164.06 ± 41.5*	165.56 ± 40.1*
* p < 0.001 using independent samples *t* test				

ERG- Estrogen receptor in gland; ERS - Estrogen receptor in stroma
PRG - Progesterone receptor in gland; PRS – Proges receptor in stroma

Table 5. Comparison of ER/ PR expression in various HP patterns in patients with DUB using Median test

H P pattern	Mean ± SD ERG	Mean ± SD ERS	Mean ± SD PRG	Mean ± SD PRS
Proliferative Endometrium n = 1 5	143.03 ± 52.92	140.14 ± 52.09	142.00 ± 49.87	143.31 ± 46.42
Secretory Endometrium n = 1 6	116.8 ± 37.93	112.31 ± 34.02	122.51 ± 35.43	120.96 ± 39.96
Hyperplasia without atypia n = 1 7	197.5 ± 44.82	197.89 ± 37.03	159.56 ± 34.92	160.17 ± 29.63
Atypical Hyperplasia n =2	111.5 ± 13.43	121.5 ± 14.85	112.00 ± 19.79	111.50 ± 0.707
Total = 50				

ER/ PR expression across various HP pattern in normal group: Variation of ER and PR across different histologic patterns in normal group also follow a trend. There was an increasing trend in both ERG and ERS in proliferative phase and decreasing trend in secretory phase (Fig. 9). The concentration of ER in both glands and stroma almost paralleled in both phase. Similar trend was noted in PRG and PRS (Fig. 10).
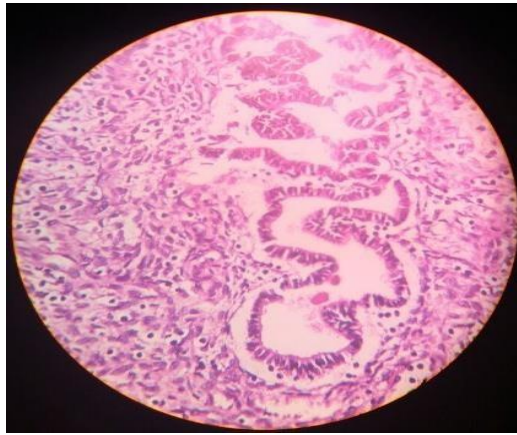
Secretory Endometrium

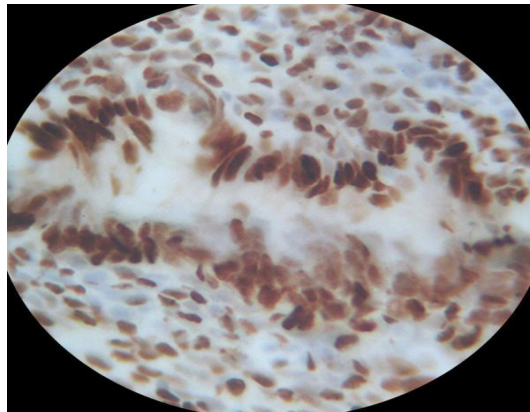**Fig. 8. Secretory Endometrium (H&E, 100x mag.)**

Fig. 9. Secretory phase (ER, 400x mag.). Endometrium showing variable staining of glandular and stromal nuclei glands shows (1+ to 2+) nuclear staining. Stromal nuclei show 0 to 2+ staining
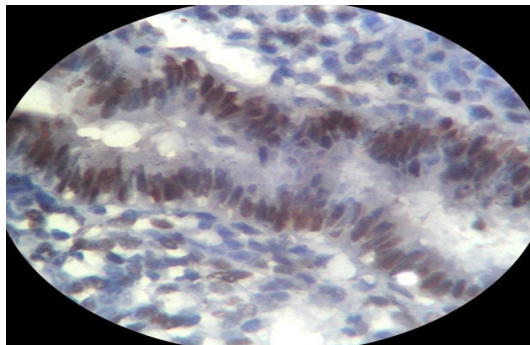

Fig. 10. Secretory phase (PR, 400x mag.). Endometrium showing variable staining of glandular and stromal nuclei glands shows 2+ to 3+ staining. Stromal nuclei show 0 to 1+ staining

4. DISCUSSION

For the past twenty years, many studies had been done on estrogen and progesterone receptors and also other hormonal receptors in the endometrium. Depending upon the presence of s steroid receptors in the target organs, steroid hormones shows response. These receptors are studied by different methods in different studies. Biochemical methods and tissue homogenization are complicated, time consuming, and less reliable techniques. 48 Immunohistochemistry allows the visualization of receptors in each cell and study their distribution [9].

