Original Research Article

Immunohistochemical study of ER, PR, p53 and Ki67 expression in patients with endometrial adenocarcinoma and atypical endometrial hyperplasia

Ravi M Swami¹, Rachana Lakhe¹*, Preeti Doshi¹, Manjiri N Karandikar¹, Ravindra Nimargi¹, N S Mani¹

1Dept. of Pathology, Bharati Vidyapeeth Medical College, Pune, Maharashtra, India

ARTICLE INFO

Article history:
Received 26-11-2020
Accepted 02-12-2020
Available online 30-12-2020

Keywords:
Endometrial adenocarcinoma
Hyperplasia
Immunohistochemistry

ABSTRACT

Objective: To study Immunohistochemistry pattern of ER, PR, p53 and Ki67 expression in patients with endometrial adenocarcinoma (EC) and endometrial hyperplasia.

Materials and Methods: A retrospective study of 78 cases were studied for a period of 02 year from June 2018 to May 2020, where in the clinical data included age, presenting complaints, menopausal status, history of hormonal treatment, ultrasound examination. Histopathological parameters were analysed as per WHO 2014 classification of endometrial hyperplasia into typical and atypical endometrial hyperplasia / Endometrial Intraepithelial Neoplasia (EIN) and endometrial carcinoma. ER, PR, p53 and Ki 67 IHC markers were done on cases diagnosed as endometrial adenocarcinoma and endometrial hyperplasia.

Results: Among 78 cases, there were 20 cases of EC and 58 cases of endometrial hyperplasia. EC was most commonly seen in sixth to seventh decade and hyperplasia was seen in fourth to fifth decade. There were 11 surgical specimens and 09 biopsies of EC. Out of total 20 cases, there were 17 cases of endometrioid adenocarcinoma and 03 cases were of papillary serous adenocarcinoma.

ER, PR expression was seen in 10 cases of grade 1 endometrial carcinomas and 5 cases of grade 2 EC. p53 expression was seen in 01 case of grade 1 EC and 03 cases grade 3 EC. ER, PR, p53 all were negative in one case. Ki67 was <10% in 11 cases, between 10-20% in 5 cases and >20% in 04 cases. We followed up 15 out of 20 cases, out of which only 01 patient died of disease who was grade 1 endometrial carcinoma. Rest 14 had progression free survival (PFS) till date.

All 58 cases of endometrial hyperplasia were reclassified complex and simple hyperplasia to typical and atypical hyperplasia (as per WHO Classification 2014). Out of 58 cases, 56 cases were typical hyperplasia and 02 cases atypical hyperplasia. ER, PR expression was positive in all the cases of typical and atypical hyperplasia. p53 expression was absent in typical and atypical hyperplasia with low Ki 67 index (<10%).

Conclusion: High ER, PR expression along with low p53 was seen in type 1 endometrial carcinomas compared to low or absent ER, PR along with strong p53 expression in papillary serous carcinoma (Type 2). The expression of ER, PR was high in typical hyperplasia as against high p53 expression observed in atypical hyperplasia.

© This is an open access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. Introduction

Endometrial carcinoma (EC) is the second most common gynecologic malignancy with an incidence of 5.9 per 100,000 women in the developing countries. In India, the incidence is 4.3 per 100,000 women.¹ Endometrial hyperplasia (EH) implies an overgrowth of the endometrium. It constitutes a spectrum of irregular morphological alterations, due to which abnormal proliferation of the endometrial glands occurs resulting in an increase in gland to stroma ratio when compared to endometrium from the proliferative phase of the cycle.²

*Corresponding author.
E-mail address: rachanalakhe@gmail.com (R. Lakhe).

