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Research Article

IMMUNOHISTOCHEMICAL EXPRESSION OF CYCLIN D1 IN ENDOMETRIAL DISORDERS

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Endometrial carcinoma is a common gynaecological malignancy all over the world. It typically occurs in elderly women, approximately 80% are post menopausal at the time of diagnosis[1]. Endometrial carcinoma can be broadly divided in to two groups, based on differences in their clinical presentation, behaviour and pathogenesis. Endometrial carcinoma is often preceded by characteristic histopathologic lesions designated as endometrial hyperplasia.[2] Currently it is accepted that there is continuum of changes that evolve to endometrioid carcinoma.[3]
Hyperplasia is usually associated with exogenous estrogen stimulation, and thus estrogen is considered as an endometrial carcinogen[4,5,6].Other mechanisms of endometrial carcinogenesis include mutations in p53 and PTEN tumor suppressor genes and over expression of cyclin D1 .Over expression of cyclin D1 has been observed in endometrial carcinoma[7].
The cyclin D1 proto-oncogene is an important regulator of G1 to S-phase transition and an important cofactor for several transcription factors in numerous cell types[8].
This study was done to investigate the pattern of cyclin D1 expression in normal,, metaplastic, and neoplastic endometrium, and thereby evaluate the possibility of a role in the genesis of endometrial neoplastic and preneoplastic lesions.
Aims and Objectives
To investigate the role of cyclin D1 in simple hyperplasia, complex hyperplsia and endometrial carcinoma.
To recognize the subset of endometrial lesions that may be precancerous.
To exclude the lesions that may be responsive to harmonal manipulation.
To know the prognosis and treatment by drug induced cyclin D1 chemo- ablation.
Materials and Methods: This study was conducted in the department of pathology of hostipals attached to the osmania medical college and Gandhi Medical College . This is a restrospective study done for a period of 2 years . A total of 60 uterine resection and endometrial biopsy specimens from 60 patients were studied. Cases for the study were selected on the basis of adequacy of tissue material on paraffin embedded blocks ensuring minimal amount of necrosis and haemorrhage. They were examined and categorized under the different groups on haematoxylin and Eosin section. Immunohistochemical stains were performed on freshly cut 4microns thick paraffin embedded tissue section. Cyclin D1 staining was evaluated in the glandular epithelium component and in the superficial epithelium component (except carcinoma cases) in each group of, simple hyperplasia, complex hyperplasia and adenocarcinoma. No attempt was made to separate complex hyperplasias as those with and without nuclear atypia.
Results: The present study was conducted to evaluate the expression of cyclin D1 in simple hyperplasia, complex hyperplasia and endometrial carcinoma. None of the cases of simple hyperplasia showed cyclin D1 immunopositivity. 6 out of 15 (40%) cases of complex hyperplasia.
Conclusion: Endometrial carcinoma is often preceded by characterstic histopathological lesions known as endometrial hyperplasia. Over expression of cyclin D1 in endometrial glands increases progressively in intensity and extent from simple hyperplasia to complex hypjerplasia and carcinoma, suggests that it may play a role in endometrial carcinogenesis. The present study support the significance of complex hyperplasia as a precancerous lesion, but apparently do not support the notion that simple hyperplasia is precancerous.

INTRODUCTION

Endometrial cancer refers to several types of malignancy which arise from the endometrium, or lining of the uterus. Endometrial cancers are the most common gynecologic cancers. The most common sub type endometrioid adenocarcinoma, typically accurs within a few decades of menopause, is associated with excessive estrogen exposure, often develops in the setting of endometrial hyperplasia, and presents most often with vaginal bleeding[2,3].

Most endometrial cancers are carcinomas (usually adenocarcinomas), meaning that they originate from the single layer of epithelial cells which line the endometrium and form the endometrial glands[4]. There are many microscopic subtypes of endometrial carcinoma, including the common endometriod type, in which the cancer cells grow in patterns reminiscent of normal endometrium, and the far more aggressive papillary serous carcinoma and clear cell endometrial carcinomas[11]. Some authors have proposed that endometrial carcinomas be classified into two pathogenetic groups.[9]

Type I: These cancers occur most commonly in pre- and peri-menopausal women, often with a history of unopposed estrogen exposure and/or endometrial hyperplasia. They are often minimally invasive into the underlying uterine wall, are of the low grade endometrioid type, and carry a good prognosis.

