



ORIGINAL RESEARCH PAPER

Medical Science

EXPRESSION OF CYCLIN STUDY D1 IN THE INTERPRETATION OF ENDOMETRIAL LESIONS

KEY WORDS: Endometrial carcinoma, complex hyperplasia, simple hyperplasia, cyclin D1.

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ABSTRACT

Introduction: Endometrial carcinoma is often preceded by characteristic histopathological lesions known as endometrial hyperplasia. Over expression of cyclin D1 has been linked to the development and progression of cancer. cyclin D1 is an important regulator of cell cycle progression and can function as transcriptional co-regulator.

Aims and objectives: To investigate the role of cyclin D1 in simple hyperplasia, complex hyperplasia and endometrial carcinoma and to recognize the subset of endometrial lesions that may be precancerous.

Materials and Methods: This is a retrospective and prospective study carried out at Kakatiya Medical College Warangal. All specimens received in the department of Histopathology over a period of 5 years. i.e., from March 2009 to May 2014 taking into account relevant clinical data and relative information were included in the study. cyclin D1 immunohistochemical analysis (IHC) was used to evaluate 60 fixed, paraffin-embedded endometrial samples.

Results: Out of 60 cases of endometrial lesions 37% (22/60) were simple hyperplasia, 33% (20/60) were complex hyperplasia, 30% (18/60) were endometrial carcinoma and control group included 10 proliferative endometrium and 11 secretory endometrium. Among 18 cases of endometrial carcinomas, majority of carcinomas were of endometriosis type (62%) followed by adenosquamous (22%), serous papillary type of carcinomas (17%). Most of the simple hyperplasia's were reported in the age group of 40-49 years, while most of the complex hyperplasia and carcinomas were above the age of 40years. The mean age of the above cases was 47.1 years (range 25-66years). cyclin D1 immunoreactivity was predominantly seen in endometrial carcinomas followed by a complex hyperplasia and simple hyperplasia. The highest extent of (3+) of positivity was seen in majority of carcinomas and complex hyperplasia.

Conclusion: Cyclin D1 is an important regulator of cell cycle progression and can function as transcriptional co-regulator. The over expression of cyclin D1 has been linked to the development and progression of cancer.

INTRODUCTION

Human endometrium is a special tissue of great proliferative and regenerative potential^[1].The ovarian and pituitary hormones cause changes typical for hormonal, two-phase endometrium with its breakdown during menstruation. Endometrial proliferation rearrangements caused by prolonged estrogen stimulation compose a wide range of abnormalities with a corresponding variety of morphological patterns. Several types of pathological proliferation beginning with the ones slightly different from the late proliferative phase endometrium to the complex types, difficult to differentiate from carcinoma^[2].The most probable hypothesis of endometrial cancer etiology is based on the prolonged estrogen stimulation of endometrium of genetically prone women, characterized by histopathologic lesions designated as endometrial hyperplasia. Currently it is accepted that there is continuum of changes that evolve to endometriosis Carcinoma^[3-6].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]. Cyclin dependent kinases and tumor suppressor gene products interact and regulate the normal cell cycle. Moreover the c cyclin which is associated with cell progression in the cellcycle has recently drawn attention in carcinogenesis. Among the cyclin, Cyclin D1 is associated with onset of cell progression in G1 phase of the cell cycle. Cyclin D1 is located on chromosome 11q13 and exhibits many characteristics of cellular oncogenes^[9-10]. Cyclin D1 over expression may be one of the several mechanisms involved in endometrial neoplasia. Proliferative endometrial glands and stromal, even when actively mitotic do not over express Cyclin D1^[7].This study was undertaken to investigate the pattern of Cyclin D1 expression in normal, hyperplastic, and neoplastic endometrium and thereby evaluate the possibility of a role in the genesis of endometrial neoplastic and Para neoplastic lesions.

MATERIALS AND METHODS

This is a retrospective and prospective study carried out at Kakatiya medical college Warangal. All specimens received in the department

of Histopathology over a period of 5 years. i.e., from March 20013to May 2018 taking into account relevant clinical data and relative information were recorded from the biopsy records, statistical books and standard journal.

INCLUSION CRITERIA

Endometrial curettage & hysterectomy specimens of females of all age groups were included. Only samples with adequate tissue material and definite histopathological diagnosis were included. Representative areas in the biopsies were only included. Only endometrial epithelial lesions were considered.