https://doi.org/10.18231/j.achr.2020.060
2581-5725/© 2020 Innovative Publication, All rights reserved. 274
WHO 2014 classification differentiates endometrial hyperplasia into typical hyperplasia and atypical hyperplasia/endometrial intraepithelial neoplasia (EIN). EC is one of the most frequent causes of abnormal uterine bleeding, which leads to EC if left untreated. In 10% of premenopausal women with abnormal uterine bleeding, histological findings show endometrial hyperplasia and in 06% of postmenopausal women with uterine bleeding EC are found.4

Despite of these facts the general population is not aware of EC which is reflected by increased use of endogenous and exogenous estrogen in the treatment of post menopausal symptoms there by resulting in EC.5-7 The two types of EC, Type-1 and Type-2 which are different in their etiology, clinical behavior and treatment modalities were originally described by Bokhman Jv et al. in 1983.8 High grade serous carcinoma are found to be less or non-responsive to usual chemotheraphy.9 One study shows that serous carcinoma forms 10% of all EC.10

Estrogenic action unopposed by progesterone induces sequential malignant changes in the endometrium by atypical hyperplasia. Decreased expressions of estrogen receptor (ER) and progesterone receptor (PR) are observed in EC which increases in both grade and stage when compared to atypical hyperplasia. Therefore absence of ER and PR expression is important in the progression of type 1 EC,11,12 In type II EC, p53 mutation is common phenomenon.

Traditional histopathological classification system of endometrial hyperplasia exhibited variable degree of diagnostic reproducibility. WHO classifications 2014 have simplified the categorization and improve the reproducibility which clearly distinguishes between clinicopathologic entities that are managed differently. The aim of this study was to assess the role of immunohistochemical expression of ER, PR, Ki67 and p53 in EC and atypical hyperplasia.

2. Materials and Methods

This retrospective study included total 78 cases of which 58 cases were endometrial hyperplasia (atypical hyperplasia and typical hyperplasia) and 20 cases of EC between June to May 2020.

Ethical clearance was obtained from the Institutional Ethical Committee (Letter number: BVDUMC/IEC/78E). The tissues for histopathology (surgical specimens, dilatation and curettage, biopsies) were received in the Department of Pathology. All routinely processed paraffin embedded tissue blocks and Hematoxylin and Eosin (H&E) stained slides of these 78 cases were retrieved. The slides were reviewed. Hyperplasias were reclassified as typical and atypical as per WHO classification.

IHC was performed on all cases of EC and atypical hyperplasia. The primary antibodies used were ER (Clone EP1; Dako), PR (Clone PgR636; Dako), p53 (Clone DO-7; Dako) and Ki-67 (Clone MIB-1; Dako). Section from normal breast tissue was used as positive control for ER and PR. Section from and skin and tonsil was used as positive control for p53 and Ki67 respectively. Scoring of ER, PR in EC and hyperplasia were done as positive and negative while percentage labeling index was followed for p53 and Ki67.

3. Results

Total 78 cases were studied for a period of 01 year from June 2019 to May 2020. There were 20 cases of EC and 58 cases of endometrial hyperplasia as seen in Table 1. EC was most commonly seen in sixth to seventh decade and hyperplasia was seen in fourth to fifth decade (Table 2).

There are 11 surgical specimens and 09 biopsies of EC. Out of total 20 cases, there were 17 cases of endometrioid adenocarcinoma and 03 cases were of papillary serous adenocarcinoma. ER, PR expression was seen in 10 cases of grade 1 endometrial carcinomas and 5 cases of grade 2 EC. p53 expression was seen in 01 case of grade 1 EC and 03 cases grade 3 EC (Table 3) ER, PR, p53 all were negative in one case. Ki67 was <10% in 11 cases, between 10–20% in 5 cases and >20% in 04 cases (table 4). We followed up 15 out of 20 cases, out of which only 01 patient died of disease who was grade 1 endometrial carcinoma. Rest 14 had progression free survival (PFS) till date.