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Type II: These cancers occur in older, post-menopausal women, are more common in African-Americans, are not associated with increased exposure to estrogen, and carry a poorer prognosis. They include: the high grade endometrioid carcinoma, the uterine papillary serous carcinoma, the uterine clear cell carcinoma.

In contrast to endometrial carcinomas, the uncommon endometrial stromal sarcomas are cancers which originate in the non-glandular connective tissue of the endometrium. Uterine carcinosarcoma, formerly called Malignant mixed mullarian tumour, is a rare uterine cancer which contains cancerous cells of both glandular and sarcomatous appearance - in this case, the cell of origin is unknown.

Endometrial carcinoma is surgically staged using the FIGO cancer staging system.

- Stage IA: tumor limited to the endometrium
- Stage IB: invasion of less than half the myometrium
- Stage IC: invasion of more than half the myometrium
- Stage IIA: endocervical glandular involvement only
- Stage IIB: cervical stromal invasion
- Stage IIIA: tumor invades serosa or adnexa, or malignant peritoneal cytology
- Stage IIIB: vaginal metastasis
- Stage IIIC: metastasis to pelvic or para-aortic lymph nodes
- Stage IVA: invasion of the bladder or bowel
- Stage IVB: distant metastasis, including intra abdominal or inguinal lymph nodes.

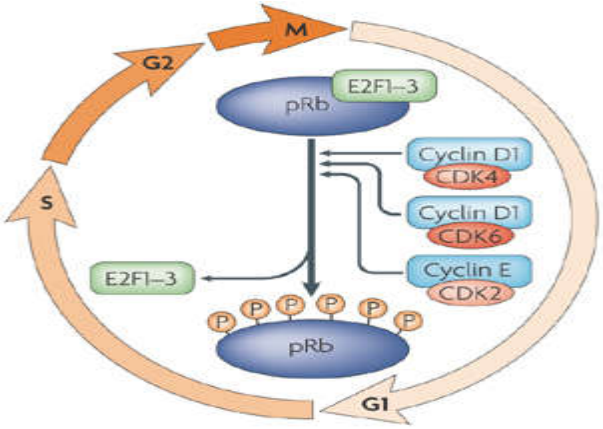
The histopathology of endometrial cancers is highly diverse. The most common finding is a well differentiated endometrioid adenocarcinoma, which is composed of numerous, small, crowded glands with varying degrees of nuclear atypia, mitotic activity, and stratification. This often appears on a background of endometrial hyperplasia. Frank adenocarcinoma may be distinguished from atypical hyperplasia by the finding of clear stromal invasion, or "back-to-back" glands which represent nondestructive replacement of the endometrial stroma by the cancer. With progression of the disease, the myometrium is infiltrated[10]. However, other subtypes of endometrial cancer exist and carry a less favorable diagnosis such as the uterine papillary serous carcinoma and the clear cell carcinoma

Cyclin D1

Cyclin D is a member of the cyclin family, and comprises three closely related proteins, cyclin D1, D2 and D3. These are expressed in an overlapping, redundant fashion in all proliferating cell types and collectively control the progression of cells through the cell cycle[2]. Since the D-cyclins are essential to cell division, they are also dysregulated in cancer.

Cyclin D1 is an important regulator of cell cycle progression and can function as a transcriptionl co-regulator. The overexpression of cyclin D1 has been linked to the development and progression of cancer. Deregulated cyclin D1 degradation appears to be responsible for the increased levels of cyclin D1 in several cancers. Recent findings have identified novel mechanisms involved in the regulation of cyclin D1 stability. A number of therapeutic agents have been shown to induce cyclin D1 degradation. The therapeutic ablation of cyclin D1 may be useful in the treatment and prognosis of carcinoma.

The cyclin D1 proto-oncogene is an important regulator of G1 to S-phase transition in numerous cell types from diverse tissues. Binding of cyclin D1 to its kinase partners, the cyclin dependent kinases 4 and 6 (CDK4\6) results in the formation of active complexes that phosphorylate the Retinoblastoma tumor suppressor protein (RB)[12].



