

**EVALUATION OF EXPRESSION OF CYCLIN D1 IN ENDOMETRIAL HYPERPLASIA  
AND ENDOMETRIAL CARCINOMA**Anjali Sindhu<sup>1</sup>, Swaran Kaur<sup>2</sup>, Parveen Rana Kundu<sup>3</sup>, Ruchi Agarwal<sup>3</sup>, Yudhvir Singh<sup>4</sup>, Prerna Mahajan<sup>1</sup><sup>1</sup>Post -Graduate, Department of Pathology, B.P.S. Government Medical College For Women, Khanpur Kalan, Sonipat, Haryana.<sup>2</sup>Professor and Head, Department of Pathology, B.P.S. Government Medical College For Women, Khanpur Kalan, Sonipat, Haryana.<sup>3</sup>Associate Professor, Department of Pathology, B.P.S. Government Medical College For Women, Khanpur Kalan, Sonipat, Haryana.<sup>4</sup>Senior Resident, Department of Pathology, B.P.S. Government Medical College For Women, Khanpur Kalan, Sonipat, Haryana.**\*Corresponding Author: Anjali Sindhu**

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**ABSTRACT**

**Objective:** Cyclin D1 over expression may be one of the several mechanisms involved in endometrial neoplasia. The present study was undertaken to examine the expression of Cyclin D1 in endometrial hyperplasia and carcinoma. **Materials and Methods:** A cross sectional study was conducted over a period of 1 year. We evaluated and compared the expression profile of Cyclin D1 in 60 endometrial samples that were diagnosed as simple hyperplasia (n=24), complex hyperplasia (n=12) and endometrial carcinoma (n=24). **Results:** An increasing expression of Cyclin D1 was seen from simple hyperplasia to carcinoma endometrium. Moreover, complex hyperplasia showed the maximum positivity for Cyclin D1. **Conclusion:** Cyclin D1 overexpression is an early event in development of endometrial neoplasia. Also maximum deregulation occurs at the stage of complex hyperplasia. Both simple and complex hyperplasia need to be followed up closely due to their premalignant potential.

**KEYWORDS:** Endometrial carcinoma, cyclin D1, simple hyperplasia, complex hyperplasia.**INTRODUCTION**

Endometrial hyperplasia involves the proliferation of endometrial glands resulting in a greater than normal gland-to-stroma ratio which leads to varying degrees of architectural complexity and cytologic atypia. It is clinically significant as it can progress to endometrial adenocarcinoma.<sup>[1]</sup> The most commonly used classification system for Endometrial Hyperplasia is the World Health Organization (WHO) 1994 classification system, in which architectural disruption and cytological atypia are used to identify four types of endometrial hyperplasia, including simple or complex hyperplasia with or without atypia. Cytological atypia is of great consideration, not only for the progression to endometrial carcinoma, but also for the risk of a coexistent endometrial carcinoma in women with endometrial hyperplasia.<sup>[2]</sup> In the new WHO classification of endometrial hyperplasia 2014, only two categories are included namely hyperplasia without atypia and atypical hyperplasia/endometrioid intraepithelial neoplasia.<sup>[3]</sup>

The risk of progression of endometrial hyperplasia to endometrial carcinoma varies from 1% to 29% (1% in Simple hyperplasia without atypia, 3% in Simple hyperplasia with atypia, 8% in complex hyperplasia without atypia and 29% in complex hyperplasia with atypia).<sup>[4]</sup> Endometrial stromal invasion, increased degrees of nuclear atypia, mitotic activity, cellular stratification, and epithelial necrosis are associated with a greater likelihood of endometrial carcinoma.<sup>[5]</sup>

Various genetic alterations are known to be associated with endometrial hyperplasia like microsatellite instability and defects in DNA mismatch repair genes (MLH1, MSH2, MSH6, PMS2) associated with the Lynch Syndrome. PTEN (Phosphatase and tensin homologue) tumor suppressor gene mutations have also been found in 55% of hyperplasia cases and 83% of hyperplasia cases once it has progressed to endometrial cancer.<sup>[6]</sup>

Cyclin D1 belongs to a family of three closely related D-type cyclins, Cyclin D1, D2 and D3. These three proteins

are expressed in proliferating cells in an overlapping and redundant fashion. D-cyclins selectively control cell cycle progression by activating their cyclin dependent kinases (CDK) partners, CDK4 and CDK6, which phosphorylate retinoblastoma (RB) protein and move the cell cycle into the S-phase through the G1-phase. Hence, Cyclin D1 is considered an essential sensor and activator of cell cycle initiation and progression.<sup>[7]</sup>

Cyclin D1 over expression may be one of the several mechanisms involved in endometrial neoplasia. Proliferative endometrial glands and stroma, even when actively mitotic do not over express Cyclin D1. Over expression of Cyclin D1 has been observed in endometrial carcinoma.<sup>[8]</sup> In the present study we evaluated the pattern of cyclin D1 expression in hyperplastic endometrium and endometrial carcinoma.