Exclusion criteria

All cases of stromal lesions were excluded. All inflammatory lesions were excluded. All hemorrhagic and necrotic samples were excluded.10 cases of normal i.e., proliferative and secretory endometrial were taken for the control group.

Specimen Handling

All curettage and hysterectomy specimens were fixed in 10% neutral buffered formalin .after adequate fixation; examination of the specimen for gross details was done. Then representative tissue bits were taken and subjected for routine processing and paraffin embedding .3-4 microns thick sections were taken from paraffin embedded blocks. These sections were routinely stained with hematoxylin and eosin (H/E) and were examined Histopathological features were noted and the tumors typed according to WHO classification system.The paraffin blocks of the samples which had met the inclusion criteria were collected .the details of each case such as biopsy number, age, histopathological diagnosis were noted.A total of 60 cases were collected and subjected to immunohistochemistry.10 control cases of normal proliferative and secretory endometrium, 22 cases of simple hyperplasia, 20 cases of complex hyperplasia and 18 cases of endometrial carcinomas were analyzed.

Procedure for IHC for cyclin D1 using FLEx monoclonal rabbit human cyclin clone EP 12

From selected blocks 3-4 micrometer thick sections were taken on Polylysine coated slides Deparaffianistaion with xylene and

hydration in alcohols and tap water was performed .Heat induced epitope retrieval using Tris /EDTA Ph 9.0buffer was done using microwave method for 20minutes .Endogenous peroxidase inactivation using 1drop of 3%aqueous hydrogen peroxide was done for 5minutes.Incubation with primary antibody anti human cyclin D1 clone EP12 was done for 20 minutes at room temperature in a moist chamber. Incubation with secondary antibody horseradish peroxidase was done for 20 minutes Incubation with freshly prepared diaminobenzediene (DAB) chromogen was done for 5 minutes.Counter staining was performed using hematoxylin .The slides were then subjected to dehydration, clearing, and mounting.

RESULTS

TABLE1 Age wise distribution of cases

Age Group	Simple Hyperplasia	Complex Hyperplasia	Carcinoma	Total
20-29	2	0	0	2
30-39	7	1	0	8
40-49	13	9	7	29
50-59	0	10	6	16
>60	0	0	5	5
5	22	20	18	60

Most of the simple hyperplasias fell in the age group of 40-49 years, while most of the complex hyperplasia and carcinomas were above the age of 40years .the mean age of the above cases was 47.1 years (range 25-66years).

Distribution of total cases

Majority of the cases were simple hyperplasia (37%) followed by complex hyperplasia (33%) and carcinomas (30%) Among 18 cases of endometrial carcinomas, majority of carcinomas were of endometrioid type (61%) followed by adenosquamous (22%), serous papillary type of carcinomas (17%).

TABLE 2: Distribution of total cases with cyclin D1immunostaining

Lesion	Positive %	Negative %	Total
Simple hyperplasia	4(18%)	18(82%)	22
Complex hyperplasia	9(45%)	11(55%)	20
Endometrial carcinoma	9(50%)	9(50%)	18
Total	22	38	60

TABLE3: Extent of cyclin D1 immunoreactivity

Lesion	0	1+	2+	3+	Total
Complex hyperplasia	11	1	3	5	20
Endometrial carcinomas	9	1	3	5	18
Total	38	5	7	10	60

TABLE5 comparison with various studies

Study	sample size	cyclin + % proliferative endometrium	cyclin + % secretory endometrium	cyclin + % simple hyperplasia	cyclin + % complex hyperplasia	cyclin + % atypical endometrial hyperplasia	cyclin + % endometrial carcinoma
Toshio Nikaido et al	74	week	0	-	-	-	40.5%
SemaOzuysal, et al	89	3.3	-	0	-	7.1%	26.6%
Gemamoreno-bueno et al	110	0	-	-	-	0	13.8%
MonishaChowdary et al	30	-	-	0	33%	-	50%
Present study	60	0	-	18%	45%	-	50%

DISCUSSION

In this study immunohistochemistry was done to demonstrate that cyclin D1 is over expressed in hyperplastic lesions, which are considered to be precursors of endometrial carcinoma and this over expression of cyclin D1 may be associated with actual gene amplification or transcriptional dysregulation in cancers.in this study the expression of cyclin D1 was evaluated in simple hyperplasia, complex hyperplasia and endometrial carcinoma. In the present study most of the simple hyperplasias were observed in

TABLE 4: Intensity of cyclin D1 immunopositivity

Lesion	0	1+	2+	3+	Total
Simple hyperplasia	18	2	2	0	22
Complex hyperplasia	11	1	3	5	20
Endometrial carcinomas	9	2	2	5	18
Total	38	5	7	10	60

Distribution of cyclin D1 immunostaining among carcinomas showed positive immunoreactivity for Cyclin D1 all were of endometrioid type. Intensity of cyclin D1 immunopositivity among various Carcinomas.