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Hyperphosphorylation of RB results in the release of RB-sequesterd E2F transcription factors and the subsequent expression of genes required for entry into S-phase. More recently, cyclin D1 has also been shown to act a cofactor for several transcription factors. Initial studies indicated that cyclin D1 is localized predominantly in the nucleus of asynchronously growing cells . During cell cycle progression, protein levels of the cyclin begin to rise early in G1, prior to its rapid nuclear export and degradation within the cytoplasm. Interestingly, the nuclear export and\or degradation of cyclin D1 is required for S-pahse progression as failure to remove the cyclin results in G1 arrest.[13,14]

Overexpression of cyclin D1 is associated with mantle cell lymphoma,[15]squamous cell carcinoma of the uterine cervix,[16] ovarian carcinoma,[17] breast cancer,[18] transitional cell carcinoma of the urinary bladder,[19] and head and neck squamous cell carcinoma.[20]

MATERIALS AND METHODS

This study was conducted in the department of pathology of hostipals attached to the osmania medical college and Gandhi Medical college. This is a restrospective study done for a period of 2 years.

A total of 60 uterine resection and endometrial biopsy specimens from 60 patients were studied. Cases for the study were selected on the basis of adequacy of tissue material on paraffin embedded blocks ensuring minimal amount of necrosis and haemorrhage. They were examined and categorized under the following groups on haematoxylin and Eosin section.

Normal proliferative and secretory endometrium, 60 control cases, simple hyperplasia 25 cases, complex hyperplasia 15 cases and endometrial carcinoma 20 cases were analysed.
Complex hyperplasia, both typical and atypical, were grouped together, as the rate of reproducibility for diagnosis of these lesions is low.

Immunohistochemical studies

Immunohistochemical stains were performed on freshly cut 4microns thick paraffin embedded tissue section. Slides with representative tissue were deparaffinized and brought to water by successive changes in graded alcohol. For antigen retrieval, slides were pressure cooked in citrate buffer at pH. 6.0, transferred to Tris buffer and endogenous peroxidase quenching was done by adding 1 drop of 3% H2O2.

Sections were then washed in Tris buffer and primary antibody was added and incubated in a moist chamber overnight at 4-8 degree C.An avidin-biotin peroxidase complex (LSAB Kit, DAKO) with DAB as chromogen was used for detecting antibody biding.

Cyclin D1 staining was evaluated in the glandular epithelium component and in the superficial epithelium component (except carcinoma cases) in each group of, simple hyperplasia, complex hyperplasia, and adenocarcinoma. No attempt was made to separate complex hyperplasias as those with and without nuclear atypia

Two parameters were taken into consideration, the intensity of nuclear staining and the extent (percentage of positive cells). Fields for calculating percentage of immunoreactive nuclei were selected based on best tissue preservation and, where there was less artifact, wrinkling or folding. Since the immunoreactivity may not be uniform among nuclei in any given case, we determined grade as the most frequently observed pattern.

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%. Percentages were estimated by counting 50 nuclei and then establishing the ratio of immunoreactive 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, 10% to 30% cell positivity was scored as 1, 31% to 60% positivity was scored as 2, and more than 60% positive cells was labeled as 3

Statistical Analysis

The statistical analysis was based on the data distribution using a continuous range from 1% to 100% reactive cells.

All pair wise comparisons were performed by using chi-square test, the Mann-Whitney *U* test; all comparisons across multiple groups were performed by Kruskal-Wallis nonparametric 1-way analysis of variation.

RESULTS

The present study was conducted to evaluate the expression of cyclin D1 in simple hyperplasia, complex hyperplasia and endometrial carcinoma. None of the cases of simple hyperplasia showed cyclin D1 immunopositivity. 6 out of 15 (40%) cases of complex hyperplasia also negative.

Table 1 Distribution of total cases with cyclin D1 immunostaining.