### MATERIALS AND METHODS

The present study was conducted over a period of one year in the Department of Pathology, Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonapat. A total of 60 cases including twenty four cases of simple hyperplasia and twelve cases of complex hyperplasia and twenty four cases of endometrial carcinoma were considered in the study. Cases included were ranging in age from 27 years to 78 years with the maximum number of cases in the age group of 41-50 years. The study material comprised of hysterectomy and endometrial biopsies/curettings specimen. Tissues were routinely fixed in buffered formalin and embedded in paraffin wax followed by routine haematoxylin and eosin staining. Standard procedure for Immunohistochemistry for Cyclin D1 Using Flex Monoclonal Rabbit Human Cyclin Clone EP 12 was performed. From selected blocks 3-4 micron thick sections were taken on polylysine coated slides followed by deparaffinisation with xylene and hydration in alcohols. Heat induced epitope retrieval using Tris /EDTA PH 9.0 buffer was done using pressure cooker method for 15 minutes. Endogenous peroxidase inactivation using one drop of 3% aqueous hydrogen peroxide was done for 5 minutes. Incubation with primary antibody anti human cyclin D1 clone EP12 was done for 30 minutes at room temperature in a moist chamber. Incubation with secondary antibody horseradish peroxidase was done for 30 minutes. Incubation with freshly prepared diaminobenzidine (DAB) chromogen was done for 5 minutes. Counter staining was performed using hematoxylin. The slides were then subjected to dehydration, clearing, and mounting.

### Interpretation of Immunohistochemistry

Cyclin D1 staining was evaluated in the glandular epithelium component. Two parameters were taken into consideration: the intensity of nuclear staining and the extent (percentage of positive cells). The intensity of nuclear staining was graded as no staining (0), weak (1+), moderate (2+), or strong (3+). The extent was semi-quantitatively estimated with a range of 0% to 100%.

Percentage was estimated by counting at least 50 nuclei and then calculating the ratio of immune-reactive nuclei to total number of nuclei multiplied by 100; percentages were rounded to the nearest 10%. When less than 10% of cells were positive, a score of 0 was used, 11% to 30% cell positivity was scored as 1+, 31% to 60% positivity was scored as 2+, and more than 60% positive cells were labelled as 3+ grade.

### Data Analysis

Data was analysed using the statistical package SPSS version 22. Chi-square was used to analyse the data and *P* value was calculated wherever required. *P* value of 0.05 or less was considered as statistically significant.

### RESULTS

**Distribution of cases:** Out of total cases, twenty four cases were of simple hyperplasia and endometrial carcinoma each along with twelve cases of complex hyperplasia. Out of twelve cases of complex hyperplasia, five cases were complex hyperplasia without atypia and seven cases were complex hyperplasia with atypia.

**Demographics:** Cases included were ranging in age from 27 years to 78 years with the maximum number of cases in the age group of 41-50 years. The mean age for simple hyperplasia was 43.9 years with maximum number of cases in the age group of 41-50 years. For complex hyperplasia, the mean age of 52.3 years was calculated while maximum number of cases fall in the age group of 41-50 years. The maximum number of cases with diagnosis of endometrial carcinoma were in the age group 61-70 years and the mean age calculated was 59.4 years.

**Clinical presentation:** The most common presenting feature was vaginal bleeding which was found in twenty three cases each of simple hyperplasia and endometrial carcinoma and in all the twelve cases of complex hyperplasia. One case of simple hyperplasia presented with uterovaginal prolapse. One case of endometrial carcinoma presented with pelvic mass.

### Cyclin D1 immunostaining

Out of twenty four cases of simple hyperplasia of endometrium, extent of staining in ten cases (41.7%) was graded 2+. Eight cases (33.3%) had zero extent out of which seven cases showed no staining at all while one case showed less than 10% nuclear staining. Three cases each (12.5%) had an extent of grade 1+ and 3+ respectively. Intensity of staining was found to be of grade 1+ in ten cases (41.7%), grade 2+ in five cases (20.8%) and grade 3+ in two cases (8.3%). Seven cases (29.2%) had zero intensity.

In case of complex hyperplasia of endometrium, eleven cases out of total twelve cases were positive. Maximum number of cases i.e. six (50%) showed an extent of 3+, followed by four cases (33.3%) of 2+ extent and one case (8.3%) of an extent of 1+. Only single case (8.3%) was

found with zero extent. In terms of intensity of staining, maximum number of cases i.e. six (50%) showed 2+ intensity. Four cases (33.3%) showed 1+ intensity while one case (8.3%) had 3+ intensity. Only one case (8.3%) was found with zero intensity.

Out of total twenty four cases of endometrial carcinoma, maximum i.e. nine cases (37.5%) showed an extent of 3+ followed by seven cases (29.2%) of 2+ extent and three cases (12.5%) with an extent of grade 1+. Five cases (20.8%) were found to have zero extent. In terms of

intensity of staining, seven cases (29.2%) each showed an intensity of 2+ and 1+ respectively whereas six cases (25%) had an intensity of grade 3+. Zero intensity was seen in four cases (16.6%).

In terms of extent percentage positivity of Cyclin D1 expression, (as shown in table 1) out of total cases, forty six i.e. 76.6% showed Cyclin D1 positivity. These included eleven cases (91.6%) of complex hyperplasia, Sixteen cases (66.7%) cases of simple hyperplasia and nineteen (79.2%) cases of carcinoma endometrium.

