Endometrial estrogen and progesterone receptors in infertility and abnormal uterine bleeding

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Abstract

Introduction: Evaluation of endometrial histology can be made more meaningful with the study of Estrogen and Progesterone receptor (ER and PR) status. Present study aimed at studying ER and PR status of normal endometrium and endometrium in infertility and abnormal uterine bleeding and elucidating its relevance and utility.

Materials and Methods: This case control study was carried out on prospectively registered patients. It included 12 cases of infertility, 24 cases of abnormal uterine bleeding, 14 cases of uterine leiomyoma and 22 cases of age matched controls undergoing endometrial sampling for causes other than above mentioned conditions. The cases were subjected to thorough clinical evaluation. The study of endometrium was done with routine histology. Endometrial ER and PR expression was studied immunohistochemically. The data collected was analyzed to find out the variability of expression of endometrial ER and PR in normal and abnormal endometrium with student's t-test and correlation t-test (SPSS software version 11).

Results: The ER and PR expression in normal endometrium showed cyclical variation. It was highest in the proliferative phase followed by the periovulatory phase followed by a significant decline in the late secretory phase. In cases of infertility and abnormal uterine bleeding with endometrial dysfunction; late secretory endometrium showed increased ER expression as compared to controls.

Conclusion: Variations noted in endometrial ER and PR expression in infertility and abnormal uterine bleeding provided insight in its pathogenesis and helped in contemplating treatment options.

Keywords: ER, PR, Endometrium, Infertility, Abnormal uterine bleeding, Uterine leiomyoma.

Introduction

Estrogen receptor (ER) and Progesterone receptor (PR) status of neoplastic breast tissue bears therapeutic implication and its immunohistochemical determination is now an established procedure in routine management of carcinoma breast. However this is not the case with endometrial ER and PR despite the fact that endometrium is a prime target organ for actions of estrogen and progesterone. Evaluation of endometrial histology is aptly called as bioassay of hormones at tissue level.[1] It can be made more meaningful with the study of ER and PR status of endometrium. Present study was an attempt to elucidate the relevance and utility of endometrial ER and PR in evaluation of infertility and abnormal uterine bleeding.

Materials and Methods

Present case-control study of prospectively registered patients was carried out over a period of two years in Sassoon General Hospitals, Pune; which is a general purpose tertiary care center attached to government medical college run by state government of Maharashtra. After obtaining informed consent, 12 patients of infertility, 24 cases with probable diagnosis of endometrial dysfunction and 14 cases with uterine leiomyoma, posted for Dilatation and Curettage (D&C) or endometrial biopsy or hysterectomy were enrolled in the study. Relatively young, fertile, women in the age group of 30-42 years who underwent surgical procedures for non-hormonal causes like uterovaginal prolapse or chronic cervicitis were enrolled as controls. The inclusion criteria for control group necessitated normal menstrual history. The peri menopausal patients were excluded from control group. The post menopausal status formed the most important exclusion criterion both for control and study group. Thorough pertinent clinical evaluation of control as well as study group was done with special reference to menstrual history. The specimens were obtained by hysterectomy (n=46), D & C (n=24) and endometrial biopsy (n=2). They were fixed in 10% formalin and processed routinely. Endometrial evaluation was done on slides stained by Hematoxylin and Eosin stain for phase of menstrual cycle and local abnormalities, if any.

The antigen retrieval was done with pressure cooker treatment. Sections for immunohistochemical studies were treated with monoclonal rabbit anti-human ER α antibody; clone SP1 (Dako) and monoclonal mouse anti-human PR antibody; clone PgR 636 (Dako). ER, PR expression was quantitatively scored based on percentage nuclear positivity (Quick score) of the whole tissue. To avoid interpretive variation, intensity of staining was not taken into consideration. The data was analyzed by applying student's t-test and correlation t-test using SPSS software version 11.

Results

The distribution of cases according to endometrial changes was as shown in Table 1.

