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Original Research Article

A Study on Immunohistochemical Expression of Ki-67 in Endometrial Hyperplasia and Endometrial Carcinoma

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Abstract

Background and Objective: Endometrial carcinoma is the most common gynaecologic malignancy in perimenopausal, and menopausal women. It is often preceded by endometrial hyperplasia. Estrogen appears to be involved in the development of endometrial carcinoma. Other mechanisms of endometrial carcinogenesis include mutations in p53, PTEN, and proliferation markers like Ki-67. However, the pattern of Ki-67 expression is not well established in hyperplastic and neoplastic endometrium. Thus, objective of our study was to determine the immunohistochemical expression of Ki-67 in hyperplastic endometrium and endometrial carcinoma.

Methods: Immunohistochemical analysis of Ki-67 was done in 60 fixed, paraffin-embedded endometrial biopsy specimens and uterine resections obtained from patients. Specimens included simple hyperplasia, complex hyperplastic lesions, and endometrial adenocarcinoma.

Results: Ki-67 expression showed strong positivity in complex hyperplasia with atypia when compared to simple hyperplasia, and complex hyperplasia without atypia. Though high grade endometrial carcinomas expressed more Ki-67 positivity, but there was no statistical significance found between expression of Ki-67 and tumour grading. Strong positivity was seen in advanced tumours with positive correlation between expression of Ki-67 and tumour stage.

Conclusion: Theexpression of Ki-67 may be used as diagnostic and prognostic marker in cases of endometrial carcinoma. Further studies with larger samples are needed to validate these findings.

Keywords: Endometrial Carcinoma; Immunohistochemistry; Ki-67.

Key Messages: Ki-67 expression was positive in 74.3% cases of endometrial hyperplasia. Statistical association was observed between Ki-67 expression and simple hyperplasia, and between Ki-67 expression and complex hyperplasia with atypia.

Ki-67 expression was positive in 80% cases of endometrial carcinoma. Statistical association was noted between tumor staging and Ki-67 expression.

Introduction

Endometrial carcinoma (EC) is the most common malignancy of the female genital tract. Its most frequent subtype, endometrioid carcinoma ("Type I" cancers), is often preceded by a histologically evident precursor lesions, which generally have fallen under the diagnostic umbrella of "hyperplasias".[1] Accurate and sensitive recognition of these precursors has a great clinical value as an early warning of increased risk of cancer and in preventive treatment. Public awareness of the

need for early diagnosis is also on the rise, in part because of widespread media coverage of the endometrial tumorigenic effects of hormonally active medications, such as tamoxifen.

It occurred in 320,000 women and caused 76,000 deaths globally.[1] The exact cause of endometrial cancer remains unknown, although several risk factors have been identified. These risk factors include nulliparity, late menopausal age (after 50 years old), diabetes mellitus, hypertension, obesity, BMI, early menarche, ethnicity and family history of endometrial cancer. [2-8]

Recent studies were mainly focused on the function of gene products which determines the cell fate. Antigen Ki-67 is a nuclear protein, a cellular marker for proliferation which is encoded by the MKI 67. Furthermore, it is associated with ribosomal RNA transcription. Inactivation of antigen Ki-67 leads to inhibition of ribosomal RNA synthesis.[9] During interphase, the Ki-67 can be exclusively detected within the cell nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. This protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent in resting cells (G0). The fraction of Ki-67-positive tumor cells (the Ki-67 labelling index) is often correlated with the clinical course of cancer.

"Thus, objective of our study was to determine the immunohistochemical expression of Ki-67 in endometrial hyperplasia (EH) and endometrial carcinoma."

Materials and Methods

This prospective study was conducted in the department of Pathology; from June 2015 to May 2017. Sixty cases were included in the study.

Inclusion Criteria

- Patients with history of abnormal uterine bleeding for a period of 6 months or above.

Exclusion Criteria

- Patients with clinically and radiologically detectable lesions in the uterus including polyp, adenomyosis, leiomyoma, coagulopathy, ovulatory dysfunction and other endocrine abnormalities.
- Patients who were on Hormone replacement therapy and Oral contraceptive pills.