**Table 1: Percentage Positivity of Cyclin D1 expression in different endometrial lesions.**

Diagnosis	No. of Cases (n)	Cyclin D1 positive	Percentage Positivity (%)
Simple hyperplasia	24	16	66.7
Complex hyperplasia	12	11	91.6
Endometrial Carcinoma	24	19	79.2
<b>Total</b>	<b>60</b>	<b>46</b>	<b>76.6</b>

**Table 2: Extent of Cyclin D1 expression in different endometrial lesions.**

Diagnosis	0	1+	2+	3+
Simple Hyperplasia endometrium	08	03	10	03
Complex Hyperplasia endometrium	01	01	04	06
Endometrial carcinoma	05	03	07	09

Comparison of extent of Cyclin D1 expression: In all the three groups, simple vs complex hyperplasia ( $p=0.102$ ), simple vs carcinoma ( $p=0.329$ ) and complex vs

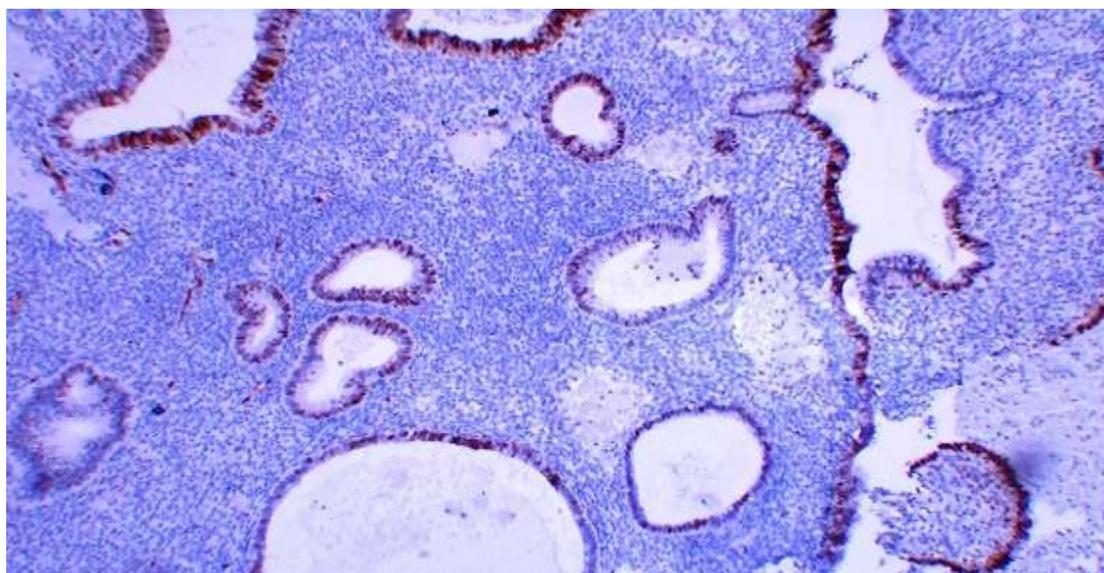
carcinoma ( $p=0.342$ ), no significant difference in extent of Cyclin D1 expression was found using Chi square test.

**Table 3: Intensity of Cyclin D1 expression in different endometrial lesions.**

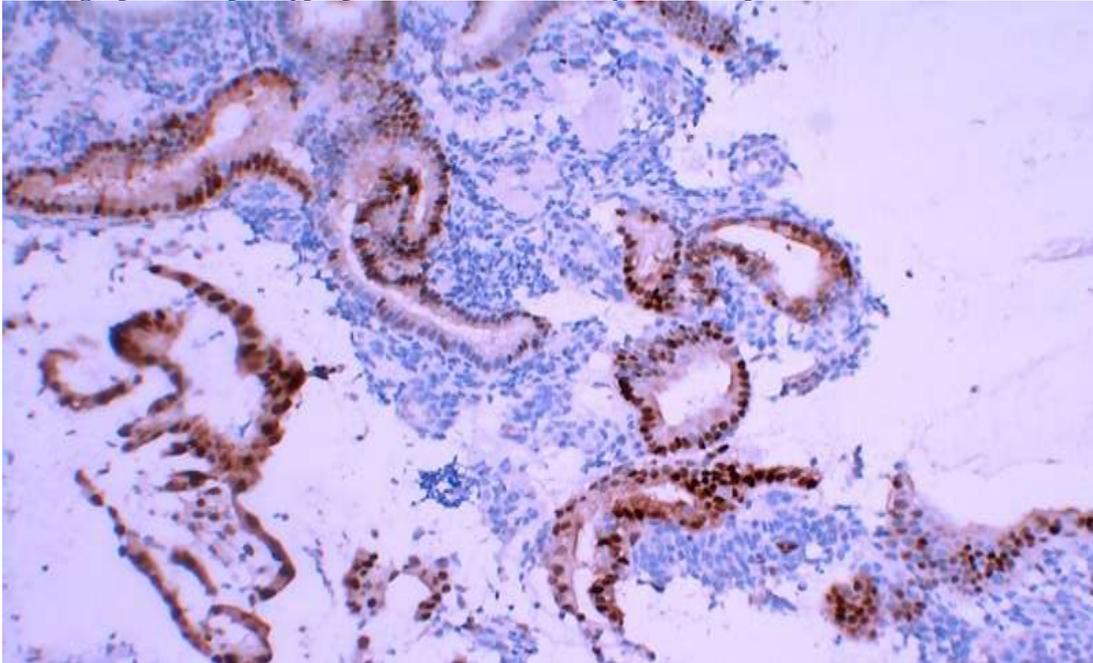
Diagnosis	0	1+	2+	3+
Simple Hyperplasia endometrium	07	10	05	02
Complex Hyperplasia endometrium	01	04	06	01
Endometrial carcinoma	04	07	07	06

Comparison of intensity of Cyclin D1 expression: In all the three groups, simple vs complex ( $p=0.156$ ), simple vs carcinoma ( $p=0.302$ ) and complex vs carcinoma

( $p=0.495$ ), no significant difference in intensity of Cyclin D1 expression was found using Chi square test.