	Number of cases	Positive %	Negative %
Simple hyperplasia	25 (41.7%)	0/25 (0%)	25/25 (100%)
Complex hyperplasia	15(25%)	6/15 (40%)	9/15 (60%)
Endometrial carcinoma	20 (33.3%)	12/20 (60%)	8/20 (40%)

12 out of 20 cases (60%) casees of endometrial carcinoma showed positive cyclin D1 immuno reactivity, in which 8 out of 12 (66.6%) cases were 3+ in extent of cycline D1 positivity. Analysis of extent of Cyclin D1 immuno reactivity showed that in endometrial carcinoma and simple hyperplasia the difference was statistically significant. (p= 0.029).

There was no difference in intensity of cyclin D1 immunoreactivity between complex hyperplasia and endometrial carcinoma.(=0.55) but there was a statistically significant diffference in intensity of cyclin D1 immuno reativity between simple hyperplasia and endometrial carcinoma. (p=0.0355)

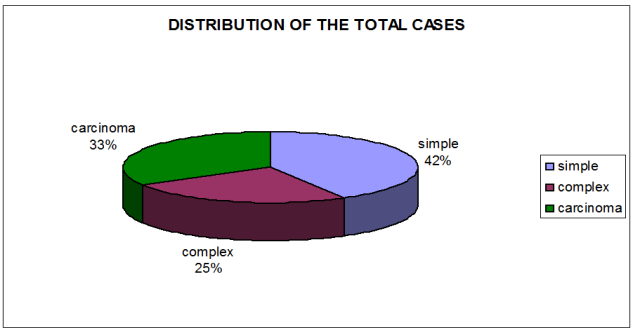


Table 2 Extent of cyclin D1 immmunoreactivity

	0	1+=	2+	3+	Total
Simple hyperplasia	25	0	0	0	25
Complex hyperplasia	9	1	2	3	15
Endometrial carcinoma	8	0	4	8	20
Total	42	1	6	11	60

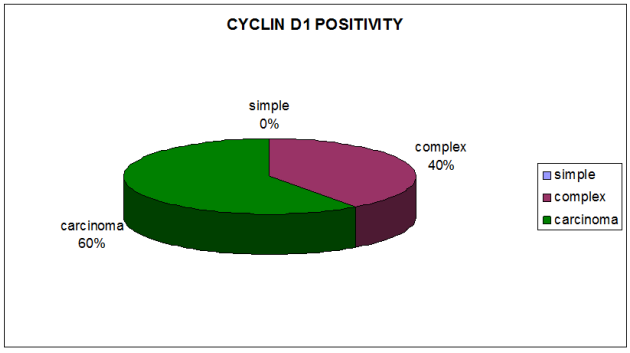
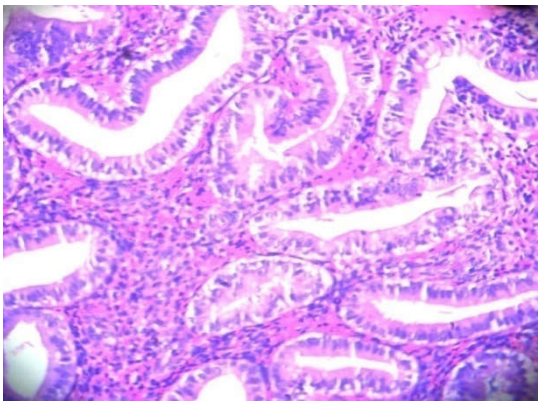
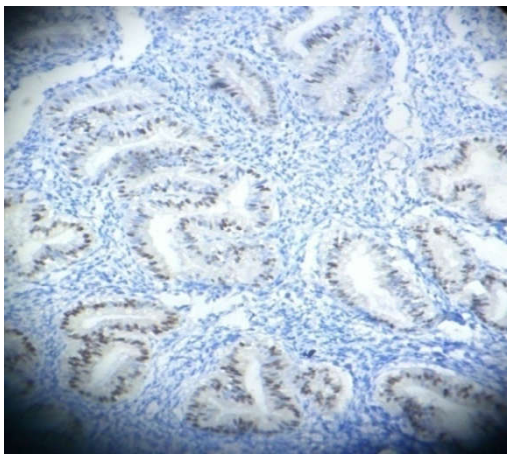