Fig.1 Hysterectomy specimen –grossly shows growth in the endometrium

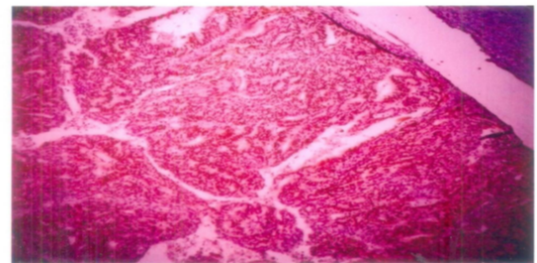


Fig.2 40X-Endometrioid adenocarcinoma ,IHC-Cyclin D1 Positivity ,Extent +3,Intensity 3+

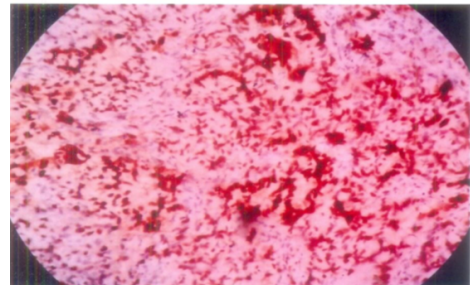


Fig.3 100X-Complex hyperplasia, IHC-Cyclin D1 Positivity, Extent +3,Intensity 3+

the age group of 40-49 years, while most of the complex hyperplasia and carcinomas were above the age of 40years.The mean age of the above cases was 47.1 years (range 25-66years). In the study by Nishimura et al mean age of patients was 57 years (range 30-83yrs)^[10]. In the study by SemaOzuysal, et al median age of the patients of endometrial carcinoma was 59.3yrs (range 47-75yrs)^[11]. In the study by Gema Moreno-Bueno et al mean age of all cases of endometrioid endometrial carcinomas was 62.47+/- 12.04yrs (range 31-89 years)^[12]. In the present study out of 60

cases of endometrial lesions 37% (22/60) were simple hyperplasia, 33% (20/60) were complex hyperplasia, 30% (18/60) were Endometrial carcinoma and control group included 10 proliferative endometrium and 11 were secretory endometrium. In study by M. RuhulQuddus et al out of 108 cases 13 patients with normal proliferative endometrium, 11 with normal secretory endometrium, 26 with simple hyperplasia, 21 with complex hyperplasia with and without atypia, and 37 with endometrioid adenocarcinoma^[13]. In study by SemaOzuysal et al out of 89 cases 30 patients with normal proliferative endometrium, 15 with simple hyperplasia, 14 atypical hyperplasia, and 30 with endometrial adenocarcinoma^[11]. In Gema Moreno-Bueno et al study 60 cases endometrial adenocarcinoma^[12]. In the study by Toshio Nikaido et al, the control group included 20 proliferative endometrium 23 secretory endometrium, 15 post menopausal endometrium, 74 endometrioid endometrial carcinomas^[7]. In MonishaChowdary et al study, out of 30 cases simple hyperplasia was observed in 10 cases, complex hyperplasia in 6, and the rest endometrial adenocarcinoma^[14]. In Mitselou A et al study 20 cases with normal endometrium, 32 hyperplastic endometrium, 66 with endometrial adenocarcinomas^[15].

Cyclin D1 Immunoreactivity

In the present study none of the proliferative and secretory endometrial showed cyclin D1 immunoreactivity. Nishimura et al no cyclin D1 expression was observed in secretory phase focal staining detected in proliferative endometrium.[10]SemaOzuysal et al 1 out of 30 cases (3.3%) of proliferative endometrium were found to be immunoreactive to cyclin D1^[11]. M. RuhulQuddus et al analysis of extent of Cyclin D1 positivity out of 13 cases of Proliferative endometrium 8 were negative and 5 were 1+ positive, among total 11 Secretory endometrium 5 were negative and 6 were 1+ positive. Analysis of intensity of Cyclin D1 positivity out of 13 cases of Proliferative endometrium 8 were negative and 5 were 1+ positive, among total 11 Secretory endometrium 4 were negative and 6 were 1+ positive^[13].

