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Immunohistochemical expression of Cyclin D1 and p53 in normal, hyperplastic and malignant endometrium in a rural tertiary care hospital of central India

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Original Article

Abstract

BACKGROUND: Endometrial carcinoma (EC) is the most common gynecological malignancy worldwide. Type I EC is often preceded by atypical endometrial hyperplasia (EH), while type II EC results from a series of genetic alterations involving activation of proto-oncogene (cyclin D1) and inactivation of tumor suppressor genes (p53). The purpose of this study was to evaluate prognostic significance of cyclic D1 and p53 expression in cases of EC by immunohistochemistry (IHC).

METHODS: This laboratory-based observational study was conducted in the histopathology section of the Department of Pathology at a rural tertiary care hospital in Central India from 2017 to 2019. The study comprised 100 cases, of which 50 cases were of EC and 38 and 12 cases of EH and normal endometrium, respectively. Relevant clinical details, histopathological diagnosis, treatment, and follow-up were retrieved from the Hospital Information System (HIS) and pathology records. IHC evaluation of cyclin D1 and p53 was done in all cases. Statistical analysis was done by descriptive and inferential statistics using a chi-square test through SPSS software.

RESULTS: Cyclin D1 was significantly over-expressed in atypical EH and type I EC. Overall, the positivity of cyclin D1 in EC was 88%. The high expression of cyclin D1 was significantly associated with poorly-differentiated tumors (P = 0.023). Overexpression of p53 was strongly associated with type II histology and poorly-differentiated type I EC, the presence of lymphovascular invasion (LVI) (P = 0.059), and advanced International Federation of Gynecology and Obstetrics (FIGO) stage (P = 0.0011).

CONCLUSION: Cyclin D1 expression increased from normal endometrium to hyperplasia and EC, thus suggesting its role in the pathogenesis of type I EC, while p53 overexpression was significantly higher in type II EC and poorly-differentiated EC. Therefore, cyclin D1 and p53 can be used as a marker for endometrial carcinogenesis and tumor cell proliferation.

KEYWORDS: Endometrium; Carcinoma; Cyclin D1; p53 Genes

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Introduction

Incidence of endometrial carcinoma (EC) has been rising in recent years, and has become the second most common and fourth leading cause of death due to gynecological malignancies among women worldwide.[1] As per the GLOBOCAN 2022 estimates, there are nearly 420368 new cancer cases and 97723 deaths occurred worldwide.[1] Endometrial cancer is the fifth most common cancer in women (4.8% of cancers in women), who have a cumulative risk of 1% of developing the

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disease by age of 75 years. In developing countries, cervical cancer still remains the leading cause of gynecological cancers, but there is a recent increase in the incidence of endometrial cancer.[1,2] In India, the total number of estimated new cases of endometrial cancer in 2022 was 17240 with an estimated 6845 deaths. The age-standardized incidence rate (ASIR) of endometrial cancer in India is 2.5/100000 women.[2,3]

EC has been classified into type 1 and type 2 endometrial cancer on the basis of histological and molecular characteristics. Type 1 EC is more common, with 80% of all endometrial cancers of endometrioid origin. Type II EC is primarily of serous or clear cell origin.[4] Type I EC is often preceded by characteristics of proliferative lesions designated as endometrial hyperplasia (EH), which is currently accepted as continuum of changes that evolve to EC.[5] EH often results from chronic estrogenic stimulation unopposed by the counterbalancing effect of progesterone.[4,5] Obese women and women with chronic ovulatory disorders are at increases risk for EH as well as women with mismatch repair abnormality and Lynch syndrome.[4]

Apart from EH, recent advances in molecular carcinogenesis revealed that EC also arised from the accumulation of a series of genetic alterations involving activation of proto-oncogenes (cyclin D1) and inactivation of tumor suppressor genes (p53).[6] Overexpression of cyclin D1 induces cellular proliferation leading to progression of EH to EC.[6,7] p53 mutation is a negative prognostic factor and frequently overexpressed in type II EC, but has also been identified in high-grade endometrioid and undifferentiated carcinomas.[8] Studies have reported increased cellular proliferation co-existing with progressive derailment of cyclin D1 leading to progression of EH to EC.