All 58 cases of endometrial hyperplasia were reclassified complex and simple hyperplasia to typical and atypical hyperplasias (as per WHO Classification 2014). Out of 58 cases, 56 cases were typical hyperplasia and 02 cases atypical hyperplasia. ER, PR expression was positive in all the cases of typical and atypical hyperplasia. p53 expression was absent in typical and atypical hyperplasia with low Ki 67 index (<10%) (Tables 4 and 5).

Table 1: Table showing different endometrial lesions (n =78)

| Type of lesion                     | Number of cases | Percentage |
|-----------------------------------|-----------------|------------|
| Typical hyperplasia               | 56              | 71.79      |
| Atypical hyperplasia              | 02              | 2.56       |
| Endometrioid carcinoma            | 17              | 21.79      |
| Papillary serous carcinoma        | 03              | 3.84       |
| Total                             | 78              | 100        |

4. Discussion

The molecular classification of EC that has emerged from the Cancer Genome Atlas (TCGA) study provides additional potentially superior prognostic information to traditional histologic typing and grading. This classifier does not, however, replace clinicopathologic risk assessment based on parameters other than histopathology type and grade.
Table 2: Age wise distribution of endometrial carcinoma, atypical and typical hyperplasia

| Age range (total number of cases) | Number of cases of Endometrial carcinoma | Number of cases of atypical hyperplasia/EIN | Number of cases of typical hyperplasia |
|-----------------------------------|------------------------------------------|-------------------------------------------|----------------------------------------|
| 21-30 (n=6)                       | 0                                        | 0                                         | 6                                      |
| 31-40 (n= 18)                     | 2                                        | 0                                         | 16                                     |
| 41-50 (n= 30)                     | 2                                        | 1                                         | 27                                     |
| 51-60 (n=12)                      | 6                                        | 1                                         | 5                                      |
| 61-70 (n = 9)                     | 7                                        | 0                                         | 2                                      |
| >70 (n= 3)                        | 3                                        | 0                                         | 0                                      |
| Total                             | 20                                       | 2                                         | 56                                     |

Table 3: Grade wise distribution of immunohistochemical markers in EC

| Endometrial carcinoma | ER, PR positive | p 53 positive | P value   |
|-----------------------|------------------|---------------|-----------|
| Grade 1 (n=11)        | 10 (90.9%)       | 01 (9.09%)    | P value = 0.005 |
| Grade 2 (n= 5)        | 05 (100%)        | 0             |           |
| Grade 3 (n = 3)       | 0                | 03 (100%)     |           |

Table 4: Ki67 index in various lesions of endometrium

| Lesions                              | Ki67 <10 | Ki67 10-20 | Ki67 >20 | P value   |
|--------------------------------------|----------|------------|----------|-----------|
| Typical Endometrial hyperplasia (n=56) | 56       | 0          | 0        |           |
| Atypical Endometrial hyperplasia (n=2) | 0        | 02         | 0        |           |
| Grade 1 EC ( n= 11)                  | 7        | 4          | 0        | P value < 0.001 |
| Grade 2 EC (n= 5)                    | 4        | 1          | 1        |           |
| Grade 3 EC (n= 3)                    | 0        | 0          | 3        |           |
| Total                                | 67       | 07         | 04       | 78        |

Table 5: Distribution of ER, PR and p53 in various endometrial lesions

| Lesion Type (Number) | ER | PR | p53 |
|----------------------|----|----|-----|
| Typical Endometrial hyperplasia (n= 56) | 56 | 56 | 00  |
| Atypical endometrial Hyperplasia(n = 02) | 02 | 02 | 00  |
| Endometrioid adenocarcinoma (n= 17) | 15 | 15 | 01  |
| Papillary serous carcinoma (n = 03) | 0  | 0  | 03  |

