ORIGINAL ARTICLE

Expression of Cyclin D1 in Hyperplastic and Neoplastic Endometrial Lesions

Summayya Shawana[1], Saleha Masood[2], Zamir Ali Siddiqui[3]

ABSTRACT:
Objective: To observe and evaluate the expression of Cyclin D1 in hyperplastic and neoplastic endometrial lesions.
Methodology: This .retrospective analytical study was conducted at BMSI, JPMC over a five year period i.e. from 1-1-2008 to 31-1-2012 and was based on the analysis of endometrial samples comprising of hysterectomies and curettage. 55 endometrial samples including 25 malignant endometrial lesions, 6 complex hyperplasia with atypia, 14 complex hyperplasia without atypia, 6 simple hyperplasia without atypia, and 4 normal proliferative endometrium were analyzed for results of immunohistochemical staining.
Results: 44% (11 out of 25) cases of malignant endometrial lesions showed Cyclin D1 over expression. 66.66% (4 out of 6) cases of endometrial hyperplasia with atypia and 50% (7 out of 14) cases of endometrial hyperplasia without atypia showed Cyclin D1 overexpression.
Conclusion: Cyclin D1 overexpression was seen in a significant number of well differentiated endometrial adenocarcinomas and complex hyperplasia with atypia suggesting it to be an early event in endometrial carcinogenesis.
Keywords: Endometrial carcinoma, Hyperplasia, Cyclin D1 overexpression, Atypia, Early event.

INTRODUCTION:

Endometrial carcinoma is one of the most common malignancies of the female genital tract.[1] 7,406 new cases of endometrial cancers are registered in the UK, 88,068 in the European Union and 40,102 in North America every year.[2] According to the Shaukat Khanum Cancer Hospital & Research Centre collective cancer registry report (1994-2011), malignancies of the corpus uteri comprised 3.02% of all neoplasms in adult females.[3] Endometrial carcinomas have been classified as type1 and type 2 tumors on the basis of light microscopic appearance and clinical behavior. Endometrial hyperplasia usually precedes type 1 or endometroid endometrial carcinomas. A prolonged unopposed estrogen exposure is seen to confer a 2-10 fold increased risk for endometrial carcinoma.[4] Endometrial hyperplasia have been classified according to two systems: the WHO system and the more recent EIN system.[5,6] The WHO classification comprises of four categories: simple hyperplasia, complex hyperplasia, simple hyperplasia with atypia and complex hyperplasia with atypia. The EH-EIN-CA classification was developed by the "The Endometrial Collaborative Group" and it proposes terms endometrial hyperplasia, endometrial intraepithelial neoplasia and adenocarcinoma. The terms "Endometrial hyperplasia" (EH) or "Benign Endometrial Hyperplasia" apply to diffuse architectural and proliferative changes due to excess estrogen stimulation. EIN has been defined as a clonal proliferation of architecturally and cytologically altered premalignant endometrial glands which are prone to malignant transformation to endometroid (type 1) endometrial carcinoma. Mutations of different genes like PTEN, K-ras, Cyclin D1 and â-catenin genes and microsatellite instability along with others are the common genetic alterations observed in cases of endometrial carcinoma. Cyclin D1, a member of the cyclin G1 family, controls the transition from G1 to S phase in the cell cycle. The gene for Cyclin D1, CCND1, is a proto-oncogene localized on chromosome 11q13.[7] Cyclin D1 acts as a cell cycle promoter by binding with cyclin dependent kinases 4 and 6 (CDK4/6) and phosphorylating the retinoblastoma tumor suppressor gene. This results in the release of Rb-bound E2F members and the subsequent expression of genes required for entry into the S-phase. Further studies have shown Cyclin D1 to be an important cofactor for several transcription factors. This function of Cyclin D1 is independent of its CDK activity.[8,9] Cyclin D1 overexpression has been observed as a potential biomarker for precancerous and cancerous endometrial lesions,[10,11] while few studies contradict this finding.[12] However it is still to be determined whether Cyclin D1 participates in a causative or incidental manner in endometrial tumor progression.