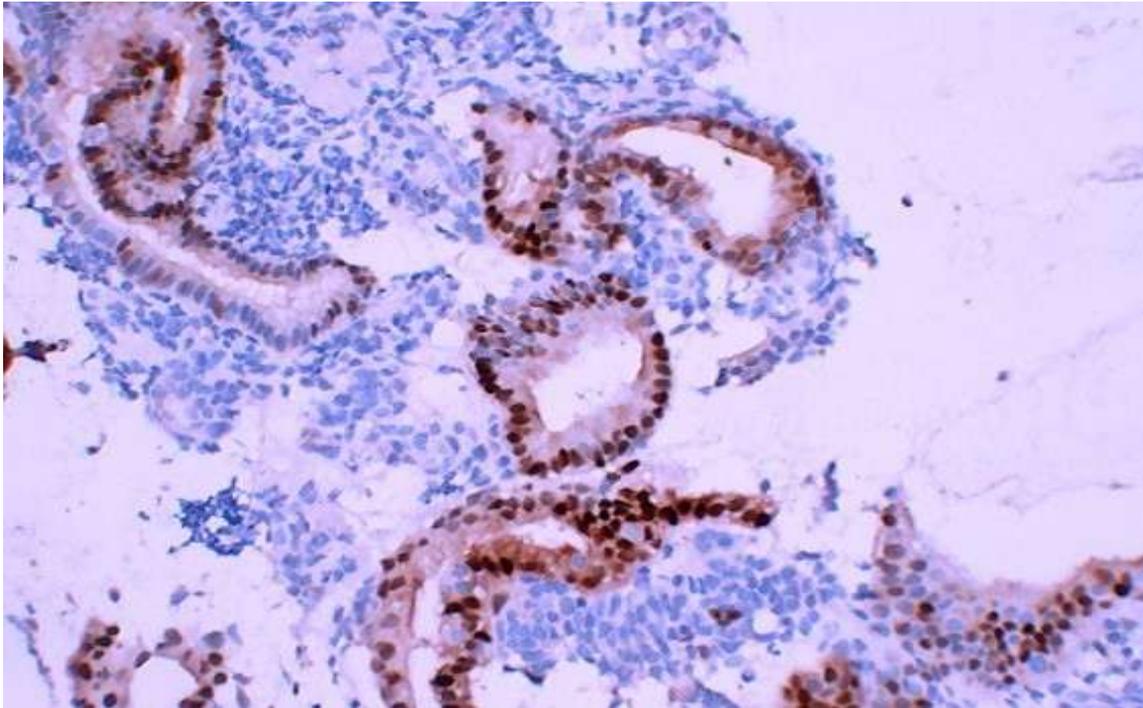
**Photomicrograph 1: Simple Hyperplasia Endometrium: Cyclin D1 Expression Show Extent 2+ (Ihc 100x).**



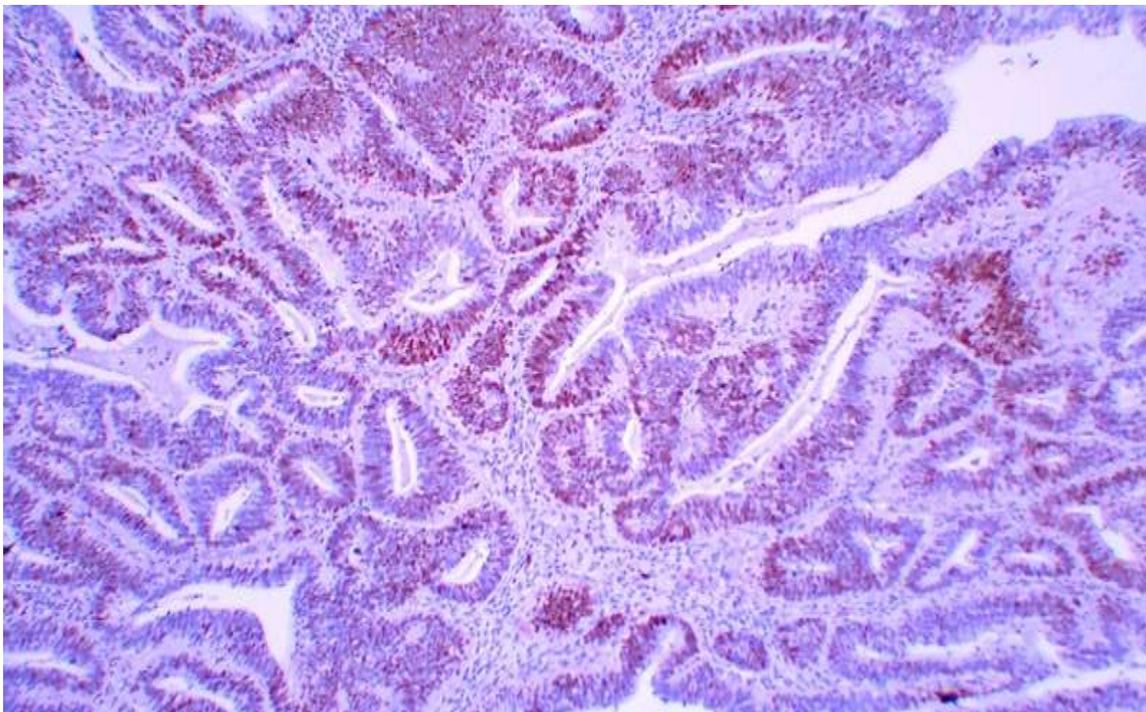
**Photomicrograph 2: Simple Hyperplasia Endometrium: Cyclin D1 Expression Show Extent 3+ (Ihc 100x).**