Table 6: Comparison studies of various variables in EC:

| Various studies                        | N [number of cases] | Age range (mean age ) | Endometriod type | Serous type | Grade 1 | Grade 2 | Grade 3 |
|---------------------------------------|---------------------|-----------------------|------------------|-------------|---------|---------|---------|
| Our study                              | 78                  | 31-70 (50.5)          | 17               | 03          | 11      | 06      | 03      |
| Somssubhra Goswami et al9              | 34                  | 35-58 (60)            | 32               | 2           | 16      | 7       | 11      |
| Trovik et al10                         | 1192                | 28-94 (66)            | 954              | 107         | 458     | 343     | 143     |
| Amany Salama et al11                   | 109                 | 37-79 (59.8)          | 89               | 12          | 36      | 39      | 30      |
| Geoffroy et al12                       | 69                  | 58-77 (67.5)          | 56               | 02          | 43      | 6       | 17      |

Table 7: Comparison studies of expression of Immunohistochemical markers in EC:

| Various studies                        | ER expression | PR expression | p 53 expression |
|---------------------------------------|---------------|---------------|-----------------|
| Our study                              | 15            | 15            | 04              |
| Trovik et al10                         | 492           | 492           | 150             |
| Nayar et al13                          | 17            | 18            | 27              |
| Suthipintawong C et al14               | 50            | 47            | 31              |
4.1. ER and PR receptors and functions

ER and PR belong to the nuclear receptor superfamily. They are ligand-dependent transcriptional factors, which can bind to different DNA sites to initiate the expression of specific genes. ER exists in 2 isoforms, ER-α and ER-β, which have a distinct pattern of expression in the tissues,\(^{13}\) that varies during cellular proliferation and differentiation.\(^{14,17}\)

Progesterone receptor (PR) also has 2 isoforms PR-A and PR-B which are functionally distinct transcription factors.\(^{18}\) According to the literature, the transcription of PR gene is induced by estrogen and inhibited by progesterone in the majority of estrogen responsive cells, so the expression of ER and PR is considered to be coordinated.\(^{19,20}\)

4.2. ER and PR in endometrial carcinoma

The expression of steroid receptors in EC has been quantitatively associated with histologic differentiation.\(^{21,22}\) The loss of steroid receptors is an early event in EC.\(^{23}\) ER-α expression is decreased in EC,\(^{24}\) in both glands and stroma in relation to EH and is further decreased as EC grading advances.\(^{23–25}\)

PR-A is associated with cell and promoter specific repression of PR-B\(^{26}\) and imbalance in PR-A to PR-B ratio is frequently associated with carcinogenesis.\(^{27}\)
4.3. **ER and PR status relationship with disease progression and prognosis**

Molecular tumor classification, which includes PR and ER expression, is an integral part of the EC. Five-year survival rate (stage I) and median survival time (stages II-IV, recurrences) for patients with ER+/PR+ and ER-/PR+ EC i.e. PR+ were reported to be significantly better than for ER-/PR- and ER+/PR- i.e. PR- patients. Thus, PR/ER immunohistochemistry appears to be a reliable means for predicting survival in EC, independent of other clinicopathological parameters.

In the present study, 58 cases of EH and 20 cases of EC were distributed in the age range of 21-81 years. It is observed that peak incidence of endometrial hyperplasia was in fourth to fifth decade and that of EC was in the sixth decade. Commonest histomorphology was endometrioid type of adenocarcinoma in 17 cases followed by papillary serous carcinomas in 3 cases.

Our study correlates with various studies as seen in table number no 6 in all the variables described. Our study show that grade 1 and 2 endometrial carcinomas have different ER, PR, Ki67 and p53 immunostaining profiles compared with grade 3, as also shows a significant p value of less than 0.005. Out of 20 cases of endometrial carcinomas, 15 cases showed strong expression of ER and PR indicating type 1 pathogenetic mechanism and 4 cases showed strong p53 expression which had serous papillary morphology confirming the pathogenesis of type 2 EC.

