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Estrogen receptor expression in complex and/or atypical endometrial hyperplasia and endometrial adenocarcinoma

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Abstract
Endometrial cancer affects approximately 142,000 women worldwide each year, and an estimated 42,000 women die from this cancer. Endometrial carcinoma is categorised into type I endometrial carcinoma and type II endometrial carcinoma. Endometrial hyperplasia and unopposed estrogenic stimulation are a significant risk factor in endometrial carcinogenesis. Type II carcinomas especially serous carcinomas are known to develop in the atrophic endometrium in an older age group and are not associated with hyperestrogenism. Early stage and well differentiated tumors are primarily estrogen and progesterone positive. Expression of estrogen receptors correlates with low tumor grade, low recurrence rate and higher survival. Whereas absence of estrogen receptor expression is associated with a worse prognosis

Keywords: Estrogen Receptors, Atypical Hyperplasia, Type I endometrial adenocarcinoma, Type II endometrial adenocarcinoma

Introduction
Endometrial cancer affects approximately 142,000 women worldwide each year, and an estimated 42,000 women die from this cancer [1]. Endometrial cancer holds fourth position after breast, colon and lung cancers [2, 3]. In different parts of the world endometrial carcinoma accounts for 4-8% of all cancers. The incidence of endometrial cancer is very low in India as compared to the western countries. The Age Standardised Rate (ASR) is approximately 4.3 cases per 1, 00,000 women (Delhi) [4]. Creaseman *et al*. (2007) [5] reported in the year 2007 that 75% patients are postmenopausal and only 3-10% are less than 40 years of age. Themedian age group for diagnosis of endometrial cancers is around 60 years.[6]Most patients present at early stage because of abnormal bleeding hence the overall morbidity and mortality of endometrial carcinoma is low [7]. Endometrial hyperplasia is a significant risk factor in endometrial carcinogenesis as atypical endometrial hyperplasia represents the early precursor of most of the endometrial carcinomas.[7]The most common subgroup of the endometrial carcinomas are adenocarcinoma of the endometrial type (Type I carcinoma) [8, 9]. Feig *et al*. (2004) [8, 2] reported in a study that ninety percent of endometrial cancers are endometroid adenocarcinomas with approximately 70% being grade 1,15% grade 2 and remaining 15% being grade 3 endometrioid endometrial cancers.
Significant risk factors for Type I endometrial carcinoma are endometrial hyperplasia and unopposed estrogenic stimulation. Uterine serous carcinoma, clear cell carcinoma and anaplastic carcinoma are segregated into type II group [10]. Type II carcinomas especially serous carcinomas are known to develop in the atrophic endometrium in an older age group and are not associated with hyperestrogenism [10, 11]. Unopposed estrogen influence is the primary cause for endometrial carcinogenesis in most cases [12]. Early stage and well differentiated tumors are primarily estrogen and progesterone positive [13].
Estrogen receptors are activated by the hormone estrogen (17β-estradiol). They are a group of proteins found inside cells. There are two classes of estrogen receptors
1. Estrogen receptors belonging to the nuclear hormone family of the intracellular receptors.
2. GPER belonging to the rhodopsin-like family of G protein-coupled estrogen receptors [14].
The ERα is found in endometrium, ovarian stromal cells, hypothalamus, breast cancer cells and epithelium of the efferent ducts in males [15, 16]. ERβ protein is found in ovarian granulosa

cells, kidney, brain, lungs, bone, heart, prostate, intestinal mucosa and endothelial cells. Hu *et al.* (2005) [17] studied the expression of estrogen receptors ERα and ERβ in 114 endometrial hyperplasia and adenocarcinoma cases. The ERα expression increased from normal proliferation to simple and complex hyperplasia, while ERβ expression did not change much. In atypical hyperplasia and adenocarcinoma ERα and ERβ expression was less as compared to normal endometrial proliferation. Most endometrial adenocarcinomas expressed ERα alone or in combination with ERβ and the ERβ/ERα ratio decreased as compared to normal endometrial proliferation. Bircan *et al*. (2005) [18] concluded in their study that gradual loss of estrogen receptors is correlated with increasing malignant transformation.