Ruhul Quddus et al. studied the pattern of cyclin D1 expression in normal, metaplastic, hyperplastic, and neoplastic endometrium, and thereby evaluated the possibility of a role in the genesis of endometrial neoplastic and preneoplastic lesions. They concluded that overexpression of cyclin D1 in endometrial glands was independent of overexpression of cyclin D1 in surface metaplastic epithelium.[9]

Ozuysal et al. studied 50 cases in order to evaluate different expression profiles of cyclin D1 in different endometrial lesions. On comparison of cyclin D1 expression in different endometrial lesions, they found that a statistically significant difference was observed between the results of proliferative phase versus complex hyperplasia without atypia (HwA) ($P = 0.017$), proliferative phase versus complex hyperplasia with atypia ($P = 0.014$), proliferative phase versus EC ($P = 0.021$), secretory phase versus complex HwA ($P = 0.020$), and secretory phase versus complex hyperplasia with atypia ($P = 0.280$). They concluded that cyclin D1 expression in endometrial glands increased progressively in extent from normal endometrium to hyperplasia to carcinoma.[10]

Therefore, the aim of this study was to evaluate the expression of cyclin D1 and p53 in EH, EC as well as normal endometrium by immunohistochemistry (IHC) and to assess the prognostic significance in case of EC.

Methods

This was a laboratory-based observational study conducted in histopathology section of Department of Pathology of Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha, India, from 2017 to 2019.

Department of Pathology regularly receives specimen of endometrial biopsy (around 750/year) and hysterectomy following a diagnosis of EH or EC on biopsy (14-18/year). The Department of Pathology also runs a Population-Based Cancer Registry since 2010 which collects data of cases of cancer. Follow-up data of cases of endometrial cancer are maintained in the registry.

Sampling method: This pilot exploratory study comprised a total of 100 cases out of which, 50 were cases of EC, 38 were cases of EH (which included 30 cases of HwA and 8 cases of hyperplasia with atypia), and 12 were cases of normal endometrium (which included 6 cases of endometrium in proliferative phase and 6 cases of endometrium in secretory).

Sampling was done by three authors. The study has been approved by institutional ethical committee prior to commencement.

Appropriate tissue blocks of all these cases were retrieved from pathology records. Hematoxylin and Eosin (H&E)-stained sections were carefully reviewed, their diagnosis was confirmed, and representative sections were selected for IHC staining.

Relevant clinical and pathological data, regarding age, menopausal status, histological diagnosis, treatment, and follow-up were noted. In cases of EC, additional features such as histological subtype, grade, depth of myometrial invasion (MI), cervical involvement, lymphovascular invasion (LVI) and stromal invasion, lymph node metastasis (LNM), and International Federation of Gynecology and Obstetrics (FIGO) stage were recorded from the Hospital Information System (HIS) and also from gross records and pathological reports of these patients.

IHC: IHC staining was performed on 4 µ-thick, representative tissue sections. The primary antibody used were rabbit anti-human monoclonal antibody, clone EP12 for cyclin D1 and CloneBP53-12 for P53. Positive controls were sections from known case of breast carcinoma for cyclin D1 and serous ovarian carcinoma for p53.

Cyclin D1 staining was evaluated in the epithelial component in EH, EC, and also in normal endometrium. Two parameters were taken into consideration: the intensity of nuclear staining and extent (percentage of positive cells). The extent is semi-quantitatively estimated with a range of 0 to 100%. 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+.[6]

Evaluation of p53 staining was done by Fadare and Parkash method.[8] A score of 0 was assigned when < 10% of tumor cells were p53 positive, and 1+, 2+, and 3+ when 10%-30%, 31%-50%, and > 50% tumor cells were p53 positive, respectively.

IHC-stained slides were examined by two investigators (Vitaladeuni Shivkumar and Manisha Atram) blinded to patient clinical details. In case of discrepancies between the two, the slides were re-examined and a final score agreed by both was assigned.

Statistical analysis: Statistical analysis was done by descriptive and inferential statistics using chi-square test by SPSS software (version 24.0, IBM Corporation, Armonk, NY, USA). P-value < 0.05 was considered as level of significance. Univariate analyses were performed to study significance of cyclin D1 and p53 immunostaining with variable prognostic factors such as histological type, grade, depth of MI, LVI, LNM, and FIGO staging.

Results

A total of 100 cases were included in the study. The mean age at the time of presentation for EC was 61 years and the majority, i.e., 17 (34%) cases, were found in sixth decade. While for EH, mean age of the patients was 46 years.

Commonest clinical presentation in EC cases was postmenopausal bleeding (n = 25, 50%), whereas 22 (57.8%) cases of EH presented with abnormal uterine bleeding. Most common risk factors for both EC and EH were obesity (n = 18, 20.45%), diabetes mellitus (DM) (n = 12, 13.63%), and estrogen therapy (n = 10, 11.36%).