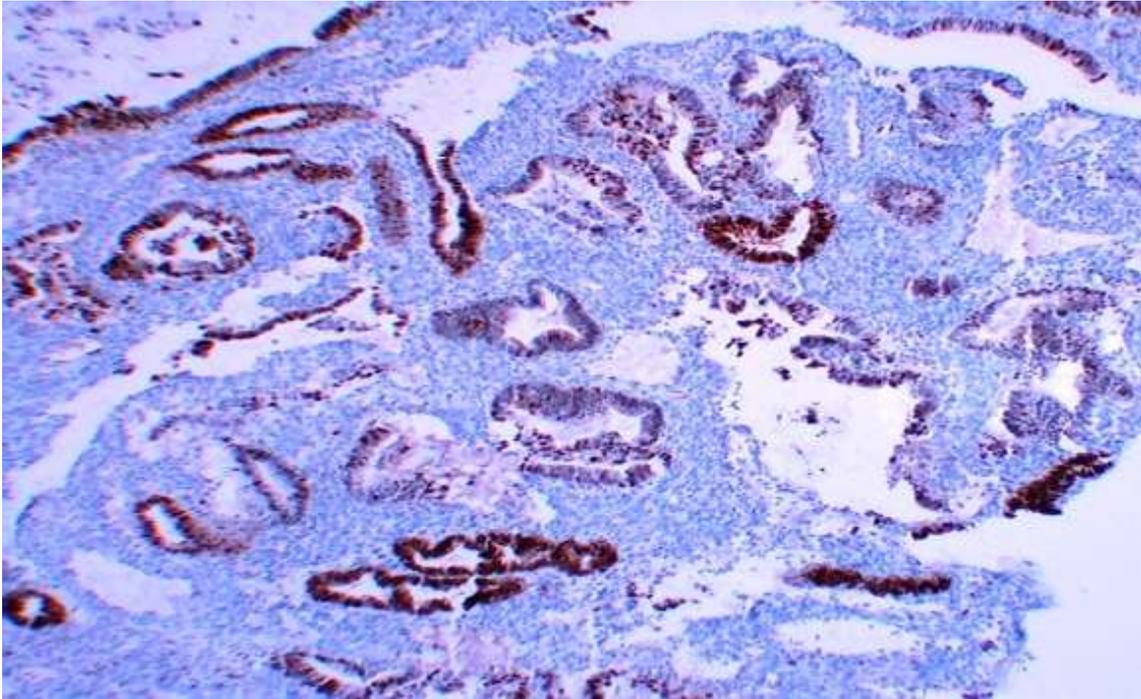
**Photomicrograph 3: Simple Hyperplasia Endometrium: Cyclin D1 Expression Show Intensity 2+ (Ihc 200x).**



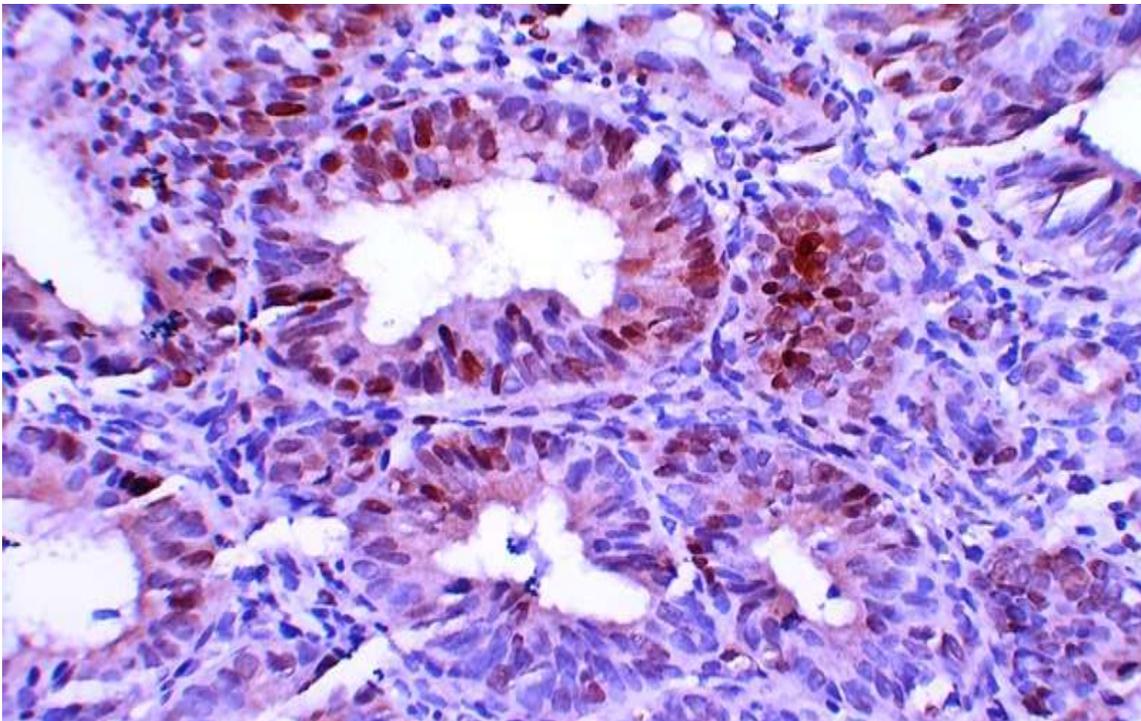
**Photomicrograph 4: Simple Hyperplasia Endometrium: Cyclin D1 Expression Show Intensity 3+ (Ihc 200x).**