Table 3 intensitiy of cyclin D1 immunopositivity

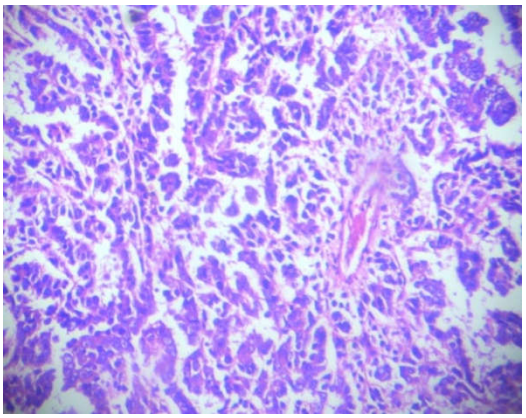
	0	*1+*	*2+*	*3+*	*Total*
Simple hyperplasia	*25*	*0*	*0*	*0*	*25*
Complex hyperplasia	*9*	*1*	*2*	*3*	*15*
Endometrial carcinoma	*8*	*1*	*2*	*9*	*20*
Total	*42*	*2*	*4*	*12*	*60*



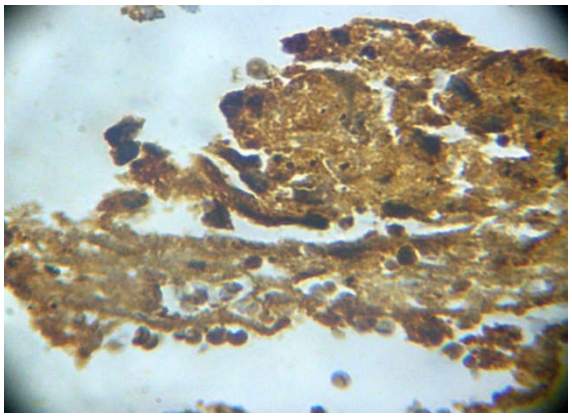
H&E Stained: Complex hyperplasia hyperplasia



Cyclin D1 positivity in Complex



H&E Stain: endometrial carcinoma

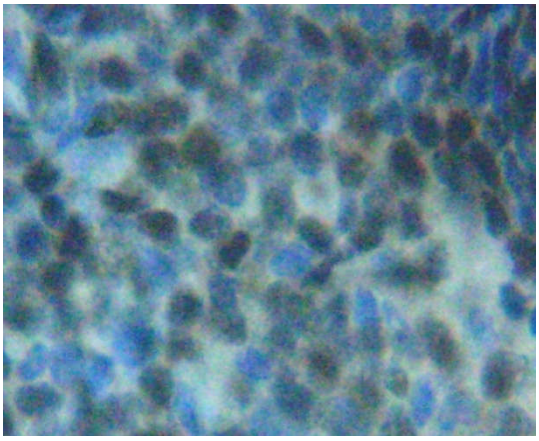


More than 60% of the cells show cyclin D1 positivity in Cancer cells

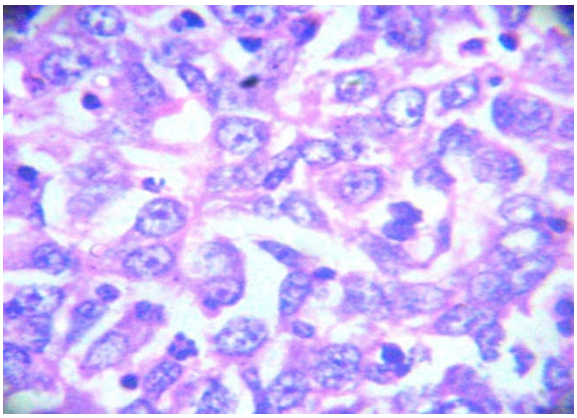
Overexpression of cyclin D1 may be associated with actual gene amplification or transcriptional dysregulation in cancers. In the study immunohistochemistry was done to demonstrate that cyclin D1 is over expressed in hyperplastic lesions, which are considered to be the precursors of endometrial adenocarcinoma.

Nikaido *et al*[18] detected cyclin D1 over expression in 40% (30/74) of cases with endometrial adenocarcinoma compared with 60% (12/20) reported in the present study.