Of the 50 cases of EC, 48 cases were of type I EC and 2 cases of type II EC. We found 38 (79%) cases of well-differentiated (WD) EC, 7 (15%)

and 3 (6%) cases of moderately and poorly differentiated EC, respectively. In type I EC, less than half of MI was present in 29 (60.41%) cases. Moreover, LVI and LNM were found in 9 (18.75%) and 6 (12.5%) cases of type I EC, respectively. Fourteen (28%) cases of EC had stage I disease, and only two cases had stage IV disease. Clinicopathological features of endometrial lesions are mentioned in table 1.

Table 1. Clinicopathological features of endometrial lesions

Clinicopathological features	Total cases
Endometrial cases	
Normal endometrium	12 (12.00)
Endometrial hyperplasia	38 (38.00)
Hyperplasia without atypia	30 (30.0)
Atypical hyperplasia	8 (8.0)
EC	50 (50.00)
Endometriod (type I)	48
Non-endometrioid (type II)	2
Serous papillary	1
Clear cell	1
Tumor grade	
FIGO grade I	38 (79.00)
FIGO grade II	7 (15.00)
FIGO grade III	3 (6.00)
MI in type I tumors (n = 48)	
< inner half	29 (60.41)
> inner half	19 (39.48)
MI in type II tumors (n = 2)	
< inner half	1 (50.00)
> inner half	1 (50.00)
LVI in type I tumors (n = 48)	
Present	9 (18.75)
Absent	39 (81.25)
LVI in type II tumors (n = 2)	
Present	1 (50.00)
Absent	1 (50.00)
LNM in type I tumors (n = 48)	
Present	6 (12.50)
Absent	42 (87.50)
LNM in type II tumors (n = 2)	
Present	1 (50.00)
Absent	1 (50.00)
FIGO stage	
Stage I	14 (28.00)
Stage II	18 (36.00)
Stage III	16 (32.00)
Stage IV	2 (4.00)

Data are presented as number and percent.
EC: Endometrial carcinoma; FIGO: International Federation of Gynecology and Obstetrics; MI: Myometrial invasion; LVI: Lymphovascular invasion; LNM: Lymph node metastasis; MI: Myometrial invasion

Of the 30 cases of HwA, 25 (83.3%) cases were negative for cyclin D1 expression and 2 (6.6%) cases were positive with a score of 2+ (Figure 1), while 7/8 (87.5%) cases of atypical hyperplasia (AH) showed positive staining with a score of 3+ reported in two (Figure 2).

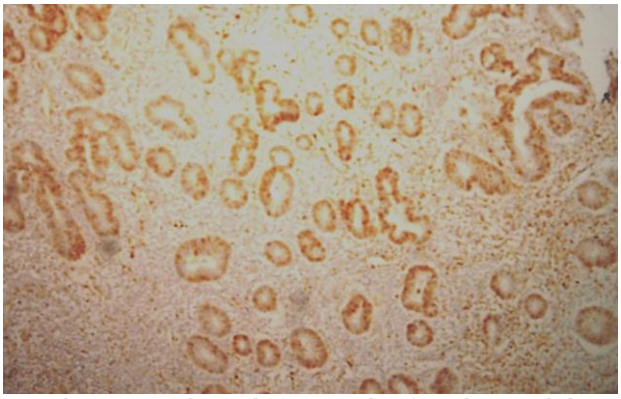

Figure 1. Microphotograph of endometrial hyperplasia (EH) without atypia showing positive reaction with cyclin D1 [Extent 2: (30% to 60% of cells expressing cyclin D1); Intensity: 1+ (cyclin D1, 100X)]

The difference in expression of cyclin D1 between AH and HwA was significant (P = 0.00045). All the 12 sections from normal endometrium were negative for cyclin D1 expression. We found the increased expression of cyclin D1 in AH compared to normal endometrium statistically significant (P = 0.0011).