Meng *et al*. (2001) [21] conducted a study on 37 specimens of endometrial lesions. There were12 cases of endometrial hyperplasia. All the 12 (100%) cases were strongly positive for estrogen and progesterone receptors.

Many reports suggest that expression of ER and PR correlates with low tumor grade, low recurrence rate and higher survival [20, 21]. Whereas absence of ER and PR expression is associated with a worse prognosis [22]. In patients where the disease is clinically confined to the uterus, the 5-year survival is 86% with ER and PR-positive tumors and only 52% in ER-positive and PR-negative tumors [20]. Poorly differentiated endometrial carcinoma and type II endometrial tumors such as serous and clear cell carcinoma are negative for ER and PR [22-24].

Aim and Objectives

1. To correlate histology of endometrial carcinoma with tissue estrogen receptor status.
2. To correlate complex and/or atypical endometrial hyperplasia with tissue estrogen receptor status.

Material and Methods

Study setting

This study titled as "Estrogen receptor expression in atypical/complex endometrial hyperplasia and endometrial adenocarcinoma" was carried out in a teaching hospital in Maharashtra. Tissues were obtained from all the specimens like biopsy, hysterectomy with or debulking surgery.

- Trained pathologist evaluated these samples and determined the pathological information required for further management of patients.
- This was a retrospective study in which available samples without any extra cost to the patient were further evaluated for expression of estrogen receptor. Sample size
- This was a pilot exploratory study.
- 50 cases diagnosed to have atypical endometrial hyperplasia or endometrial carcinoma were selected in retrospective manner from records of Department of Pathology

Study Subjects

Women admitted in gynaecology in-patient wards satisfying following Inclusion criteria were included in the study:

• Histologically confirmed diagnosis of atypical endometrial hyperplasia or endometrial adenocarcinoma.

Patients were excluded from the study if:

They have uterine malignancies other than epithelial as diagnosed by histopathology.

Cases diagnosed to have atypical endometrial hyperplasia or endometrial. Adenocarcinoma elsewhere and with no samples (paraffin sections/blocks/ formalin fixed tissue) available from prior surgeons.

Study procedure

The study protocol was approved by Institutional ethical committee prior to commencement of study. Hematoxylin and eosin stained slides were retrieved and the cases were classified into different grades as follows: Endometrioid endometrial adenocarcinomas (Type I) are composed of predominantly glands which formed the architectural Grade 1, admixture of glands and masses of solid epithelium forming the architectural Grade 2 or majorly solid proliferations which belonged to architectural Grade 3. The nuclear grade was classified as grade 1, having little variation in size and shape with evenly distributed chromatin and inconspicuous nucleoli - mild nuclear atypia. Grade 2 nucleus had moderate variation in size and shape with clumping of chromatin and single nucleolus - moderate nuclear atypia. Grade 3 nucleus had pleomorphism, hyperchromatic nucleus with presence of irregular coarse chromatin and eosinophilic nucleoli - severe nuclear atypia. The final grading was done by addition of 1 to tumors with architectural Grade 1 or 2 if they showed severe, Grade 3 nuclear pleomorphism. Type II endometrial carcinomas were composed of majorly papillary proliferations alongwith solidand glandular patterns. The tumor cells were observed to be loosely cohesive and cuboidal with presence of severe nuclear atypia. The glands in endometrioid carcinomas were lined by epithelium with either Grade 1 or 2 nuclei. In cases of serous carcinomas there was Grade 3 nuclei [10].

- Further the cases were screened to obtain the best section for immunohistochemistry.
- As far as possible, sections having both normal as well as tumor were selected so as to have an internal control.
- Formalin-fixed paraffin embedded tissue blocks were retrieved. Sections of 3-5 microns in thickness were obtained.
- Immunohistochemical stain for estrogen receptor was performed on these sections. Attempts were made to obtain informal consent from patients. Although, this was not feasible in most cases as patients were not traceable or available for consent. Patient confidentiality however, was maintained for all research procedures.