Cyclin D1 over expression in endometrial glands increases progressively in intensity and extent from simple hyper plasia to complex hyperplasia. Since there was no difference in cyclin D1 expression between complex hyperplasia and endometrioid adenocarcinoma, it appears that the deregulation is maximal at the complex hyperplasia state and that other alterations may be responsible for the different morphologic features and behavior of complex hyperplasia and carcinoma.

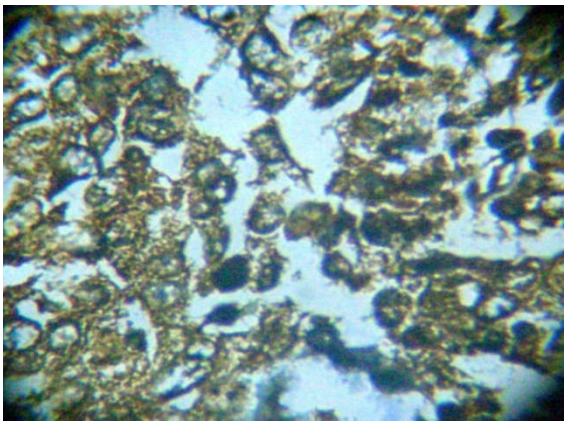


Positive control of cyclin D1



Expression

H&E stain: endometrial carcinoma.



Endometrial carcinoma with 3+ intensity and 3+ extent of cyclinD1 positivity

This pattern of expression suggests that cyclin D1 overexpression may be an early event in endometrial carcinogenesis.

The present study support the significance of complex hyperplasia as a precancerous lesion, but apparently do not support the notion that simple hyperplasia is precancerous.

Mutter *et al*[19], proposed that simple hyperplasia should not be considered as a precancerous lesion but rather a reaction to unopposed estrogen or anovulation. In this study, complex hyperplasia with and without atypia are combined together because it is well known that the rate of reproducibility for the

diagnosis of these lesions is low; similarly excluded simple hyperplasia with atypia are excluded because it is a rare lesion cyclin D1 overexpression may be an informative biomarker to recognize subsets of endometrial lesions that may be precancerous and, thus, susceptible to surgical therapy and at the same time exclude lesions that may be responsive to hormonal manipulation and might involute with progestin therapy.

The mechanisms of cyclin D1 dysregulation in endometrial neoplasia are not well defined, but it is likely that the dysregulation contributes to an increase in the proportion of cells in transition from G1 to S phase. Cyclin D1 over expression may be one of several mechanisms involved in endometrial neoplasia. proliferative endometrial glands and stroma, even when actively mitotic, do not overexpress cyclin D1.[20]

The positivity rate for cyclin D1 overexpression in endometrial hyperplasia ranges from no positivity as reported by Tsuda *et al* 6 to 83% reported by Cao *et al*. Cyclin D1 immunopositivity was 0% in simple hyperplasia, 7.1% in atypical hyperplasia as reported by ozuysal *et al* [21], 57% in simple hyperplasia and 71% in complex hyperplasia as reported by Quddus *et al*. The percentage positivity rate for cyclin D1 overexpression in endometrial carcinoma ranges from 13.8% as reported by Moreno Bueno *et al* 9 to 68% as reported by Quddus *et al*.

In the present study the cyclin D1 expression is 0% in simple hyperplasia, 40% in complex hyperplasia, 60% in endometrial carcinoma. The intensity and extent of cyclin D1 immunoreactivity is progressively increased from simple hyperplasia to complex hyperplasia and endometrial carcinoma.

DISCUSSION

Present study is conducted in the department of pathology of hospitals attached to the osmania medical college and Gandhi Medical College, for a period of 2 years. This study was conducted to evaluate the expression of cyclinD1 in simple hyperplasia, complex hyperplasia and endometrial carcinoma.

Overexpression of cyclin D1 may be associated with actual gene amplification or transcriptional dysregulation in cancers. In this study immunohistochemistry was done to demonstrate that cyclin D1 is overexpressed in hyperplastic lesions, which are considered to be the precursors of endometrial adenocarcinoma.

Nikaido *et al*[18] detected cyclin D1 overexpression in 40% (30/74) of cases with endometrial adenocarcinoma compared with 60% (12/20) reported in the present study.