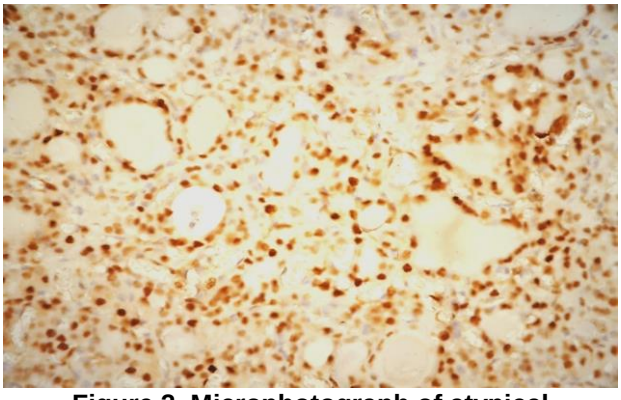

Figure 2. Microphotograph of atypical endometrial hyperplasia (EH) showing positive reaction with cyclin D1 [Extent: 3 (> 60% of cells expressing cyclin D1); Intensity: 2+ (cyclin D1, 100X)]

Cyclin D1 expression was absent in five (10.41%) cases of type I EC. Scores of 2+ and 3+ were noted in 9 (18.75%) and 18 (37.5%) cases of type I EC, respectively. Cyclin D1 expression was absent in 5/38 (13.15%) cases of WD carcinoma, while 15 (39.47%) cases showed 3+ score (Figure 3). However, all the 3 cases of poorly-differentiated EC had strong cyclin D1 expression (3+).

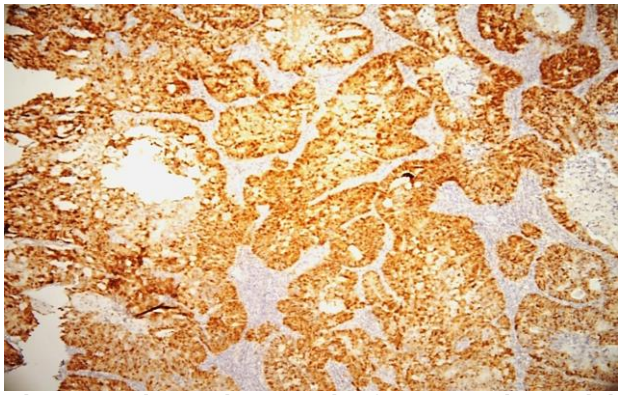

Figure 3. Photomicrograph of type I endometrial carcinoma (EC) showing positive reaction with cyclin D1 [Extent: 3 (> 60% of cells expressing cyclin D1); Intensity: 2+ (cyclin D1, 100X)]

One (50%) case of type II EC showed 1+ score (Figure 4). There was no significant association between cyclin D1 expression and type of EC (P = 0.280).

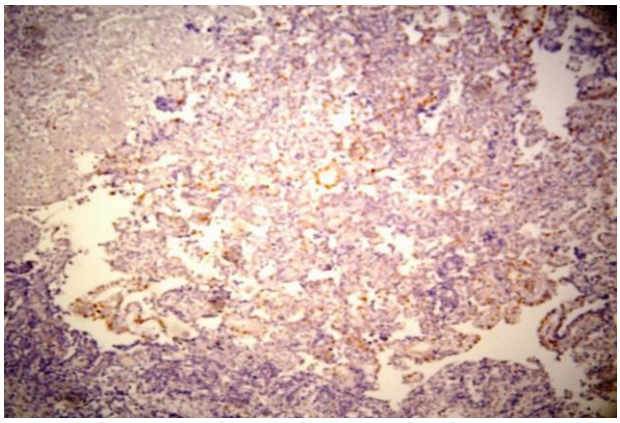

Figure 4. Photomicrograph of type II endometrial carcinoma (EC) (clear cell type) showing positive reaction with cyclin D1 [Extent: 1 (10%-30% of cells expressing cyclin D1); Intensity: 1+ (cyclin D1, 100X)]

Eleven (57.8%) cases of type I EC with more than half of MI showed positive staining (3+) of cyclin D1. All the nine cases of type I EC with presence of LVI showed positive expression of cyclin D1 with score of 3+ in 5/9 cases. However, the differences in scores of cyclin D1 expression in type I EC cases with or without LVI and LNM were insignificant (P = 0.150 and P = 0.062, respectively). Moreover, we did not find any association between percentage of cyclin D1 expression and FIGO stage of EC (P = 0.060). Correlation of cyclin D1 expression with pathological variables is shown in table 2.

None of the normal endometrium and HwA cases showed positive p53 immunostaining, while 2/8 cases (25%) of AH showed 2+ positivity of p53. No significant differences were noted between HwA versus AH (P = 0.06) and AH versus EC (P = 0.14) for p53 immunostaining.

Twenty-one (43.75%) cases of type I EC were p53 positive, whereas both cases (100%) of type II EC showed overexpression (3+) of p53. Overexpression of p53 was seen in 12/38 cases of WD EC (Figure 5). However, 3 (100%) cases of poorly-differentiated type I EC showed overexpression of p53 (Figure 6). All the nine cases of type I EC with presence of LVI showed overexpression of p53.