Atypical hyperplasia is considered a potential precursor lesion for endometrial carcinomas; however it is still not clear whether hyperplasia without atypia poses a potential risk for developing into endometrial carcinoma. In case of endometrial hyperplasia without atypia the recommended treatment is cyclical progestins therapy whereas hysterectomy is the recommendation in patients with hyperplasia with atypia. Patients who are young

✉ **Dr. Summayya Shawana**
Assistant Professor
Department of Pathology
Bahria University Medical and Dental College
Karachi
Email: shawana75@yahoo.com
✉ **Dr. Saleha Masood**
Assistant Professor
Department of Pathology
Jinnah Medical and Dental College
Karachi
✉ **Dr. Zamir Ali Siddiqui**
Assistant professor
Department of Pharmacology
Poonch Medical College, Rawlakot,
AJK
Received: 01-12-2016
Revised: 12-12-2016
Accepted: 20-12-2016

and wish to conceive, high dose progestin therapy may be considered as an alternate in cases of atypical hyperplasia.[4] Researches have been carried out worldwide to come up with methods for early diagnosis of endometrial carcinomas and premalignant endometrial lesions for proper therapeutic interventions. However limited work in Pakistan has been done in this regard, therefore it was decided to carry out a study to observe the differential expression of Cyclin D1 in different endometrial morphologies ranging from normal proliferative endometrium to endometrial cancers.

METHODOLOGY:

This retrospective analytical study was based on the analysis of endometrial samples comprising of both hysterectomies and curettage, received at the department of pathology BMSI, JPMC, Karachi over a five year period i.e. from 1-1-2008 to 31-12-2012. Over the five year study period we came across 294 endometrial lesions. These included 144 simple hyperplasia without atypia, 107 complex hyperplasia without atypia, 8 complex hyperplasia with atypia, and 35 malignant endometrial tumors. 55 samples were selected for immunohistochemical analysis. These included 25 malignant endometrial lesions, 6 complex hyperplasia with atypia, 14 complex hyperplasia without atypia, 6 simple hyperplasia without atypia and 4 normal proliferative endometrium. Sample size was calculated using the survey system sample size calculator. Poorly fixed tissue, inadequate material and samples of foreign nationals and Pakistanis living abroad for more than ten years were excluded. H&E stained slides were reviewed to confirm the diagnosis and the most representative section was used for immunohistochemical analysis. Anti-Cyclin D1, rabbit monoclonal antibody procured from Cell Marque, was used in all immunohistochemical analysis. Antigen detection was done using HiDef detection HRP polymer system kit (ready to use) procured from Cell Marque. Ductal carcinoma breast was taken as positive control for Cyclin D1. PBS substituted primary antibody for negative control. Sections of approx. 5µm were cut on to poly L-lysine coated slides and were deparafinized and rehydrated. Antigen retrieval was achieved by steamer method using citrate buffer, slides were allowed to cool for 20 minutes and were then placed in UV block for 5 minutes. Tissues were covered with primary antibody at dilution 1:50 and were incubated for 1 hour at room temperature. Slides were then incubated first with Amplifier and then with HRP polymer for 10 minutes. Chromogen was applied for 20 minutes and all the slides were counter stained with haematoxylin, dehydrated and mounted. Between each step the slides were washed with phosphate buffer solution (PBS).

The intensity of staining was graded as no nuclear staining (0), weak nuclear staining (1+), moderate nuclear staining (2+) and strong nuclear staining (3+). The extent of staining was estimated in percentage by counting at least 50 nuclei, calculating the ratio of reactive nuclei to total number of nuclei and multiplying it by 100. A score of 0 was used when less than 10% cells were positive, 10 to 30% immunoreactive cells were scored as 1, 31 to 60% positive cells were scored as 2 and more than 60% immunoreactive cells were scored as 3. After observing Cyclin D1 immunostaining in normal proliferative endometrium, strong staining in more than 30% nuclei was taken as overexpression of Cyclin D1. Data was collected on specially designed proforma. Statistical analysis was performed using SPSS version 21. Mean and standard deviation were calculated for quantitative variables while percentages and frequencies were calculated for qualitative variables.

RESULTS:

The 55 cases selected for immunohistochemical analysis, included 4 normal proliferative endometrium, 6 simple hyperplasia without atypia, 14 complex hyperplasia without atypia, 6 complex hyperplasia with atypia, and 25 malignant endometrial tumors. The 25 cases of malignant endometrial tumors included 18 well differentiated adenocarcinomas, 1 moderately differentiated endometrial carcinoma, 4 poorly differentiated carcinomas and two spindle cell tumors. Table-1 distributes different endometrial lesions according to age groups along with the mean age for every lesion. Table-2 shows the extent and intensity of nuclear staining of Cyclin D1 in different endometrial samples. 11 out of 25 cases of malignant endometrial tumors showed strong staining for Cyclin D1 with 9 cases showing strong reactivity in more than 60% of neoplastic cells while 2 showed strong reaction in more than 30% of cells. 10 cases of malignant endometrial tumors showed positive staining in less than 10% of nuclei. 4 out of 6 cases of atypical complex hyperplasia showed strong expression of Cyclin D1 with 2 cases showing expression in more than 60% of cells and 2 cases showing strong reaction in almost 50% of cells. Complex non-atypical hyperplasia showed strong nuclear staining for Cyclin D1 in 7 out of 14 cases with all 7 cases showing positive staining in more than 60% of cells. 2 out of 6 cases of simple hyperplasia without atypia showed strong nuclear staining for Cyclin D1 in more than 60% of cells. 2 out of 4 cases of normal proliferative endometrium showed moderate staining with Cyclin D1 in less than 30% of nuclei while 2 showed no staining at all. The 9 cases of malignant endometrial tumors which were negative for Cyclin D1 expression included 2 spindle cell tumors and 1 poorly differentiated adenocarcinoma.

Table-3 compares the expression of Cyclin D1 in different degrees of endometrial adenocarcinomas. 8(44.4%) out of 18 well differentiated endometrial adenocarcinomas showed strong intensity of staining for Cyclin D1 with 7 cases showing strong staining in more than 60% of cells. The only case of moderately differentiated carcinoma showed no staining at all. 3 out of the 4 cases of poorly differentiated carcinomas showed strong Cyclin D1 expression with positive staining in more than 60% of cells while one case showed no staining at all.

Table: 1
Distribution of different endometrial lesions according to age (n=294)

Lesions	Total no. of cases	(21-30) Yrs	(31– 40) Yrs	(41- 50) Yrs	(51-60) Yrs	(61-70) Yrs	> 70 Yrs	Mean (±S.D.)
Simple Hyperplasia without atypia	144	5 (3.97%)	50 (34.72%)	75 (52.08%)	7 (4.86%)	7 (4.86%)	0 (0%)	43.88(±8.09)
Complex hyperplasia without atypia	107	10 (9.343%)	51 (47.66%)	38 (35.51%)	6 (5.6%)	2 (1.86%)	0 (0%)	41.90(±8.04)
Complex hyperplasia with atypia	8	0 (0%)	1 (12.5%)	5 (62.5%)	2 (25%)	0 (0%)	0 (0%)	47.01(±8.05)
Malignant endometrial tumors	35	0 (0%)	3 (8.57%)	7 (20%)	15 (42.85%)	9 (25.71%)	1 (2.85%)	57.04(±8.08)

Table: 2
Nuclear intensity and extent of Cyclin D1 immunoreactivity in normal and hyperplastic endometrium and endometrial tumors (n=55)

Lesions	Total no. of cases	Extent*				Intensity**			
		0	1+	2+	3+	0	1+	2+	3+
Proliferative endometrium	4	2 (50%)	2 (50%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	2 (50%)	0 (0%)
Simple hyperplasia without atypia	6	1 (16.66%)	1 (16.66%)	2 (33.33%)	2 (33.33%)	1 (16.66%)	3 (50%)	0 (0%)	2 (33.33%)
Complex hyperplasia without atypia	14	0 (0%)	2 (14.28%)	4 (28.57%)	8 (32%)	0 (()%)	1 (7.14%)	6 (42.85%)	7 (50%)
Complex hyperplasia with atypia	6	0 (0%)	1 (16.66%)	3 (50%)	2 (33.33%)	0 (0%)	0 (0%)	2 (33.33%)	4 (66.66%)
Malignant Endometrial tumors	25	10 (40%)	3 (12%)	3 (12%)	9 (36%)	9 (36%)	2 (8%)	3 (12%)	11 (44%)

*Extent of reactivity (% of immunoreactive nuclei) was as follows: 0, <10%; 1+, 11-30%; 2+, 31-60%; 3+, >60%.
**Intensity of reactivity was as follows: 0, no staining; 1+, weak nuclear staining; 2+, moderate nuclear staining; 3+, strong nuclear staining

Table-3
Nuclear intensity and extent of ptenimmunoreactivity in different grades of endometrial adenocarcinomas (n=23)

Grades of adenocarcinomas	Tota no. of cases	Extent*				Intensity**			
		0	1+	2+	3+	0	1+	2+	3+
Well differentiated carcinoma	18	11 (61.11%)	0 (0%)	3 (16.66%)	4 (22.22%)	11 (61.11%)	4 (22.2%)	3 (16.66%)	0 (0%)
Moderately differentiated carcinoma	1	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)
Poorly differentiated carcinoma	4	0 (0%)	0 (0%)	1 (25%)	3 (75%)	0 (0%)	0 (0%)	3 (75%)	1 (25%)

