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IMMUNOEXPRESSION OF CYCLIN D1 AND KI-67 IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM

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ABSTRACT

Background-various cell cycle promoting and inhibiting factors have important role in proliferation and differentiation of malignant cells

Aim- The study was done to examine the expression of cell cycle regulatory protein Cyclin D1 in endometrial hyperplasias and endometrial carcinomas in a quantitative manner & to assess correlations of Cyclin D1 expression with Ki-67 ,a proliferation marker.

Materials and methods- We evaluated and compared the expression of Cyclin D1 and Ki-67 in 50 endometrial samples submitted as either endometrial curetting or hysterectomy specimens, which were diagnosed as Hyperplasia without atypia(n = 23), atypical hyperplasia (n = 16), and endometrial carcinoma (n = 11).

Results- There was increased expression of Cyclin D1 and Ki-67 in patients with endometrial carcinoma compared to endometrial hyperplasia. Cyclin D1 expression had a positive correlation with Ki-67 expression.

Conclusion- Cyclin D1 together with Ki-67 may be used as a marker for endometrial carcinogenesis and tumor cell proliferation.

KEYWORDS : Endometrial Hyperplasia, Endometrial Carcinoma, Cyclin D1, Ki-67.

INTRODUCTION-

Carcinoma of the endometrium is the most common gynecologic malignancy in developed countries. It typically occurs in elderly individuals, 80% of the patients being postmenopausal at the time of diagnosis. It is currently believed that endometrial carcinomas can be divided in two distinct types on the basis of their pathogenesis: one – by far the more common – occurring as a result of excess estrogenic stimulation and developing against a background of endometrial hyperplasia, and the other developing de novo. Atypical hyperplasia (AH) is considered as a precursor of endometrioid type of Endometrial Carcinoma(EC) and endometrial intraepithelial carcinoma (EIC) is considered the precursor of serous carcinoma.[1]

Endometrial hyperplasia on the other hand is defined as an increased proliferation of the endometrial glands relative to the stroma, resulting in an increased gland to-stroma ratio when compared with normal proliferative endometrium. Recently WHO classified endometrial hyperplasia into 2 categories-Hyperplasia without atypia (HWA) and Atypical hyperplasia(AH).

Immunohistochemical methods have been very useful in recent times for detecting several biomarkers of possible diagnostic and prognostic value for various types of malignancies[2].

Cyclin D1 is a key protein in the regulation of the cell cycle at the G1 to S phase transition, and is essential for regulation of proliferation, differentiation and transcription[3] .Over expression of Cyclin D1, induces excessive cellular proliferation and is often over-expressed in various neoplasias such as infiltrating ductal breast carcinoma, colorectal carcinoma, bladder carcinoma, head and neck, lung and prostate carcinomas.

Several studies in recent past have reported increased cellular proliferation co-existing with progressive derailment of Cyclin D1 leading to progression of endometrial hyperplasia to Endometrial carcinoma.

The Ki-67 protein is a cellular marker for proliferation. It is strictly associated with cell proliferation, Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent from resting cells (G_0)[4].

The present study is aimed to evaluate the expression pattern of Cyclin D1 in Hyperplastic endometrium and Endometrial Carcinoma and to compare it with expression of Ki-67 which has a direct correlation with the degree of proliferation of cells and to explore the possibility for Cyclin D1 as a potential diagnostic marker to distinguish these endometrial disorders.

MATERIALS AND METHODS-

The present study was a retrospective study done in the Department of Pathology, ESIPGIMSR, Manicktala, Kolkata over one year 2015-2016.

Samples received either as endometrial curettage or hysterectomy specimens of the patients over 12 months which were diagnosed as endometrial hyperplasia or endometrial carcinoma on histopathological examination were included in the study. The hematoxylin and eosin (H&E) stained sections and representative blocks were retrieved from the archives of the Department of Pathology, 3 µm sections were cut and IHC was done.

Immunohistochemistry for Cyclin D1 and Ki-67 were done by using antibody manufactured by Cellmarque. The quantitative evaluation of immunostaining for Cyclin D1 and Ki-67 was done by assessing extent of nuclear positivity. Due to the heterogeneity of the reaction, the area with the highest level of nuclear staining were selected ,the number of positively stained nuclei in 1000 glandular cells were counted under 40 × magnification and results were expressed as percentage staining.

RESULTS-

Immunohistochemical analysis was performed on 50 cases which included 11 cases of EC of various types,16 cases of AH, 23cases of hyperplasia without atypia. The median age of these cases on which immunohistochemistry has been done was 50 years in the category of EC (n = 11, age range 30-80 years) as well as in the category of AH (n = 16, age range 30-75 years). The median age of the patients in the group of HWA was 40 years (n = 23, age range 30-69 years).