Table 1: Endometrial change in control and study groups

	Control	Infertility	AUB	Leiomyoma
Early and Mid Proliferative	10	2	8	6
Late Proliferative and Early Secretory (Periovulatory)	7	3	10	3
Late Secretory	5	4	4	5
Menstrual	-	3	-	-
Simple Hyperplasia	-	-	1	-
Complex Hyperplasia without atypia	-	-	1	-
Total	22	12	24	14

Experience with evaluation of ER and PR expression: It was found convenient to express the results of ER and PR expression based on Quick score (as percentage positivity) and as a total impression than noting it in different components. Nonspecific cytoplasmic staining was observed in five cases (7%). There was seen variation in intensity of staining with ER and PR. Staining for PR was more intense than that for ER in 36 (50%) cases. There was no problem in grading the intensity of staining at both extremes of very strong and very weak staining but we found it difficult to grade the subtle differences in intensity. Hence we restricted the receptor evaluation to percentage positivity (Quick score). ER and PR expression was observed in endometrial surface epithelium, glandular epithelium and stroma (Fig. 1). ER expression in glandular epithelium predominated over stroma in all phases. PR expression of stromal cells predominated over glandular epithelium in late secretory phase. Endothelial cells and vascular smooth muscle cells of blood vessels were negative for ER and PR.

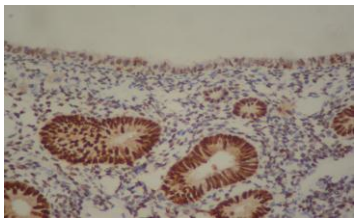

Fig. 1: 40X, ER expression of surface & glandular epithelium & stroma in late proliferative phase in control

ER and PR expression of normal endometrium: The ER and PR expression of normal endometrium showed cyclical variation. Highest mean ER expression (of glands, stroma and total) was noted in early and mid proliferative phase followed by periovulatory phase (late proliferative and early secretory phase) followed by late secretory phase. Mean PR expression (of glands, stroma and total) was highest in the periovulatory phase. Next highest values were noted in early and mid proliferative phase. It started declining after twenty first endometrial date (in late secretory phase). The findings are summarized in Fig. 2 and 3. These line scatter diagrams emphasize the chronology of ER and PR expression. The fall in ER expression (of glands, stroma and total) in the late secretory phase was statistically significant when compared with periovulatory phase (p: < 0.0001, < 0.01, < 0.0001) and early and mid proliferative phase (p: < 0.0001). For PR, it was significant for glandular and total expression (p : <0.0001) but not for stromal expression (p: > 0.05) The ER: PR ratio was highest in proliferative phase followed by periovulatory phase and fell significantly in the late secretory phase (p: < 0.001 for glands and stroma and > 0.0001 for total expression).

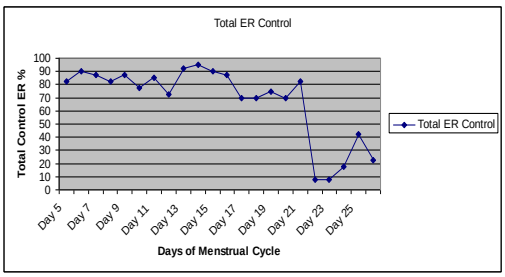

Fig. 2: Line scatter diagram showing total ER expression in normal endometrium

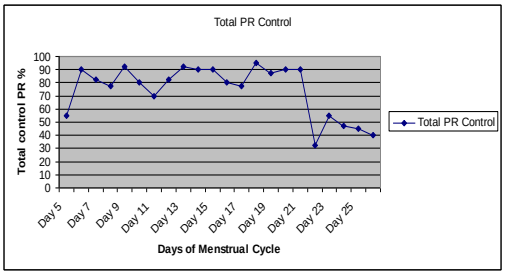

Fig. 3: Line scatter diagram showing total PR expression in normal endometrium

Infertility: On applying finer classification of endometrial changes in infertility, 2 cases each belonged to deficient and irregular proliferation, 5 cases had corpus luteal deficiency with apparent co ordinate delay and 3 cases showed adequate secretory response.