Nikaido *et al* reported s that about 40% of endometrial carcinomas over expressed cyclin d1 and proposed that cyclin D1 dysregulation may have a role in endometrial carcinogesis. Quddus *et al* in 2002 reported that overexpresion of cyclin D1 increases from normal endometrium to hyperplasia and carcinoma, suggesting that it may play a role in endometrial carcinogenesis.

Cyclin D1 over expression in endometrial glands increases progressively increases in intensity and extent from simple hyperplasia to complex hyperplasia. Since there was no difference in cyclin D1 expression between complex hyperplasia and endometrioid adenocarcinoma, it appears that the deregulation is maximal at the complex hyperplasia state and that other alterations may be responsible for the different morphologic features and behavior of complex hyperplasia and carcinoma.

This pattern of expression suggests that cyclin D1 over expression may be an early event in endometrial carcinogenesis. The present study support the significance of complex hyperplasia as a precancerous lesion, but apparently do not support the notion that simple hyperplasia is precancerous.

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The positivity rate for cyclin D1 overexpression in endometrial hyperplasia ranges from no positivity as reported by Tsuda *et al* 6 to 83% reported by Chaudary *et al*[22]. Cyclin D1 immunopositivity was 0% in simple hyperplasia, 7.1% in atypical hyperplasia as reported by ozuysal *et al*[21], 57% in simple hyperplasia and 71% in complex hyperplasia as reported by Ouddus *et al*. The percentage positivity rate for cyclin D1 overexpression in endometrial carcinoma ranges from 13.8% as reported by Moreno Bueno *et al* 9 to 68% as reported by Quddus *et al*.

In the present study the cyclin D1 expression is 0% in simple hyperplasia, 40% in complex hyperplasia, 60% in endometrial carcinoma. The intensity and extent of cyclin D1 immunoreactivity is progressively increased from simple hyperplasia to complex hyperplasia and endometrial carcinoma.

Comparative studies

Quddus	Monisha Choudhury	Present
02%	0%	0%
30%	43%	40%
68%	57%	60%

CONCLUSION

Endometrial carcinoma is often preceded by characteristic histo pathological lesions known as endometrial hyperplasia. Other mechanisms of endometrial carcinogenesis include mutations in P53 and PTEN, tumour supressor genes and over expression of cyclinD1. The cyclin D1 proto-oncogene is an important regulator of G1 to S-phase transition in endometrial

neoplasia. Protooncogene encoding cyclin D1 is located on chromosome 11 in the region of 11q13.

Cyclin D1 is an important regulator of cell cycle progression and can function as a transcriptional co-regulator. The over expression of cyclin D1 has been linked to the development and progression of cancer. Over expression of cyclin D1 in endometrial glands increases progressively in intensity and extent from simple hyperplasia to complex hypjerplasia and carcinoma suggests that it may play a role in endometrial carcinogenesis.

Since there was no difference in cyclin D1 expression between complex hyperplasia and endometrioid adenocarcinoma, it appears that the deregulation is maximal at the complex hyperplasia state. This pattern of expression suggests that cyclin D1 overexpression may be an early event in endometrial carcinogenesis. The present study support the significance of complex hyperplasia as a precancerous lesion, but apparently do not support the notion that simple hyperplasia is precancerous.

Cyclin D1 may be a marker playing a role in endometrial carcinogenesis and tumour cell proliferation. Further longitudinal studies are needed to assess prognostic impact of cyclin D1 expression in endometrial hyperplasia and carcinoma. This method is not sensitive or specific enough to be treated as significant during diagnostic procedures of endometrial pathologies.