The expression of ER, PR, p 53 was compared and correlated with other studies in the literature as shown in Table 7. One case of endometrial carcinoma in present study was negative for ER, PR and p53 (aberrant expression). These findings are clinically important, because ER/PR loss may help identify high-risk patients and its expression increased as disease progressed from presumed low-risk group for in-time and treatment. In our study, grade 1 and 2 endometrial carcinomas showed lower Ki67 index (<10- 20%) than grade 3 tumors thus Ki 67 labeling index can be closely associated with tumor grade. This also correlates with Canlborge G et al.

In study by Sirijaipracharoen et al., which included 108 patients, ER, PR and HER-2/neu expression were positive in 59.3%, 65.7% and 2.8% respectively. Out of 20 cases of endometrial carcinomas, 15 cases showed strong expression of ER and PR indicating type 1 pathogenetic mechanism and 4 cases showed strong p53 expression which had serous papillary morphology confirming the pathogenesis of type 2 EC. Ki67 labeling index can be closely associated with tumor progression and prognosis.

The p value for expression in our study for ER, PR, p53 and Ki67 was statistically significant for hyperplasia versus EC (Tables 6 and 7).

5. Conclusion

Distinguishing between hyperplasia and true precancerous lesions has significant clinical implications because distinct endometrial precancerous conditions require appropriate intervention. EH, especially atypical, increases the risk for EC and their early detection becomes mandatory under cancer prevention.

High ER, PR expression along with low p53 was more in type 1 endometrial carcinomas compared to low or absent ER, PR along with strong p53 expression in papillary serous carcinoma (Type 2) confirming the different pathogenetic mechanism for both.

6. Source of Funding

No financial support was received for the work within this manuscript.

7. Conflict of Interest

The authors declare they have no conflict of interest.

References

1. Faria SC, Sagebiel T, Devine C, Lal C, Balachandran A, Bhosale PR, et al. Imaging in endometrial carcinoma. *Indian J Radiol Imaging*. 2015;25(2):137. [d...](https://doi.org/10.1016/j.ijri.2015.01.006)
2. Sanderson PA, Critchley HOD, Williams ARW, Arends MJ, Saunders PTK. New concepts for an old problem: the diagnosis of endometrial hyperplasia. *Human Reprod Update*. 2016;12(2):232–54. [d...](https://doi.org/10.1093/humupd/dmw043)
3. Gupta A, Paitiri K, Gupta A, Gupta R, Khare P. Histopathological patterns in endometrial biopsy associated with abnormal uterine bleeding. *Asian Pac J Trop Med*. 2018;30(3):31–6.
4. Sobczuk K, Sobczuk A. New classification system of endometrial hyperplasia WHO 2014 and its clinical implications. *Menopausal Rev*. 2017;3(3):107–11. [d...](https://doi.org/10.5114/mr.2017.7089)
5. Buza N, Roque DM, Santin AD. HER2/neu in Endometrial Cancer: A Promising Therapeutic Target With Diagnostic Challenges. *Arch Pathol Lab Med*. 2014;138:343–50. [d...](https://doi.org/10.1043/arpa.2012-0416-tf).
6. Key TJ, Pike MC. The dose-effect relationship between ‘unopposed’ oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk. *Br J Cancer*. 1988;57(2):205–12. [d...](https://doi.org/10.1038/bjc.1988.7)
7. Creasman WT. Endometrial cancer: incidence, prognostic factors, diagnosis, and treatment. *InSeminars Oncol*. 1997;24:1–140.
8. Bohkman JV. Two pathogenetic types of endometrial carcinoma. *Gynecol Oncol*. 1983;15(1):10–7. [d...](https://doi.org/10.1016/0090-8258(83)90111-1)
9. El-Sahwi KS, Schwartz PE, Santin AD. Development of targeted therapy in uterine serous carcinoma, a biologically aggressive variant of endometrial cancer. *Exp Rev Anticancer Ther*. 2012;12(1):41–9. [d...](https://doi.org/10.1586/era.11.192)
10. Black JD, English DF, Roque DM, Santin AD. Targeted Therapy in Uterine Serous Carcinoma: An Aggressive Variant of Endometrial Cancer. *Women’s Health*. 2014;10:45–57. [d...](https://doi.org/10.1097/bwh.0b013e32836c197a)
11. Maniketh I, Ravikumar G, Crasta JA, Prabh u, Vallikad E. Estrogen and progesterone receptor expression in endometrioid endometrial carcinomas: a clinicopathological study. *Middle East J Cancer*. 2014;1(2):67–73.
12. Armstrong AJ, Hurd WW, Elguero S, Barker NM, Zanotti KM. Diagnosis and Management of Endometrial Hyperplasia. *J Minimally Invasive Gynecol*. 2012;19(5):562–71. [d...](https://doi.org/10.1016/j.jmig.2012.05.009)
13. Mueller S. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. *Curr Opin Pharmacol*. 2001;1(6):613–9. [d...](https://doi.org/10.1016/s1471-4892(01)00010-9)
25. Prostaglandin F may play a role in regulating expression of ER and MMP2 secretion in endometrial carcinoma cell lines. *Harefuah*. 2009;148(7):416–9.