Cyclin D1 and Ki-67 expression: **.[Figure 1, Figure 2, Figure 3]** For all cases, these are shown in **[Table 1]** The median expression of Cyclin D1 was 60% in the cases of EC (n = 11) .Minimum expression of 10% was recorded, whereas highest expression of 80% staining was recorded in 2 cases

The median expression of Ki-67 in the cases of EC was 50%, the minimum expression of 20% and maximum expression value was 80% . In the 16 cases of AH, Cyclin D1 expression varied in a range of 20% to 60% with a median value of 30%, whereas Ki-67 expression varied from 20% to 55% with the median value of 35%. The highest value of expression for Cyclin D1 and Ki-67 were 60% & 55%, respectively, in this category. In cases of Hyperplasia without atypia, immunoreactivity for Cyclin D1 varied from 5% to 20% , whereas the expression of Ki-67 varied from 10% to 30% . The median value for Cyclin D1 expression and Ki-67 expression was 10% and 20%, respectively.

For Cyclin D1 it was found that that 91% of ECs, 62.5% of AH and 0% of HWA displayed >30% nuclear staining, whereas <30% nuclear staining was observed in 9% of ECs, 37.5% of AH and 100% of SH **[Table 2].** Less than 10% nuclear staining was found in 56.5% of HWA, 0% of AH and 0% of Ecs.

More than 30% nuclear staining for Ki-67 was observed in 63.5% of ECs, 62.5% of AH, 0% of HWA whereas <30% nuclear staining for Ki-67 was observed in 36.5% of ECs, 37.5% of AH, 100% of HWA**[Table 1][Table 2].**

Between group analysis: By comparing the expression profile of Cyclin D1 in paired groups a statistically significant difference was observed between the results of EC and HWA (P = 0.00001), HWA and AH (P = 0.00001), EC and AH (P = 0.00071). Comparing the expression profile of Ki-67 among the various (paired) groups, a statistically significant difference was observed among the results of EC and HWA (P = 0.00003), between EC and AH (p=0.04664) and between AH and HWA (p=0.00001) **[Table 3].**

DISCUSSION-

Endometrial carcinoma is often preceded by histopathologic lesions designated as endometrial hyperplasia and currently it is accepted that there is a continuum of changes that evolve to Endometrioid carcinoma. Hyperplasia is usually associated with exogenous estrogen stimulation therefore estrogen is considered as an endometrial carcinogen. Other mechanisms of endometrial carcinogenesis include mutations in p53 and PTEN tumor suppressor genes.

A gradual and progressive increase in expression of both Cyclin D1 and Ki-67 levels was observed when the results were compared between hyperplasia without atypia, atypical hyperplasia and EC.

Our study revealed that expression of Cyclin D1 progressively increases from a median 10% in HWA to a median of 30% expression in AH to highest median (60%) expression in EC which was statistically significant. A simultaneous and corresponding increase in the levels of Ki-67 was also observed in these groups (median 20%, 35% and 50%, respectively). A positive correlation found between median value of median Ki-67 levels and median Cyclin D1 levels in the present study is expected with cell cycle accelerating effects of high Cyclin D1 levels.

More than 50% cases of ECs showed >30% nuclear staining for both Ki-67 and Cyclin D1 whereas 62.5% cases of AH showed > 30% expression of Cyclin D1 and Ki-67 respectively. None of the cases of AH showed <10% staining of Either Cyclin D1 for Ki-67. Statistically significant difference was observed in the expression profile of Cyclin D1 when compared between EC and HWA (P = 0.00001), HWA and AH (P = 0.00001). Analyzing the results of Ki-67 expression, statistically significant difference was seen in the category of EC and AH (P = 0.04664), EC and CH (P = 0.001), EC and SH (P = 0.001), SH and AH (P = 0.048).

Our findings suggest that both these cellular proteins are deregulated and therefore may have a role in endometrial carcinogenesis. Our findings support the significance of AH as a precancerous lesion, and the assumption that HWA not a precancerous lesion.

Ruhul Quddus et al.(2002)[5] analyzed immunoreactivity in five groups(Proliferative, Secretary, Simple hyperplasia, Complex Hyperplasia with or without atypia and EC) and have shown that they are statistically different groups and have documented no difference between CH and carcinoma and between proliferative, secretory and simple hyperplasia. They suggested that maximal dysregulation occurs at the CH state and concluded that some alterations might be responsible for the different morphologic featuresand behavior of CH and carcinoma. Authors have also suggested that Cyclin D1 over expression might be an early event in endometrial carcinogenesis.

Ozuysal et al. (2005)[6] observed higher mean Ki-67 values in Cyclin D1 immunoreactive endometrial adenocarcinoma when compared with Cyclin D1 nonreactive cases of endometrial adenocarcinoma (P < 0.05). Mean Ki-67 values were higher in cases with PE compared to EC and AH (P < 0.01) and non-significant difference was found between the mean Ki-67 values of cases with SH and PE. They reported no difference in Cyclin D1 immunoreactivity among AH, SH, and PE groups in contrast to our findings.

Liang et al.(2013)[7] observed no statistical significant difference (P > 0.05) on comparing Cyclin D1expression of SH, atypical CH, endometrioid carcinoma clear cell carcinoma and endometrial serouscarcinoma (ESC) though there was a gradual increase in the expression, except ESC. They concluded that though Cyclin D1 exhibited a promising potential to predict the prognosis of patients with EC,Cyclin D1 exhibited a poor ability to differentiate neoplastic lesions from non-neoplastic lesions; thus,the application of Cyclin D1 only is not so credible for differentiation between benign and malignant lesions.