Many studies which are available to date are based on the expression of steroid receptors in normal endometrium. There are very few studies available on the immunohistochemical analysis of hormonal receptors in the endometrium of patients with dysfunctional uterine bleeding. So, in this study we examined the hormonal status in endometrium of patients with dysfunctional uterine bleeding by immunohistochemistry using specific monoclonal antibodies against estrogen and progesterone receptors. The clinical parameters of DUB patients in this study and in other s tudies are shown in [10].

The mean age (41.08 years) of DUB patients in this study was not significantly different from that of (36 years and 36.6 years in studies by Chakraborty et al. 9 and Gleeson et al. 4 respectively, and it was almost similar to the normal group (42.3 in this study and 40.2 years 9 in another study). DUB patients in this study were also comparable with those in other studies in terms of duration of bleeding (mean of 8.04 days in this study and 7.5 days in study by Chakraborty et al.). 9 In study by C Levy et al, 45 S Chakraborty et al 9 and N Gleeson et al , 5 there was an increasing trend in ER and PR in proliferative phase and decreasing t rend in secretory phase in the DUB group. Mylonas I et al 7 and Noe et al 50 showed similar findings in normal endometrium both of which was consistent with this study [11].

These findings confirm the cyclic difference of the steroid receptors. And, it supports the concept that progesterone hormone decreases the synthesis of estrogen and progesterone receptors in secretory phase and estrogen hormone induce synthesis of both estrogen and progesterone receptors during proliferative phase. As in studies showed by, S Chakraborty et al 9 there was increased occurrence of endometrial hyperplasia and increased endometrial thickness in DUB patients compared to normal. Which was in concordance with our study [12]. Since the concentrations of both ER and PR were increased in endometrial glands and stroma of DUB patients, the above f in dings of this study support the notion that the action of ovarian hormones in DUB is increased through increased concentration of estrogen progesterone receptors in glands and stroma of endometrium and following unhampered estrogen effect leading to excess endometrial proliferation and hyperplasia. 39 This, may be responsible for increased endometrial thickness seen in DUB patients [12,13].

In study by N Gleeson et al 4 and Critchley et al 55 they found no significant difference in estrogen and progesterone receptors between normal and dysfunctional uterine bleeding endometrium. The results of this study were in contrast which showed the peak concentration of ER and PR in endometrial hyperplasia. This could be due to difference in the inclusion criteria of the patients for the study. Both Critchley et al and Gleeson et al excluded patients with histologic evidence of hyperplasia without atypia and those with difference between menstrual dating and endometrial maturation [14].

There are no studies available on correlation between the ER, PR and the endometrial thickness or parity in dysfunctional uterine bleeding. The most effective initial treatment strategy in medical management of DUB is hormonal therapy. But it is having many complications such as obesity, deep vein thrombosis, etc. Endometrial ablation and hysterectomy are reserved for intractable cases since they are invasive procedures and have many complications of surgery and anesthesia. Majority of patients with hyperplasia without atypia are amenable to medical management [15].

The development of progesterone antagonists such as Mifepristone, and selective progesterone receptor modulators, such as Mesoprogestin J 1042, 5 6 which act by inhibition of growth factors and angiogenesis By inhibiting synthesis of estrogen receptors, which are also required for synthesis of progesterone receptors. Have been widely used now a day for the management of dysfunctional uterine bleeding [16,17,18].

This is a prospective cross sectional study, for the estimation of estrogen and progesterone receptors using immunohistochemistry in endometrial tissue of 50 DUB patients and 50

normal endometrium which we received in Sree Balaji Medical College and Hospital. Apart from pelvic ultrasound and histological examination of endometrial aspirate samples, in the management of patients with dysfunctional uterine bleeding. Immunohistochemistry for estrogen and progesterone receptors in endometrial curettage samples is a very useful and important tool, which can be used as an ancillary technique. Immunohistochemistry has the benefit of tissue localization of these receptors in endometrium, which helps in assessing the distribution and intensity of these receptors in both endometrial glands and stroma.

5. CONCLUSION

It can also be concluded that there is increased concentration of estrogen and progesterone receptors in hyperplasia without atypia and decreased concentration of estrogen and progesterone receptors in atypical hyperplasia. Thus helps in identifying patients with increased concentration of estrogen and progesterone receptors, who would probably benefit from the drugs targeting these receptors (progesterone antagonists and selective progesterone receptor modulators), which would help them to avoid unnecessary invasive surgical procedures, and possibly prevent the progression to atypical endometrial hyperplasia, which is a precursor lesion of endometrial carcinoma by preventing sustained endometrial estrogenic stimulation.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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