ER and PR expression in infertility: ER and PR expression (of glands, stroma and total) of infertile endometrium in proliferative and periovulatory phase was comparable to that of control. ER expression was significantly increased in secretory phase with p: < 0.0001 for glands, < 0.01 for stroma and < 0.001 for total when compared with control. PR expression of glands, stroma and both combined remained comparable to control even in late secretory phase. The comparison of Total ER and PR expression in infertility and control is depicted in Fig. 4.

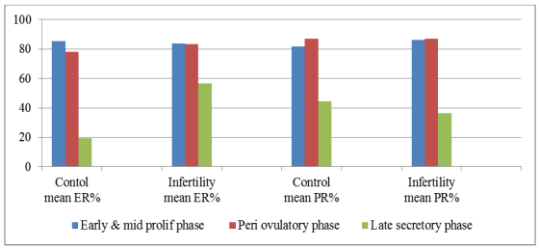

Fig. 4: Bar diagram showing total ER & PR expression in infertility vs. control

The inter phase comparison of ER and PR expression (of glands, stroma and total) of early and mid proliferative phase with periovulatory phase showed statistically comparable results. The ER expression of glands, stroma and its total was significantly higher in periovulatory phase than in late secretory phase with p: < 0.0001. Similarly PR expression in glands, stroma and for both combined was higher in periovulatory phase than in late secretory phase with p: < 0.05. When we compared ER expression of early and mid proliferative phase with late secretory phase it was significantly lower in late secretory phase for glands and total (p: < 0.01, <0.05) but not for stroma. The total PR expression and that for glands was significantly lower in late secretory phase than in early and mid proliferative phase. The values for stromal PR expression were statistically comparable in these phases.

Increased ER and normal PR expression in cases with late secretory phase when compared with control; ruled out possibility of pseudocorpus luteal deficiency. ER: PR ratio was significantly raised in late secretory phase when compared with control (p: < 0.001 for glands and stroma and < 0.0001 for total expression).

Abnormal uterine bleeding (AUB): There were 24 cases of AUB. Two cases of endometrial hyperplasia one each with simple hyperplasia and complex hyperplasia without atypia were excluded from the statistics. The case of simple hyperplasia showed total ER expression of 87.5% and total PR expression of 80% roughly comparable to control proliferative and periovulatory endometrium (Control Mean+/- SD for total ER: 81.25+/- 6.82 & PR: 81.5+/- 11.74 for early and mid proliferative phase and ER: 77.86+/- 8.71 & PR: 87.14=+/- 6.19 for peri ovulatory phase) [Fig. 5]. In a case of complex hyperplasia without atypia, both the expressions were remarkably low with ER being 55% and PR with values of 70% [Fig. 6].

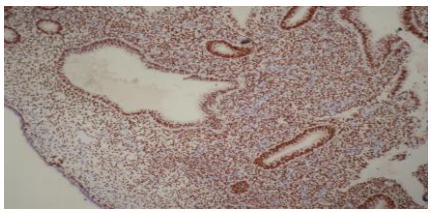

Fig. 5 A: 40X, ER expression in simple endometrial hyperplasia without atypia

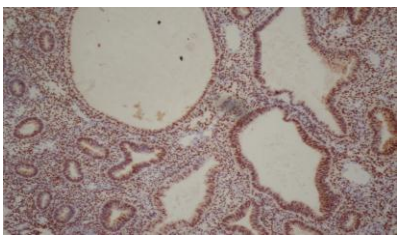

Fig. 5 B: 40X, PR expression in simple endometrial hyperplasia without atypia

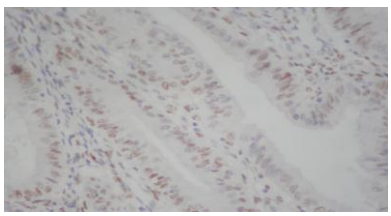

Fig. 6 A: 40X, ER expression in complex endometrial hyperplasia without atypia

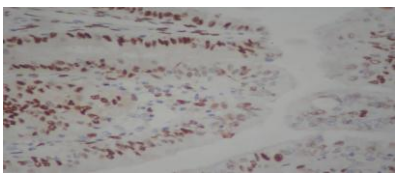

Fig. 6 B: 40X, PR expression in complex endometrial hyperplasia without atypia

ER and PR expression in AUB: The receptor expression of cases with AUB with endometrial dysfunction was comparable to control throughout the menstrual cycle, except for glandular ER expression of late secretory phase which was significantly increased when compared with control (p: < 0.0001). Periovulatory glandular expression of PR was lesser than control (p: < 0.05). The findings of comparison of AUB with control with respect to total ER and PR expression are depicted in Fig. 7. The ER: PR ratio in

glands, stroma and in both together was significantly raised in late secretory phase when compared with control (p: < 0.001, 0.001, 0.0001).