Analysis of extent of immunopositivity

Present study: out of 22 Simple hyperplasia, 18 cases show 0 positivity, 3 shows +1 positivity, 1 case showed +2 positivity, among 20 Complex hyperplasia 11 cases show 0 positivity, 1 shows +1 positivity, 3 cases showed +2 positivity, 5 cases showed +3 positivity. Among endometrial carcinomas 9 cases show 0 positivity, 1 showed +1 positivity, 3 cases showed +2 positivity, 5 cases showed +3 extent of positivity M. RuhulQuddus et al out of 26 Simple hyperplasia, 11 cases show 0 positivity, 12 shows +1 positivity, 3 cases showed +2 positivity, among 21 Complex hyperplasia 6 cases show 0 positivity, 7 shows +1 positivity, 4 cases showed +2 positivity, 4 cases showed +3 positivity. Among 37 endometrial carcinomas 12 cases show 0 positivity, 10 showed +1 positivity, 9 cases showed +2 positivity, 6 cases showed +3 extent of positivity^[13]

Analysis of intensity of cyclin d1 positivity

Present study showed out of 22 Simple hyperplasia, 18 cases show 0 positivity, 2 shows +1 positivity, 2 case showed +2 positivity, among 20 Complex hyperplasia 11 cases show 0 positivity, 1 shows +1 positivity, 3 cases showed +2 positivity, 5 cases showed +3 positivity. Among endometrial carcinomas 9 cases show 0 positivity, 2 showed +1 positivity, 2 cases showed +2 positivity, 5 cases showed +3 extent of positivity M. RuhulQuddus et al (13) 26 Simple hyperplasia, 10 cases show 0 positivity, 13 shows +1 positivity, 3 cases showed +2 positivity, among 21 Complex hyperplasia 4 cases show 0 positivity, 9 shows +1 positivity 5 cases showed +2 positivity, 3 cases showed +3 positivity. Among 37 endometrial carcinomas 5 cases show 0 positivity, 12 showed +1 positivity, 8 cases showed +2 positivity, 12 cases showed +3 extent of positivity^[13]. In the study Q.Jackie Cao et al cyclin D1 was over expressed in both endometrial hyperplasia and endometrial carcinomas^[15]. In the study by Rahul Quddus et al overexpression of cyclin D1 increases significantly from normal endometrial hyperplasia to endometrial carcinomas compared to proliferative endometrium, secretory endometrium and simple hyperplasia suggesting it may play role in endometrial carcinogenesis^[13]. In the study by SemaOzuysal et al cyclin D1 expression in endometrial carcinoma is higher than proliferative endometrium and simple

hyperplasia. These findings support that cyclin D1 may play a role in endometrial carcinogenesis^[11]. In the study by Nishimura et al results suggest that high expression of cyclin D1 may be an early event of carcinogenesis of endometrial carcinoma^[10].

In the study by RalucaBalan et al over expression of cyclin D1 increases from normal endometrium to endometrial hyperplasia and endometrial carcinoma, suggesting that it may play a role in endometrial carcinogenesis^[16]. The study by Aleksandra Bruka et al did not reveal any statistical significant correlation in cyclin D1 expression in glandular endometrial nuclei, morphological pattern of endometrium, patient age and parity^[11]. In the study by Mitselou A et al, immunoexpression of cyclin D1 does not appear to be associated with cell cycle progression in the benign or malignant endometrium.

CONCLUSION

Endometrial carcinoma is often preceded by characteristic pathological lesions known as endometrial hyperplasia. Other mechanisms of endometrial carcinogenesis include mutations in p53 and pTEN, tumor suppressor genes and over expression of cyclin D1. The cyclin D1 proto-oncogene is an important regulator of G1 to S-phase transition in endometrial neoplasia. Proto-oncogene 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 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 hyperplasia and carcinoma suggests that it may play role in endometrial carcinogenesis. Since there is no difference in Cyclin D1 expression between complex hyperplasia and endometrial carcinoma, it appears that the deregulation is maximal at the complex hyperplasia state. This pattern suggests that cyclin D1 over expression may be early event in the endometrial carcinogenesis.

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