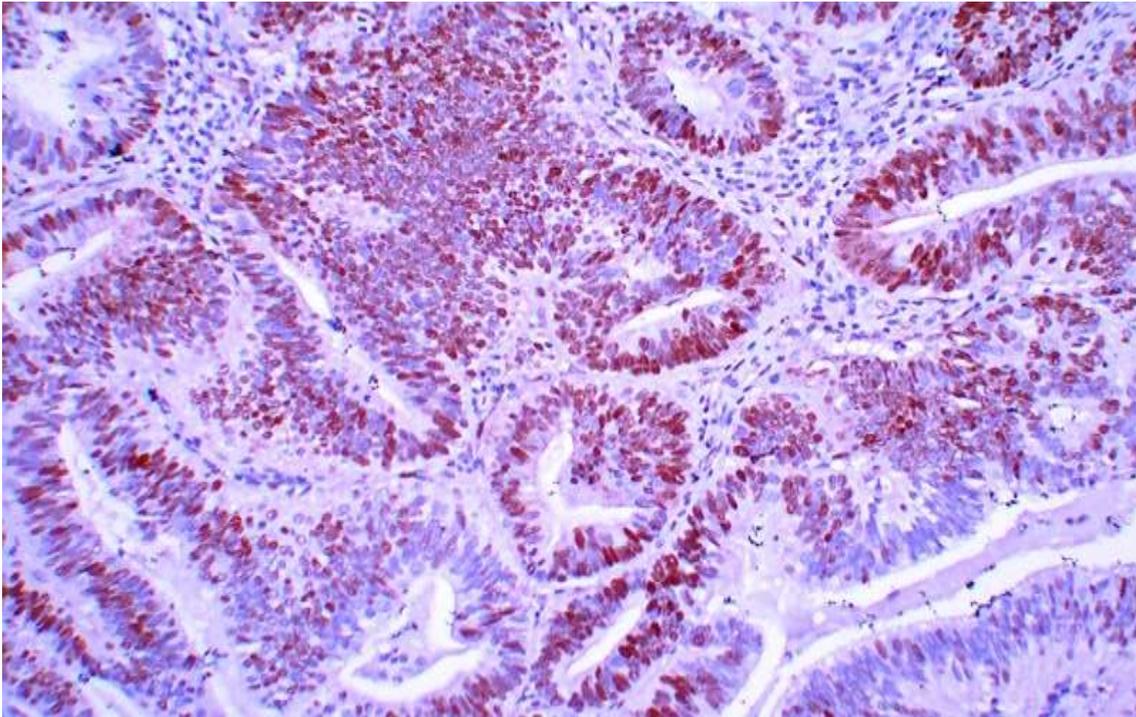
**Photomicrograph 5: Complex Hyperplasia: Cyclin D1 Expression Showing Extent 2+ (Ihc 100 X).**



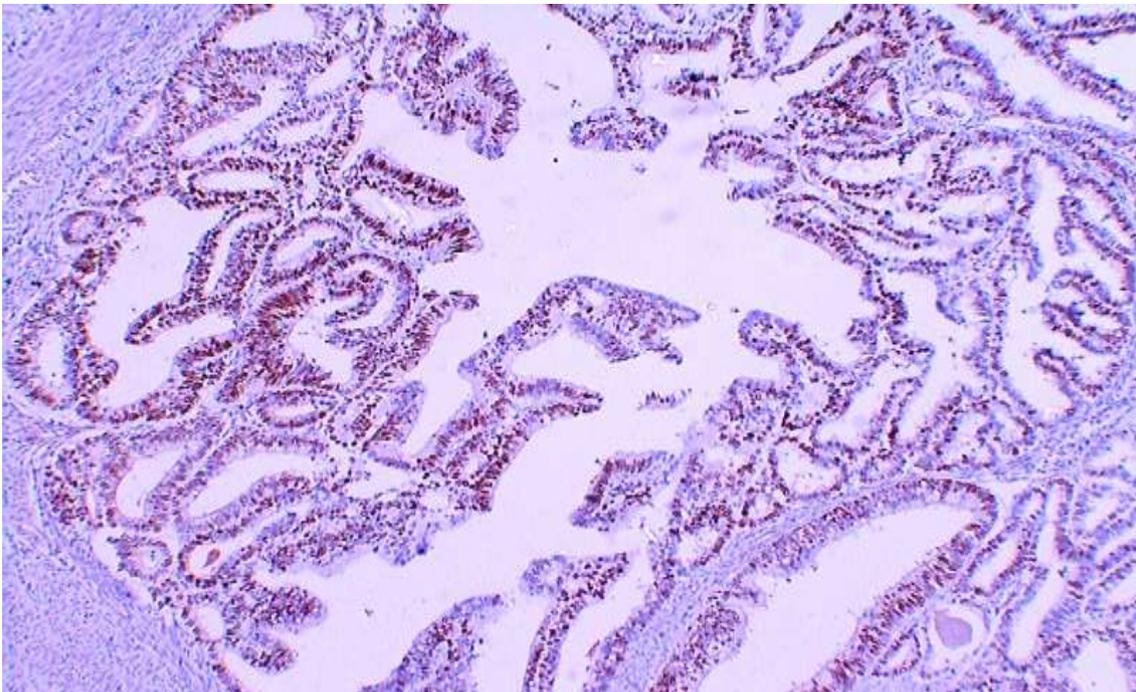
**Photomicrograph 6: Complex Hyperplasia: Cyclin D1 Expression Showing Extent 3+ (Ihc 40x).**