Evaluation of Immunohistochemical staining

The evaluation of ER and PR was performed according to the method described by Carcangiu *et al*. (1990). [24] On the basis of percentage of stained cells and the intensity of nuclear stain. The percentage of ER positive cells was graded as: 1, if 0 to 25% of the nuclei stained; 2, when 26 to 75% of nuclei stained; 3,if more than 76% of the nuclei stained. Whereas the staining intensity was scored as 1: absent or weak, 2: strong and 3: very strong. The sum of percentage and intensity gave an immune histochemical score. Tumours were categorised as follows - Category I as score of 2, Category II was assigned score of ¾ and

Category III carried a score of 5/6. Category I tumours were immune negative and Category II and III tumours were immune positive.

Observation and Results

This was a retrospective study entitled as "Estrogen Receptors expression in Complex and/or Atypical Endometrial Hyperplasia and Endometrial Adenocarcinoma" in which we selected 50 cases diagnosed to have either atypical hyperplasia or endometrial adenocarcinoma on histopathology for expression of ER Age of the patients in this study ranged from 38 years to 80 years. Majority of the cases (21 cases, 42%) were found in fifth decade. Even the fourth decade showed many cases (13 cases, 26%). In the sixth decade ten (20%) cases were reported. There was no case reported in less than 30 years of age. Whereas there were only two (4%) cases found in the third decade.

Table 2.1

Age (Years)	No. of patients	Percentage (%)
<30	0	0
30-39	02	4
40-49	13	26
50-59	21	42
60-69	10	20
≥70	04	8
Total	50	100

There were 10 cases of atypical endometrial hyperplasia and 40 cases of endometrial adenocarcinoma as shown in table No. 2.2

Table 2.2

Cases	Number	Percentage (%)
Atypical Hyperplasia	10	20
Endometrial Carcinoma	40	80
Total	50	100

In our study we found that majority of the endometrial carcinomas were of type 1(36 cases, 90%) and only four (10%) cases of type 2 were observed.

Table 2.3

Type of endometrial cancer	Number of patients	Percentage (%)
Type I	36	90
Type II	04	10
Total	40	100

In our study we found that endometrioid adenocarcinoma cases were the most common ones (36 cases, 90%). Only four (10%) cases of non endometroid adenocarcinoma were reported. In the non endometrioid adenocarcinoma two (5%) cases of serous carcinoma were observed. A single case of clear cell variant and mucinous variant were observed.

Table 2.4

Histological Subtype		No. of cases	Percentage (%)
Endometrioid		36	90
Non Endometrioid	Serous	2	5
	Clear cell	1	2.5
	Mucinous	1	2.5
	Mixed	0	0
Total		40	100

In our study we found that there were 14 cases (33.89%) of well differentiated endometrioid adenocarcinoma. There were 3 (8.33%) cases of moderately differentiated endometrioid adenocarcinoma. There were 19 (52. 78) cases of poorly differentiated endometrioid adenocarcinoma.

Table 2.5

Grade	No. of cases	Percentage (%)
Well differentiated (1)	14	38.9
Moderately differentiated (2)	3	8.3
Poorly differentiated (3)	19	52.8
Total	36	100

In our study we found that 28 (56%) cases were estrogen receptor positive. In the type 1 endometrial carcinoma 18 (50%) cases were ER positive. All the four (100%) cases of type II endometrial cancer were estrogen receptor negative. All the 10 (100%) cases of atypical endometrial hyperplasia strongly expressed for estrogen receptor. The difference was found to be statistically significant as shown in table no. 2.6.

Table 2.6

Type of endometrial cancer	Number of cases	ER Positive No.	(%)	ER Negative No.	(%)
Type I	36	18	50	18	50
Type II	04	0	0	04	100
Hyperplasia	10	10	100	0	0
Total	50	28	56	22	44

P= 0.0011

Based on the intensity of ER staining and percentage of cells involved we observed that in atypical hyperplasia there was strong positivity for estrogen receptor. Out of 10 cases, 8 (80%) cases were in category three and 2 (20%) cases showed moderate positivity for ER receptors. None of the cases of atypical hyperplasia were immune negative for ER. In well differentiated endometrioid adenocarcinoma 12 out 14 cases were immune positive for ER and 9 (64.3%) cases strongly expressed for estrogen receptors. All the 3(100%) moderately differentiated tumours were immune positive for ER. Whereas the intensity and percentage of nuclear stain reduced from moderately differentiated tumours, poorly differentiated tumours to type II non endometrioid tumors. In poorly differentiated carcinoma cases 16 out of 19(84.2%) tumors were ER negative. All (4, 100%) type II endometrial tumors were ER negative. The difference was found to be statistically significant. Table 2.7 (Fig 1, 2)