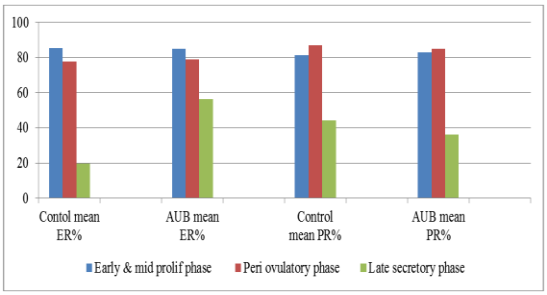

Fig. 7: Bar diagram showing total ER & PR expression in AUB vs. control

Endometrial ER and PR expression in leiomyoma uterus: It is shown in Fig. 8**.** It was statistically comparable to control in all phases. But it can be seen that though not statistically significant PR expression remained fairly high in the late secretory phase when compared with normal endometrium in same phase. By far it had highest values for late secretory phase in normal and abnormal endometrium.

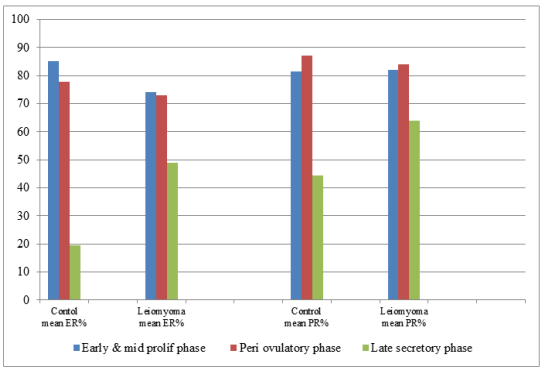

Fig. 8: Bar diagram showing total ER & PR expression in Leiomyoma vs. control

Correlation of ER and PR; ER: PR ratio and age with each other:

ER showed a significant positive correlation with PR only in the proliferative phase of normal endometrium (r : 0.43, p < 0.05). ER showed a significant positive correlation with PR in all phases of abnormal endometrium **(Infertility:** : r:1, p< 0.001; r: 0.59, p< 0.01; r: 0.77, p< 0.001 **AUB** : r: 0.44, p< 0.05; r: 0.52, p< 0.01; r: 0.99, p< 0.001 and **Leiomyoma :** r: 0.75, p< 0.01; r: 0.83, p< 0.001; r: 0.76, p< 0.001 for early and mid proliferative, periovulatory and late secretory phase respectively).

In normal endometrium, the ER expression of proliferative and periovulatory endometrium showed a positive correlation with age (r: 0.39, p< 0.05, r: 0.30, p< 0.05). It showed a negative correlation with age in late secretory phase (r: - 0.46, p< 0.05). The PR expression showed positive correlation with age in the periovulatory phase (r: 0.75, p< 0.01) but it was negative in late secretory phase (r: -0.29, p< 0.05); ER: PR ratio showed a negative correlation with age in the late secretory endometrium (r: -0.36, p< 0.05). None of these trends were followed in the abnormal endometrium.

Discussion

Hormonal dysfunction underlies many cases of female infertility and abnormal uterine bleeding and the endometrium responds to it very precisely and predictably.[2] Histological examination of endometrium is the most time tested method of judging hormonal imbalance. Supplementation of this with immunohistochemical study of ER and PR holds a great promise. Present study was an attempt to explore this possibility.