*Extent of reactivity (% of immunoreactive nuclei) was as follows: 0, <10%; 1+, 11-30%; 2+, 31-60%; 3+, >60%.
**Intensity of reactivity was as follows: 0, no staining; 1+, weak nuclear staining; 2+, moderate nuclear staining; 3+, strong nuclear staining

DISCUSSION:

In the present study we attempted to observe the differential expression of Cyclin D1 in different endometrial morphologies.The 35 malignant endometrial tumors included 2(5.4%) malignant spindle cell tumors, 2(5.4%) papillary serous carcinomas, 6(18.9%) poorly differentiated, 1(27%) moderately differentiated carcinoma and 24(67.5%) endometroid adenocarcinomas. The mean age for endometrial carcinomas was found to be 57 years and majority of the patients (67.5%) belonged to the 6th and 7th decade of life. In a study done at AKUH out of 86 cases of endometrial carcinomas 53(61.5%) belonged to the age range of 51 to 70 years.[13] In the present study Cyclin D1 immunostaining in >30% of nuclei was observed in 8 (44.4%) cases of endometroid endometrial carcinoma. Our findings correspond to those observed in other studies,[14,15,16] where strong expression of Cyclin D1 was seen in endometrial adenocarcinomas. The 2 papillary serous carcinomas in our study showed strong staining with Cyclin D1 in 60% of nuclei. Nishimurai et al[17] in one study showed significant correlation of Cyclin D1 over expression with low p53 expression, which is a frequently observed mutation in these tumors. Other studies[18,19] also observed positive expression of Cyclin D1 in 15% and 11.1% of non endometroid endometrial carcinomas while Balan et al[20] showed moderate to strong expression of Cyclin D1 in 2 out of 2 cases of papillary serous carcinomas. Immunohistochemistry was done on 6 cases of complex hyperplasia with atypia, out of which 66.6% i.e. 4 out of 6 showed Cyclin D1 overexpression. Similar higher figures were seen by Balan et al[20] who observed strong Cyclin D1 staining in 3 out of 3 cases of atypical hyperplasia. In cases of complex hyperplasia without atypia, 50% i.e.7 out of 14 cases of complex hyperplasia without atypia showed overexpression of Cyclin D1. These findings correspond to those quoted by Shevra et al.[21] Out of 6 cases of simple hyperplasia without atypia, 33.3%, i.e. 2, showed strong Cyclin D1 expression, corresponding to those observed by Quddus et al[22] and Liang et al,[16] who showed reactivity of Cyclin D1 in 30% cases of simple hyperplasia. Chaudhry and Bansal and Semczuk et al observed Cyclin D1 immunohisto-chemistry in none of the samples of simple hyperplasia without atypia.[23,24] On immunostaining, 2 out of 4 cases of normal proliferative endometrium showed moderate Cyclin D1 staining in less than 30% of nuclei while 2 showed no staining with Cyclin D1. Similar findings were observed by Shevra et al and Ozuysal et al.[21,25]

CONCLUSION:

Increasing Cyclin D1 expression was seen from normal proliferative endometrium to complex hyperplasia with atypia and carcinoma. This finding suggests that Cyclin D1 overexpression plays a role in endometrial carcinogenesis and could be an early event in endometrial carcinogenesis. Studies done worldwide suggest a potential role of Cyclin D1 in endometrial carcinogenesis. However it is emphasized that Cyclin D1 overexpression alone should not be the criteria for labeling a hyperplastic lesion as premalignant and should be correlated with other immunological and molecular markers.

REFERENCES:

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90
2. Colombo N, Preti E, Landoni F, Carinelli S, Colombo A, Marini C, et al. ESMO Guidelines Working Group: Endometrial cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2011;22:35-39
3. Collective cancer registry report (1994-2011). Shaukat Khanum Cancer Hospital and Research Centre, p6
4. Trimble CL, Method M, Leitao M, LuK, Loffe O, Hampton M et al. Management of endometrial precancers. Obstet Gynecol 2012;120:1160-75
5. Silverberg SG, Mutter GL, Kurman RJ, Kubik-Huck RA, Nogales F, Tavassoli FA. Tumors of the uterine corpus: epithelial tumors and related lesions, in WHO Classification of Tumors: Pathology and Genetics of Tumors of the Breast and Female Genital Organs. Tavassoli FA, Stratton MR, editors. Lyon, France: IARC Press; 2003. pp. 221-32
6. Mutter GL. Endometrial intraepithelial neoplasia (EIN): Will it bring order to the chaos? The endometrial collaborative group. Gynecol Oncol 2000; 76:287-90
7. Yu W, Jin FZ, Ying YL, Gui PH. An analysis of Cyclin D1, Cytokeratin 5/6 and Cytokeratin 8/18 expression in breast papillomas and papillary carcinomas. Diagn Pathol. 2013; 8: 8-10.1186/1746-1596-8-8
8. Ewen ME, Lam J. The activities of cyclin D1 that drive tumorigenesis. Trend Mol med 2004; 10:158-62
9. Coqueret O. Linking cyclins to transcriptional control. Gene 2002; 299:35-55
10. Kala M, Gill M, Gupta V, Srivastava D, Tanwar P, Goayal V. Cyclin D1 Expression in Hyperplasia and Carcinoma of the Endometrium and Its Correlation with Histologic Grade and Tumor Type. J Gynecol Surg 2012;30:1-4
11. Cao TQ, Einstein MH, Aderson PS, Runowicz CD, Balan R, Jones JG. Expression of cox-2, Ki-67, cyclin D1 and p21 in endometrial endometroid carcinoma. Int J Gynecol Pathol 2002; 21:147-54
12. Brucka A, Bartczak P, Ratynska M, Sporny S. Immuno-histochemical pattern of protein p21, Cyclin D1 and Cyclin E in endometrial hyperplasia. Pol J Pathol 2009; 1:19-25
13. Ahmed Z, Azad NS, Yaqoob N, Husain A, Ahsan A, Khan AN et al. Frequency of primary solid malignant neoplasms in both sexes as seen in our practice. J Ayub Med Coll 2007; 19:53-55
14. Bookya K, Muthyam S, Kumari K. Study of expression of Cyclin D1 in the interpretation of endometrial lesions. EJBPS 2015;2:1456-67
15. Stewart CJR, Crook ML, Leung YC, Platten M. Expression of cell cycle regulatory proteins in endometrial adenocarcinoma; variations in conventional tumor areas and in microcytic, elongated and fragmented glands. Mod Pathol 2009; 22:725-33
16. Liang S, Mu K, Wang Y, Zhou Z, Zhang J, Sheng Y et al. CyclinD1, a prominent prognostic marker for endometrial diseases. Diagn Pathol 2013;15:8-138
17. Nishimura Y, Watanabe J, Jobo T, Kato N, Fujisawa T,

Kamata Y, Kuramoto H. Cyclin D1 expression in endometroid type endometrial adenocarcinoma is correlated with histological grade and proliferative activity, but not with prognosis. Anticancer Res 2004; 24:2185-93
18. 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
19. Moreno-Bueno G, Rodrigez-Perales S, Sanchez-Estevez C, Marcos R, Hardlsson D, Cigudosa JC, Palacios J. Molecular alterations associated with cyclin D1 overexpression in endometrial cancer. Int J Cancer 2004; 110: 194-200
20. Balan R, Amaline C, Craucux E, Carunta ID, Gheorghita V, Toma O. Expression of Cyclin D1 and Ki-67 in endometrial hyperplasia and carcinomas. Sectonea Genetica Si Biologie Moleculara 2008; 9:17-23
21. Shevra CR, Ghosh A, Kumar M. Cyclin D1 and Ki-67 expression in normal, hyperplastic and neoplastic endometrium. JPGM 2014;61:15-20
22. Quddus MR, Latkovich P, Castellani WJ, Sung CJ, Steinhoff MM, Briggs RC, Miranda RN. Expression of Cyclin D1 in normal, metaplastic, hyperplastic endometrium and endometrial carcinoma suggests a role in endometrial carcinogenesis. Arch Pathol Lab Med 2002; 126:459-63
23. Choudhary M, Bansal S. Expression of Cyclin D1 in endometrial hyperplasia and endometrial carcinoma. Indian J Pathol Microbiol 2007; 50:708-10
24. Semczuk A, Miturski R, Skomra D, Jackowicki JA. Expression of cell cycle regulatory proteins in human endometrial cancers: correlation with clinicopathological features. Arch Gynecol Obstet 2004; 269:104-10
25. Ozuysal S, Ozuturk 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; 271:123-26