Table 2.7

ER Category	Atypical Hyperplasia No. (%)	Well differentiated endometrial carcinoma No. (%)	Moderately differentiated endometrial adenocarcinoma No. (%)	Poorly differentiated endometrial carcinoma No (%)	Type II endometrial cancers No. (%)
Category 1/Negative	0 0	2 14.3	0 0	16 84.2	4 100
Category 2	2 20	3 21.4	2 66.7	2 10.5	0 0
Category 3	8 80	9 64.3	1 33.3	1 5.3	0 0
Total	10 100	14 100	3 100	19 100	4 100

P=0.000008

It was observed that out of 17 low grade cases 10 (58.8%) cases strongly expressed for estrogen receptor. Moderate expression of estrogen receptor was seen in 5 (29.4%) cases hence 15 out 17 low grade tumors were ER positive and only 2 (11.8%) cases were immuno negative for ER. Whereas in high grade tumors 20 out of 23(86.9%) tumors were ER negative. The difference was found to be statistically significant as shown in Table 2.8

Table 2.8

Histological Grade	ER Category 3	%	ER Category 2	%	ER Category 1	%	No. of cases	%
Low	10	58.8	5	29.4	2	11.8	17	100
High	1	4.4	2	8.7	20	86.9	23	100
Total	11		7		22		40	100

P= 0.000011

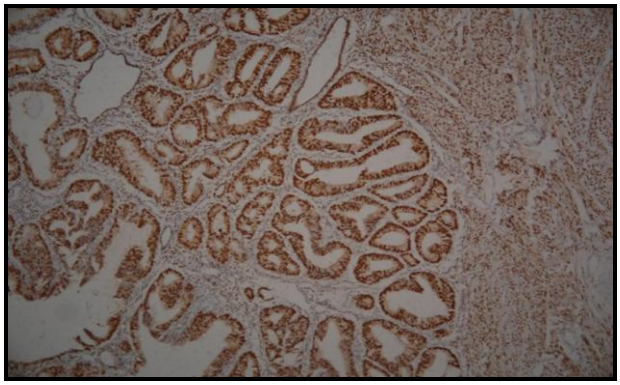

Fig 1: Category 3 Positivity for estrogen receptor in atypical endometrial hyperplasia (IHC X 100)

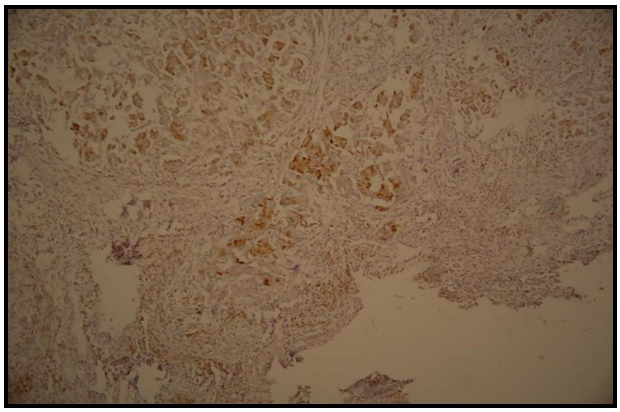

Fig 2: Category 2 positivity for estrogen receptor in moderately differentiated adenocarcinoma. (IHC X 100)

Discussion

In our study we found that lesions of endometrium including atypical endometrial hyperplasia and endometrial cancer were more common in fourth, fifth and sixth decade (Table No.1). In a study done by Kounelis *et al.* (2000) [10] it was observed that out of 61 cases of endometrial cancers the average age at presentation for type I endometrial cancer was 64.5 years and for type II endometrial cancer (uterine papillary serous adenocarcinoma) was 69.6 years respectively. Reed *et al.* (2009) [36]. Reported in their study that incidence of endometrial hyperplasia with and without atypia showed a bimodal peak present in early postmenopausal years and also in the early 6th decade. Our study was consistent with the findings of studies in literature that atypical endometrial hyperplasia and adenocarcinoma mostly occurs in fifth and sixth decade.