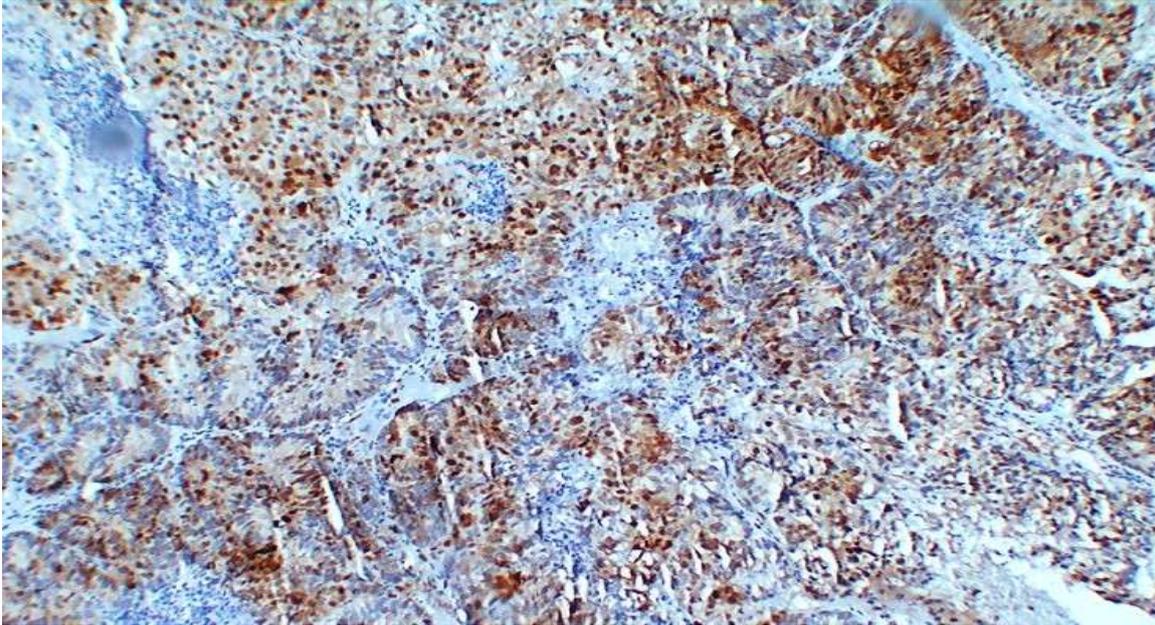
**Photomicrograph 7: Complex Hyperplasia: Cyclin D1 Expression Showing Intensity 2+ (Ihc 400x).**



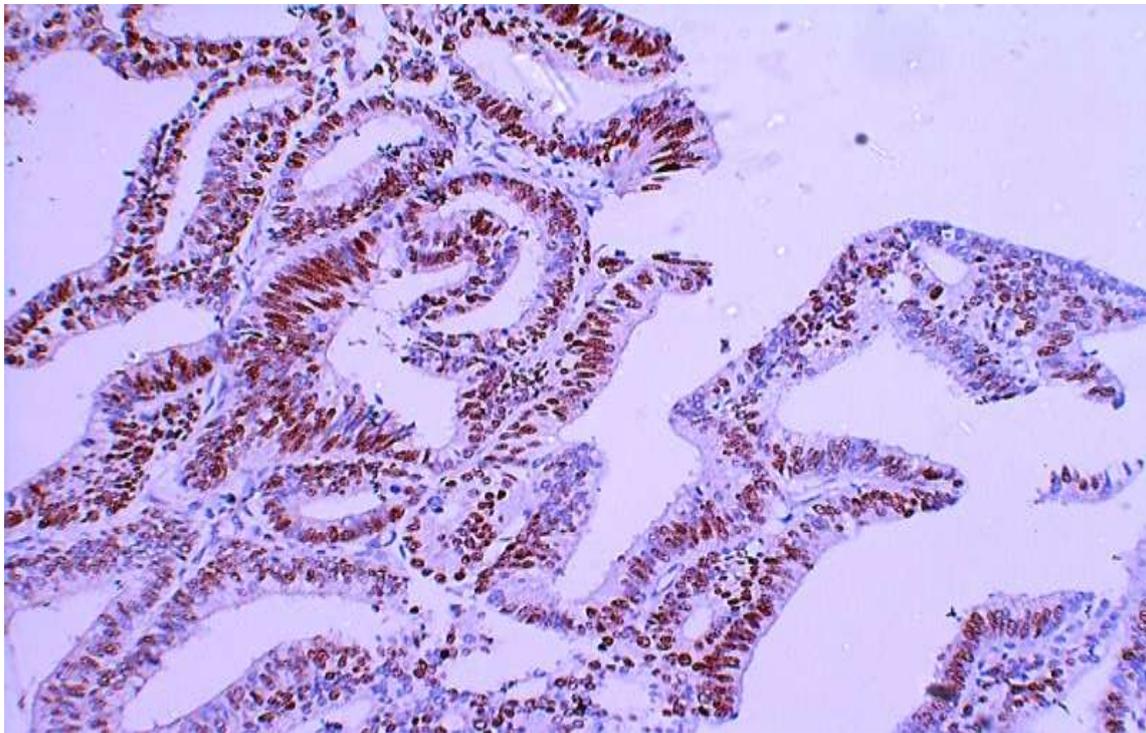
**Photomicrograph 8: Complex Hyperplasia: Cyclin D1 Expression Showing Intensity 3+ (Ihc 200x).**