26. Sen A, Goswami S, Biswas M. Association of the hormonal receptor status of endometrial carcinomas with the markers of tumor aggression: A comparison with similar studies in developed nations. *Med J Dr DY Patil Univ*. 2017;10(4):334, doi:10.14105/mdydpuru.mdypuru_276-16.

27. Trovik J, Wik E, Werner HMI, Krakstad C, Helland H, Vandenvoot I, et al. Hormone receptor loss in endometrial carcinoma predicts lymph node metastasis and poor outcome in prospective multicentre trial. *Eur J Cancer*. 2013;49(16):3431–41.

28. Salama A, Arafa M, El Zahaf E, Shebl AM, Awad AAEH, Ashamallah SA, et al. Potential Role for a Panel of Immunohistochemical Markers in the Management of Endometrial Carcinoma. *J Pathol Transl Med*. 2019;53(3):164–72. doi:10.1016/j.jptm.2019.02.012.

29. Canforbe G, Laas E, Bendifallah S, Darai E, Ballester M. Contribution of immunohistochemical profile in assessing histological grade of endometrial cancer. *Anticancer Res*. 2013;33(1):2191–99.

30. Lax SF. Pathology of endometrial carcinoma. In: Molecular Genetics of Endometrial Carcinoma. Springer Cham. 2017:p. 75–96.

31. Stelloo E, Jansen AML, Osse EM, Nout RA, Creutzberg CL, Ruano D, et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. *Ann Oncol*. 2017;28(1):96–102. doi:10.1093/annonc/mdw334.

32. Masjed NM, Khandeparkar SG, Joshi AR, Kulkami MM, Pandya N. Immunohistochemical study of ER, PR, Ki67 and p53 in endometrial hyperplasia and endometrial carcinomas. *J Clin Diagn Res*. 2017;11(8):31.

33. Suthipintawong C, Wejaranayang C, Pipupinyo C. Prognostic significance of ER, PR, Ki67, c-erbB-2, and p53 in endometrial carcinoma. *J Med Assoc Thai*. 2008;91(12):1779.

34. Khan JA, Amazit L, Bellance C, Guichon-Mantel A, Lombès M, Loosfelt H, et al. p38 and p42/44 MAPKs Differentially Regulate Progesterone Receptor A and B Isoform Stabilization. *Mol Endocrinol*. 2011;25(10):1710–24. doi:10.1210/me.2011-1042.

35. Zafran N, Levin A, Goldman S, Shalev E. Progesterone receptor’s profile and the effect of the hormone and its derivatives on invasiveness and MMP2 secretion in endometrial carcinoma cell lines. *Harefuah*. 2009;139(7):274–79.