In our study we observed that there were 36 (90%) cases of type I endometrial carcinoma whereas there were only 4 (10%) cases of type II endometrial carcinoma. (Table No.2. and Table No.3). On the basis of a prospective study conducted on 366 patients with endometrial carcinoma, Bokhman *et al.* (1983) [8] reported that out of 366 cases of endometrial cancers frequency of type 1 endometrial carcinomas was 65%, whereas the frequency of the type 2 was only 35%. Moore *et al.* (2011) [9] postulated in their study that the frequency of type II endometrial cancer was only 10% but these tumors are responsible for 40% of deaths. The type II endometrial tumors are aggressive tumors and spread early beyond the uterus. These results were consistent to our observation that type I endometrial cancer were more common than type II endometrial cancer.

In our study we found that there were 17 cases (47.2%) of well differentiated adenocarcinoma and moderately differentiated adenocarcinoma. There were 19(52.78%) cases of poorly differentiated adenocarcinoma cases (Table No. 4).

Bokhman *et al.* (1983) [8] reported in case study conducted on 366 patients suffering with endometrial cancer that the frequency of the first pathogenetic type in the study group was 65%, whereas the frequency of type II endometrial cancer was 35%. It was observed that there were 82.3% endometrioid endometrial cases which included the well differentiated or moderately differentiated endometrial cancers whereas there were only 17.7% cases of poorly differentiated adenocarcinoma. Feig *et al.* (2004) [82] reported that ninety percent of endometrial cancers were endometroid adenocarcinomas. They also reported that the frequency of Grade 1 endometrial cancers was 70% grade 1 and the frequency of grade 2 and grade 3 tumors was15% each. There were 5% to 7% cases of papillary serous carcinomas and the remaining 3% to 5% were clear cell carcinomas.

The results of previous studies varied to the results observed in our study. As poorly differentiated adenocarcinoma cases were more than well or moderately differentiated cancers.

We observed that all the endometrial atypical hyperplasia

cases (10,100%) were strongly positive for ER. It was noticed that 18 out of 36 (50%) type I endometrial cancer cases were positive for ER (P= 0.0011). Whereas all 4 cases of type II endometrial carcinoma were negative for estrogen receptor. The difference was found to be statistically significant. (Table 5, 6, 7, 8).

Kounelis *et al*. (2000) [10] conducted a study on 61 patients of endometrial cancer. Type I cases were 40 and type II cases were 21. They observed that in Type I endometrial cancer 70% cases were ER positive whereas only 23.8% of type II endometrial cancers were ER positive.

Meng *et al*. (2001) [21] conducted a study on 37 specimens of endometrial lesions. There were12 cases of endometrial hyperplasia. All the 12 cases were strongly positive for estrogen and progesterone receptors.

Conclusion

The findings of present study are summarized as follows:

1. Lesions of endometrium including atypical endometrial hyperplasia and endometrial cancer are more common in fourth, fifth and sixth decade.
2. The proportion of type I endometrial cancer cases was 90% (36 cases) as compared to 10% (4 cases) of type II endometrial carcinoma.
3. The mean age of presentation of endometrioid endometrial cancer cases was 55.5 yrs. Whereas the mean age of presentation of type II non endometrioid endometrial cancer cases was 65.5 years
4. Endometrial atypical hyperplasia cases (10,100%) were strongly positive for estrogen receptors
5. In type I endometrial cancer 18 cases (50%) were positive for estrogen receptors. Whereas all 4 cases (100%) of type II endometrial carcinoma were negative for estrogen receptors.
6. As the endometrial lesions progressed from atypical hyperplasia towards poorly differentiated endometrial carcinoma the expression for ER reduced markedly.
7. Hence we can conclude that expression of ER was seen strongly in all cases of endometrial atypical hyperplasia. ER expression was also noticed in endometrial cancer cases, but the intensity of expression reduced as the tumor progressed from well differentiated tumors to poorly differentiated tumors.

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