**Photomicrograph 9: Endometrial Adenocarcinoma: Cyclin D1 Expression Showing Extent 3+ (Ihc 100x).**



Photomicrograph 10: Endometrial Adenocarcinoma: Cyclin D1 Expression Showing Extent 3+ (Ihc 100x).



Photomicrograph 11: Endometrial Adenocarcinoma: Cyclin D1 Expression Showing Intensity 3+(Ihc 200x).

#### DISCUSSION

The most probable hypothesis of endometrial cancer (EC) etiology is based on the prolonged estrogen stimulation of endometrium of genetically prone women, characterized by histopathologic lesions designated as endometrial hyperplasia (EH) with continuum of changes that evolve to endometrial carcinoma.

Other mechanisms of endometrial carcinogenesis include mutations in p53 and PTEN tumor suppressor genes. Several authors have documented overexpression of Cyclin D1 in endometrial hyperplasia and carcinoma.

The positivity of Cyclin D1 in case of simple hyperplasia has been reported as zero percent in the studies conducted by Tsuda *et al*, Chaudhary *et al*, and Kala *et al*.<sup>[9-11]</sup> In the study by Nishimura *et al*, 22.2% positivity for Cyclin D1 in cases of simple hyperplasia was noted while Suri *et al* observed 50% positivity in cases of simple hyperplasia. Quddus *et al* reported 57% positivity in simple hyperplasia.<sup>[7,12-13]</sup> In the present study, we found out that sixteen out of total twenty four cases (66.7%) of simple hyperplasia were positive for Cyclin D1.

Cyclin D1 positivity in complex hyperplasia has been reported as zero percent by Tsuda et al. Nishimura et al reported 27.2% positivity while Chaudhary et al reported 33% positivity and Liang et al reported 49% positivity for Cyclin D1 in case of complex hyperplasia. Suri et al and Quddus et al reported 63.6% and 71% positivity respectively in cases of complex hyperplasia.<sup>[7,10,12,14]</sup> In the present study, complex hyperplasia showed 91.6%(11/12) positivity for Cyclin D1 resembling the study by Kala et al who observed 100% positivity for Cyclin D1 in cases of complex hyperplasia.<sup>[11]</sup>

According to previously done studies, Cyclin D1 expression in carcinoma has varied from 10% in the study by Tsuda et al to 85.71% as observed by Suri et al. In our study we found out 79.2% positivity for Cyclin D1 in case of endometrial carcinoma. Our findings bear close resemblance to study by Suri et al.<sup>[9,12]</sup> In our study, complex hyperplasia showed the maximum positivity. These findings support the significance of complex hyperplasia as a preneoplastic lesion and suggest that to some extent simple hyperplasia is also precancerous.

In the study by Rahul Quddus et al over expression of cyclin D1 increased significantly from normal endometrium to hyperplasia.<sup>[12]</sup> Choudhary et al analysed that there was statistically significant difference in the extent and intensity of cyclin D1 immunoreactivity between endometrial carcinoma and simple hyperplasia but there was no difference in extent and intensity of cyclin D1 expression between complex hyperplasia and endometrial carcinoma, concluding that cyclin D1 may be an early event in endometrial carcinogenesis.<sup>[10]</sup>

Results of study by Nishimura et al suggest that high expression of cyclin D1 may be an early event of carcinogenesis of endometrial carcinoma.<sup>[7]</sup> The inference of the study by Kala et al was that Cyclin D1 expression is a quantitative molecular dysregulation that increases progressively from complex hyperplasia to carcinoma of the endometrium.<sup>[11]</sup> In the study by Suri et al, an increasing gradient of Cyclin D1 expression was noted in endometrial glands from normal endometrium to hyperplasia to carcinoma concluding that Cyclin D1 overexpression is an early event in endometrial carcinogenesis.<sup>[12]</sup>

In the present study, Cyclin D1 is maximally expressed in cases of complex hyperplasia. Thus, our study supports the hypothesis that endometrial hyperplasia is a premalignant condition and maximum the de regulation is at the complex hyperplasia state suggesting that cyclin D1 over expression may be an early event in the endometrial carcinogenesis.

## CONCLUSION

Cyclin D1 overexpression may be an informative biomarker to recognize precancerous endometrial lesions thereby helping in their surgical management. In our study, apart from high expression of Cyclin D1 in

complex hyperplasia, we observed similar results in cases of simple hyperplasia. Keeping this in view, we suggest that patients with endometrial hyperplasias of all types should be followed up for early detection of transformation into endometrial carcinoma.

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