Source of Data

Among the sixty cases of endometrial samples, 26 specimens were obtained from dilatation and curettage, 34 specimens were obtained from total abdominal hysterectomy with bilateral salphingoophorectomy. Informed consent was obtained from each patient before surgery for the use of endometrial tissues. In EC cases, the standard protocol for surgical grossing was followed. Multiple representative bits were taken from the tumor and surgical margins; the blocks were cut and stained with hematoxylin and eosin. Each slide was examined by a pathologist for histopathological features.

Processing for Immunohistochemistry (IHC)

These formalin fixed, paraffin-embedded blocks were sliced in 3–4 µm thickness for IHC. The technique for IHC includes antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse anticlonal antibody against Ki-67 (Anti Ki - 67 monoclonal antibody), linking with rabbit anti mouse secondary antibody (Biogenex), enzyme labelling with streptavidin-horseradish peroxidase, developing chromogen with deaminobenzidine (DAB) and Counter-staining with hematoxylin. Sections of tonsil were taken as positive controls and Sections of adipose tissue were taken as negative controls.

Ki-67 positivity is indicated by brown staining of nucleus in tumor cells. The mean percentage of positive Ki-67 was determined by counting 1000 cells in 10 randomly selected high power fields.[10, 11, 12] A semiquantitative assessment of staining was done as follows:

Weak positive (1+) – less than 25% of tumor cells positive for Ki-67

Mild positive (2+) – 25–50% of tumor cells positive for Ki-67

Moderate positive (3+) – 50–75% of tumor cells positive for Ki-67

Strong positive (4+) – 75–100% of tumor cells positive for Ki-67

Statistical Analysis

Statistical analysis was carried out using SPSS version 21.0 (IBM SPSS, US) software with Regression Modules installed. In this study analysis were done using chi-square test, ANOVA test,

Table 1: Ki-67 Expression in Endometrial Hyperplasia.

S. No	Type of Endometrium	Sample Size	Ki 67 Positive		P Value
			No. of Cases	Percentage	
1	Simple hyperplasia without atypia	20	15	75%	0.02
2	Complex hyperplasia without atypia	8	6	75%	0.4
3	Complex hyperplasia with atypia	7	5	71.4%	0.01
	Total	35	26		

Table 2: Intensity of Ki-67 Expression in Endometrial Hyperplasia.

Ki-67 Expression	Simple Hyperplasia	Ch without Atypia	Ch with Atypia
0	5 (25%)	2 (25%)	2 (28.6%)
1+	11 (55%)	4 (50%)	0
2+	3 (15%)	2 (25%)	0
3+	0	0	4 (57.2%)
4+	1 (5%)	0	1 (14.2%)
Total	20 (75%)	8 (75%)	7 (71.4%)

CH- Complex hyperplasia

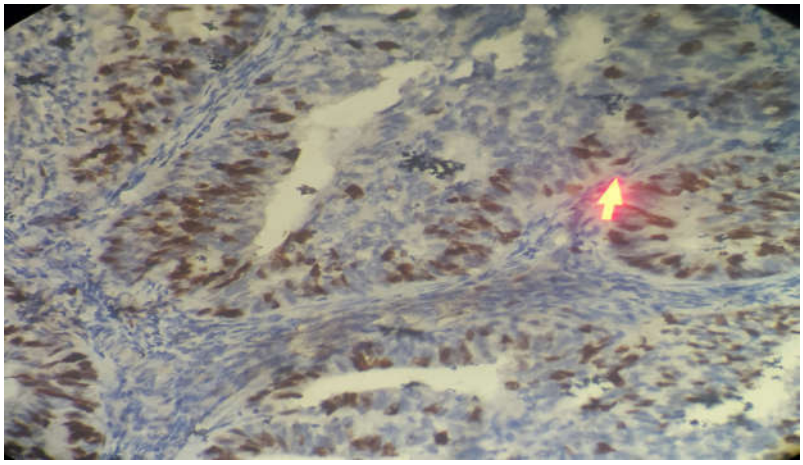

Fig. 1: (1+) Immunohistochemical nuclear staining of Ki-67 in simple hyperplasia (x40).