We were successful in establishing trends of ER and PR expression in normal endometrium which showed cyclical variation. The experience is shared by others.[3-5] It has been suggested that both ER and PR are induced by estrogen while the progesterone decreases the expression of both these receptors.[5] Some authors consider this to be an oversimplification and suggest possibilities of multiple interactions in hormones and PR in secretory phase.[6]

We have noted abnormally raised ER and normal PR expression with raised ER: PR ratio in late secretory infertile endometrium. The experience is shared by Margarit et al.[7] Considering the relationship between hormones and receptor expression and the fact that most of our cases had corpus luteal deficiency with co-ordinate apparent delay, such an occurrence can be explained on the basis of hyperestrogenism resulting from persistent Graafian follicle. This in turn can result in delayed ovulation and shortened luteal phase. The normal PR expression signifies relative rather than absolute deficiency of progesterone for abnormally proliferated endometrium of hyperestrogenism.[8] Similar alterations were also noted in patients of AUB and leiomyoma uterus. Experience is shared by others.[9, 10] Gleeson et al in their explicitly designed study have gone further and showed correlation between ER expression and amount of blood loss in cases of AUB.[9] According to studies by Xu et al in cases with luteinized unruptured follicle, there is seen failure of down-regulation due to suboptimal serum progesterone in such cases.[11] It has been seen in literature that the cases of recurrent miscarriage show low levels of ER and PR in endometrium. This again emphasizes importance of study of endometrial receptors in such cases.[12]

Spirtos et al have suggested that the use of low-dose follicular phase estrogen therapy or the anti-estrogen, tamoxifen citrate may help in increasing the content of PR in infertile women with pseudocorpus luteal deficiency.[13] Fukushima et al have noted that

tamoxifen therapy in follicular phase results in lengthening the luteal phase and improves its glycogen content in cases of infertility.[14] Considering the fact that cases of luteal phase deficiency with coordinate delay in present study showed raised ER expression, the possibility of response to tamoxifen which will act as estrogen antagonist but will not antagonize PR, can be contemplated. Tamoxifen, thus, seems to be a wonderful drug which provides hope in cases of ovulatory infertility and AUB. But the fact remains that tamoxifen acts as a partial estrogen antagonist on the endometrium and search for drugs with purely antagonist activity continues.[9] The study of endometrial ER and PR status thus holds promise of therapeutic implications. Some authors have noted significant reduction in endometrial estrogen and progesterone receptors after radiofrequency endometrial ablation therapy in patients of abnormal uterine bleeding with endometrial dysfunction and have attributed this to long term cure and prevention of AUB recurrence.[15] Chakravarthy et al have emphasized importance of evidence based stepwise approach to managing cases of AUB while discussing their findings of markedly raised endometrial ER and PR in cases of simple and complex hyperplasia, mildly raised receptors in disordered proliferation and near normal receptor status in normal proliferation and irregular shedding.[16]

Significant positive correlation was found between ER and PR in all phases of menstrual cycle in infertility and abnormal uterine bleeding. This can possibly be attributed to the high estrogen levels of the proliferative phase persisting in the late secretory phase in these cases.

We have attempted correlation between endometrial ER and PR, ER: PR ratio and age which provided observations very complex to interpret. There was a significant positive correlation between total ER and PR only in the early and mid proliferative phase of the normal menstrual cycle.

The major constraint of the present study was unavailability of blood hormone levels and moderate sample size owing to financial constraints however the major strength of our study lies in its comprehensive nature in terms of inclusion of different types of endometrial pathology, very precise classification of endometrial change and an attempt to correlate receptor status with each other and with age.

The distinct advantage of our study of endometrial ER and PR was in ruling out possibility of pseudocorpus luteal deficiency which needs to be treated differently.

It will not be too pragmatic to state that, present study of ER and PR expression of endometrium provided insight into pathogenesis of infertility and abnormal uterine bleeding. Besides this it has also helped in contemplating treatment options in such cases. Though endometrial carcinoma was not a part of this study, knowing ER and PR expression of normal and abnormal nonneoplastic endometrium will help in contemplating a new field in hormonal treatment of endometriatl carcinoma.[17]

Conclusion

The present study of endometrial ER and PR expression in normal and abnormal endometrium helped in establishing its trend in normal endometrium and providing insight in the pathogenesis of infertility and abnormal uterine bleeding.

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