References

1. Felix AS, Yang HP, Bell DW, Sherman ME. Epidemiology of endometrial carcinoma: Etiologic importance of hormonal and metabolic influences. Adv ExpMed Biol 2017;943:3-46.
2. Crosbie EJ, Zwahlen M, Kitchener HC, Egger M, Renehan AG. Body mass index, hormone replacement therapy, and endometrial cancer risk: A meta-analysis. Cancer Epidemiol Biomarkers Prev 2010;19:3119-
3. Voskuil DW, Monninkhof EM, Elias SG, Vlems FA, van Leeuwen FE. Physical activity and endometrial cancer risk, a systematic review of current evidence. Cancer Epidemiol Biomarkers Prev 2007;16:639 48.
4. Friberg E, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: A meta-analysis. Diabetologia 2007;50:1365-74.
5. Trabert B, Wentzensen N, Felix AS, Yang HP, Sherman ME, Brinton LA, *et al.* Metabolic syndrome and risk of endometrial cancer in the united states: A study in the SEER-medicare linked database. Cancer Epidemiol Biomarkers Prev 2015;24:261-7.
6. Zhou B, Yang L, Sun Q, Cong R, Gu H, Tang N, *et al.* Cigarette smoking and the risk of endometrial cancer: A meta-analysis. Am J Med 2008;121:501-8.
7. Jaishuen A, Kunakornporamat K, Viriyapak B, Benjapibal M, Chaopotong P, Petsuksiri J, *et al.* Incidence and clinical outcomes of non-endometrioid carcinoma of endometrium: Siriraj Hospital experience. Asian Pac J Cancer Prev 2014;15:29059.
8. Qie S, Diehl JA. Cyclin D1, Cancer progression, and opportunities in cancer treatment. J Mol Med 2016;94:1313-26.
9. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From discovery to therapy. Cancer Discov 2016; 6:353-67.
10. Biliran H, Wang Y, Banerjee S, Xu H, Heng H, Thakur A, *et al.* Over expression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line. Clin Cancer Res 2005;11:6075-86.
11. Harada M, Sakai S, Ohhata T, Kitagawa K, Mikamo M, Nishimoto K, *et al.* Homeobox transcription factor NKX2-1 promotes cyclin D1 transcription in lung adenocarcinomas. Mol Cancer Res 2017;15:1388-97.
12. Kumari S, Puneet, Prasad SB, Yadav SS, Kumar M, Khanna A, *et al.* Cyclin D1 and cyclin E2 are differentially expressed in gastric cancer. Med Oncol 2016;33:40-
13. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P, *et al.* Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PLoS One 2017;12:e0188068.
14. Kurman RJ, Carcangiu ML, Herrington CS. World Health Organisation Classification of Tumours of the Female Reproductive Organs. 4th ed., vol 6. Lyon: IARC; 2014. p. 121-4.
15. Besson A, Dowdy SF, Roberts JM. CDK inhibitors: Cell cycle regulators and beyond. Dev Cell 2008; 14:159-69.
16. Kato JY, Sherr CJ. Inhibition of granulocyte differentiation by G1 cyclins D2 and D3 but not D1. Proc Natl Acad Sci USA 1993;90:11513-7.
17. Poch B, Gansauge F, Schwarz A, Seufferlein T, Schnelldorfer T, Ramadani M, *et al.* Epidermal growth factor induces cyclin D1 in human pancreatic carcinoma: Evidence for a cyclin D1-dependent cell cycle progression. Pancreas 2001;23:280-
18. Nikaido T, Li SF, Shiozawa T, Fujii S. Coabnormal expression of cyclin D1 and p53 protein in human uterine endometrial carcinomas. Cancer 1996; 78:1248 53.
19. Mutter *et al* Soslow RA, Shen PU, Chung MH, Isacson C, Baergen RN. Cyclin D1 expression in high-grade endometrial carcinomas--association with histologic subtype. *Int J Gynecol Pathol* 2000;19:329-34.
20. Nishimura Y, Watanabe J, Jobo T, Kato N, Fujisawa T, Kamata Y, *et al.* Cyclin D1 expression in endometrioid-type endometrial adenocarcinoma is correlated with histological grade and proliferative activity, but not with prognosis. Anticancer Res 2004;24:2185-91.
21. Ozuysal S, Ozturk H, Bilgin T, Filiz G. Expression of cyclin D1 in normal, hyperplastic and neoplastic endometrium and its correlation with Ki-67 and clinicopathological variables. Arch Gynecol Obstet 2005; 271:123-6.
22. Choudhury M, Bansal S. Expression of cyclin D1 in endometrial hyperplasia and endometrial carcinoma. *Indian J Pathol Microbiol*. 2007; 50(04):708–710.

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