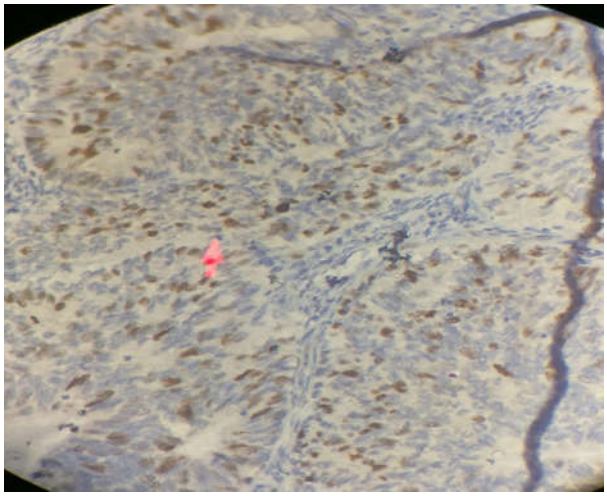

Fig. 2: (1+) Immunohistochemical nuclear staining of Ki-67 in complex hyperplasia (x40).

and Mann-Whitney U test among the groups and between the groups.

Results

A total of 60 cases were studied. 52% of the cases were in the age group below 45 years, followed by 33% of cases were in the age group of 45-60 years and 15% of cases were in the age group above 60 years. 28% of cases were postmenopausal females.

Out of 60 cases, 35 (58.3%) were cases of EH and other 25 (41.7%) were cases of EC. Among EH cases, simple hyperplasia (SH) was most common type (57.2%), followed by complex hyperplasia without atypia (CHWA; 22.8%) and complex hyperplasia with atypia (CHA; 20%). All cases of EC turned out to be endometrioid type. 44% of the carcinomas were low grade (well differentiated) and 56% were high grade (moderately and poorly differentiated) tumors. Stage I (68%) was the most common presentation followed by stage II (16%), stage III (8%) and stage IV (8%).

Overall, 76.6% (46 cases) showed Ki-67 expression. Among 35 cases of EH, 74.3% (26) of cases were positive for Ki-67. The distribution of Ki-67 expression among SH (Fig. 1), CHWA (Fig. 2), and CHA cases is shown in table 1 and 2. Statistical association was observed between Ki-67 expression and SH, and between Ki-67 expression and CHA ($p< 0.05$).

Among 25 cases of EC, 80% (20) cases expressed Ki-67 positivity. The distribution of Ki-67 expression among EC cases is shown in the table 3. 40% of low grade EC tumors were positive for Ki-67 expression and 60% of high grade tumors were positive for Ki-67. No statistical association was found between Ki-67 and tumor grading (table 4). The distribution of Ki-67 expression vs EC staging is shown in table 5. Statistical association was noted between the tumor staging and Ki-67 expression ($p< 0.05$).

Table 3: Intensity of Ki-67 Expression in Endometrial Carcinoma.

Ki-67 Expression	Endometrial Carcinoma
0	5 (20%)
1+	8(32%)
2+	5(20%)
3+	3(12%)
4+	4 (16%)
Total	25

Table 4: Ki-67 Expression with Respect to Grading of Endometrial Carcinoma.

Endometrial Carcinoma Grading	Sample Size	Ki 67 Positive	
		No. of Cases	Percentage
Low (I)	11	8	40%
High (II and III)	14	12	60%
Total	25	20	

$p = 0.12$

Table 5: Ki-67 Expression with Respect to Staging of Endometrial Carcinoma.

Endometrial Carcinoma Staging	Sample Size	Ki 67 Positive	
		No. of Cases	Percentage
I	17	15	75%
II	4	3	15%
III	2	1	5%
IV	2	2	10%
Total	25	20	

$p = 0.001$

Between Group Analysis

The expression profile of Ki-67 was compared in paired groups. Statistical correlation was found between CHWA and CHA, and between CHWA and EC (Table 6; $p< 0.05$).

Table 6: Comparison of Immunoreactivity of Ki-67 between Two Groups.