CONCLUSION-

Our findings suggest the significance of Atypical hyperplasia as a precancerous lesion, but not Hyperplasia without atypia. Further, these findings also indicate that both Cyclin D1 and Ki-67 together may serve as informative biomarkers to recognize subsets of lesions that may be precancerous (AH) and thus amenable for early surgical therapy and at the same time exclude lesions that may be responsive to hormonal therapy. Thus they have the potential to serve as an useful adjunct to categorize these lesions.

TABLE 1- Percentage expression of cyclin D1 and ki 67 in cases of HWA, AH and EC

HWA	HWA	HWA	AH	AH	AH	EC	EC	EC
Sl no.	Cyclin d1	Ki 67	Sl no	Cyclin d1	Ki67	Sl no	Cyclin d1	Ki 67
HWA1	5	10	AH1	50	55	EC1	55	20
HWA2	10	15	AH2	25	30	EC2	70	50
HWA3	5	10	AH3	20	30	EC3	60	80
HWA4	15	30	AH4	35	35	EC4	60	80
HWA5	10	20	AH5	35	30	EC5	80	50
HWA6	10	15	AH6	50	55	EC6	70	80
HWA7	10	30	AH7	60	50	EC7	50	20
HWA8	20	30	AH8	35	35	EC8	80	50
HWA9	15	20	AH9	25	20	EC9	70	60
HWA10	10	7	AH10	45	25	EC10	10	20
HWA11	20	25	AH11	50	45	EC11	50	20
HWA12	10	12	AH12	30	35			
HWA13	10	20	AH13	55	40			
HWA14	15	30	AH14	35	30			
HWA15	12	10	AH15	25	25			

HWA16	15	25	AH16	25	35			
HWA17	10	10						
HWA18	10	30						
HWA19	20	25						
HWA20	5	7						
HWA21	15	30						
HWA22	7	10						
HWA23	10	15						

TABLE 2- Expression pattern of Cyclin D1 and ki 67 in EC, AH and HWA

Expression(%)	EC	EC	AH	AH	HWA	HWA
	Cyclin D1	Ki 67	Cyclin D1	Ki 67	Cyclin D1	Ki 67
≤10	0	0 (10%)	0 (0%)	0 (0%)	13 (56.5%)	7 (30.5%)
11-30	1 (9%)	4 (36.5%)	6 (37.5%)	6 (37.5%)	10 (43.5%)	16 (69.5%)
31-60	5 (45.5%)	4 (36.5%)	10 (62.5%)	10 (62.5%)	0 (0%)	0 (0%)
≥60	5 (45.5%)	3 (27%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 3- Comparision of Immunireactivity of Cyclin D1 and ki 67 between two groups

Comparing Groups	Cyclin D1 (P value)	Ki 67 (P value)
EC & AH	0.00071	0.04664
EC & HWA	0.00001	0.00003
AH & HWA	0.00001	0.00001

Significant ($P < 0.05$), (using Student T test)

Figure 1-

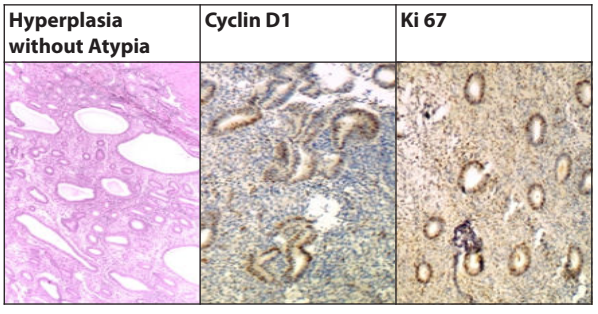


Figure 2-

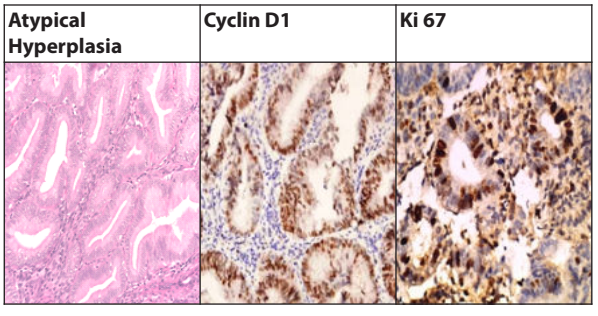
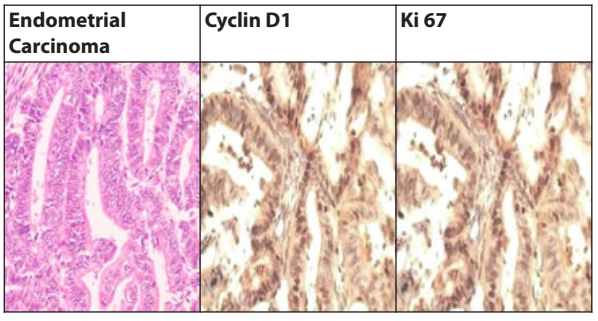


Figure 3-



DECLARATIONS-

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