Comparing Groups	Ki-67 (p value)
SH and CHWA	0.78
CHWA and CHA	0.03
CHWA and EC	0.01
CHA andEC	0.87

Significant ($P< 0.05$)

SH- Simple hyperplasia, CHWA- Complex hyperplasia without atypia, CHA- Complex hyperplasia with atypia, EC- Endometrial carcinoma

Discussion

EC is the most common female genital cancer in the developed world.[13] It does not follow a single homogenous disease process, at least two clinically distinct pathways have been found out so far. One pathway is estrogen dependent which is preceded by hyperplasia and results in low grade neoplasm with better prognosis and survival rate. In second set of pathways, it is non estrogen dependent and is not preceded by hyperplasia and results in high grade neoplasm with poor prognosis and survival rate.[14]

Among all proliferative markers, Ki-67 is more reliable and significant in assessing the proliferative index by mitotic activity. Ki-67 expression had a strong and independent prognostic impact in addition to FIGO staging which will help in identifying patients who may be benefited by post-operative adjuvant therapy. Ki-67 is also a significant indicator in assessing recurrence.[15]

In the present study, mean age of EH and EC cases was 42 years and 57 years respectively. The age wise distribution of EH and EC was comparable with previous studies.[16,17] This could be justified by the suggestion that epithelial transformation from the benign to the malignant may develop over a time period by progressive increase in the degree of abnormality.

Ki-67 Expression in Endometrial Hyperplasia

In current study, the high proliferative index was seen in CHA when compared to the SH and CHWA. Statistical significance was found between Ki-67 and SH, and Ki-67 and CHA. Our findings were in accordance with Ghosh et al study where they found sequential increase in median expression of Ki-67 from SH to CHA.[17] In contrast to our study, Daniela Ilie et al found that the expression was high in SH when compared with CHWA and CHA. It could be due to an increase in expression of NCoR (co-repressor of steroid receptors) which blocks estrogen dependent growth.[18]

In a study done by Khedr et al, the result of quantitative measurement of Ki-67 index showed a drastic decrease in cyclic proliferative endometrium to EH. This finding appeared to contrast with the expectation that hyperplasia would be associated with increased proliferation indices. This contrast may be explained, as proliferative endometrium has high mitotic activity to compensate for the regular shedding; whereas in endometrial hyperplasia, there is no regular shedding of the tissue. Thus the net result is glandular crowding which can transform into malignancy over a period of time.[19] However, the cases with proliferative endometrium were not included in the present study.

Ki-67 Expression in Endometrial Carcinoma

In the present study, the expression of Ki-67 was seen in 80% of the endometrial carcinoma. Positive correlation was noted between Ki-67 and tumor staging. Even though high grade tumors showed increased Ki-67 expression, no statistical significance was found. Study by Li et al demonstrated that the Ki-67 labelling index had no significant prognostic impact, although correlation with tumor grade was detected. In this study, tissue micro array of endometrial carcinoma was used instead of the large paraffin sections used.[20]

In Salvesen et al Ki-67 expression was found to be a significant prognostic indicator for endometrial carcinoma patients. The Ki-67 expression was significantly associated with FIGO stage, histological type, and histological grade.[21]

Gassel AM et al, investigating 224 cases, found a highly significant correlation between the percentage of the proliferating tumor cells and the survival, independent of other associated factors such as tumor grade and stage.[22]

Between Group Analysis

In the present study, the expression profile of Ki-67 was compared in paired groups. Positive correlation was found between CHWA and CHA, and between CHWA and EC. However, statistical significance was not observed between SH and EC, and between CHA and EC. Previous studies (Amit pal et al, stoian et al) compared the expression profile of Ki-67 among the paired groups and found a statistically significant difference among the results of EC and hyperplasia without atypia, between EC and atypical hyperplasia, and between atypical hyperplasia and hyperplasia without atypia; and also suggested that this cellular protein Ki-67 is deregulated and therefore may have a role in endometrial carcinogenesis.[23, 24]

Conclusion

Ki-67 expression showed strong positivity in complex hyperplasia with atypia when compared with simple hyperplasia and complex hyperplasia without atypia. Though high grade endometrial carcinomas expressed more Ki-67 positivity, no statistical significance was found between expression of Ki-67 and tumor grading. Strong positivity was seen in advanced tumors with positive correlation between the Ki-67 and tumor stage. Considering this into account, Ki-67 can have a vital role as a prognostic marker. It may require further validation using other molecular markers including ER, PR and p53 and cytogenetic methods, with more sample size for better understanding of the underlying molecular mechanisms that determine Ki-67 overexpression and other pathways in development of precancerous and cancerous lesions of Endometrium.

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