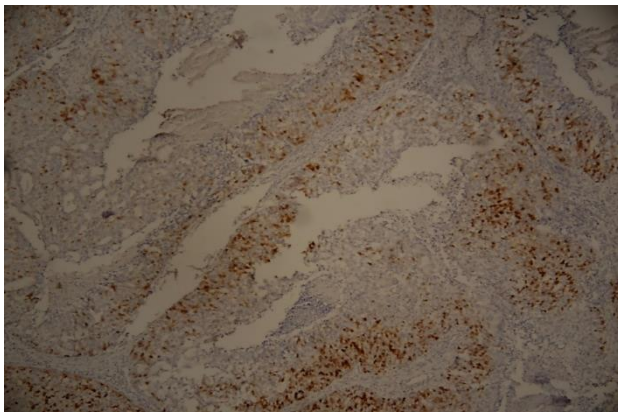

Figure 5. Photomicrograph of moderately-differentiated endometrial carcinoma (EC) showing weak positivity with p53 [Extent: 1 (1%-30% of cells expressing p53, 100X)]

Table 2. Cyclin D1 expression in endometrial lesions and its correlation with histopathological variables

Sr. No.	Histopathological parameters	Cyclin D1 score				χ^2	P
		0	1	2	3		
1.	Normal endometrium (n = 12)	12 (100)	0 (0)	0 (0)	0 (0)	16.15	0.001
	Atypical hyperplasia (n = 8)	1 (12.5)	2 (25.0)	3 (37.5)	2 (12.5)		
2.	Hyperplasia without atypia (n = 30)	25 (83.5)	3 (10.0)	2 (6.6)	0 (0)	17.77	0.004
	Atypical hyperplasia (n = 8)	1 (12.5)	2 (25.0)	3 (37.5)	2 (12.5)		
3.	EC (n = 50)	6 (12.0)	17 (34.0)	9 (18.0)	18 (36.0)	1.68	0.640
	Atypical hyperplasia (n = 8)	1 (12.5)	2 (25.0)	3 (37.5)	2 (12.5)		
4.	Type I EC (n = 48)	5 (10.4)	16 (33.3)	9 (18.7)	18 (37.5)	3.78	0.280
	Type II EC (n = 2)	1 (50.0)	0 (0)	1 (50.0)	0 (0)		
5	Grade I (n = 38)	5 (13.1)	14 (36.8)	4 (10.5)	15 (39.5)		
	Grade II (n = 7)	0 (0)	2 (28.5)	4 (57.4)	1 (14.3)	14.64	0.023
	Grade III (n = 3)	0 (0)	0 (0)	0 (0)	3 (100)		
6	MI < 1/2 (n = 30)	6 (20.0)	13 (43.3)	5 (16.6)	6 (20.0)	20.26	0.016
	MI > 1/2 (n = 20)	0 (0)	4 (20.0)	5 (25.0)	11 (55.0)		
7	LVI present (n = 10)	1 (10.0)	3 (30.0)	1 (10.0)	5 (50.0)	13.11	0.050
	LVI absent (n = 40)	4 (10.0)	15 (37.5)	8 (20.0)	1 (2.5)		
8	LNM present (n = 7)	0 (0)	1 (14.2)	1 (14.2)	5 (71.4)	15.86	0.062
	LNM absent (n = 43)	7 (16.2)	16 (37.2)	8 (18.6)	12 (27.9)		
9	FIGO stage I (n = 14)	2 (14.2)	7 (50.0)	1 (7.1)	4 (28.4)		
	FIGO stage II (n = 18)	3 (16.6)	4 (22.2)	7 (38.8)	4 (22.2)	24.26	0.060
	FIGO stage III (n = 16)	1 (6.2)	3 (18.1)	4 (25.0)	8 (50.0)		
	FIGO stage IV (n = 2)	0 (0)	0 (0)	0 (0)	2 (100)		

Data are presented as number and percent.
EC: Endometrial carcinoma; MI: Myometrial invasion; LVI: Lymphovascular invasion; LNM: Lymph node metastasis; FIGO: International Federation of Gynecology and Obstetrics

A significant association was noted between p53 overexpression and FIGO stage (P = 0.001). Correlation of expression of p53 with pathological variables is shown in table 3.

Table 3. P53 expression in endometrial lesions and its correlation with histopathological variables

Sr. No.	Histopathological parameters	p53 score				χ^2	P
		0	1	2	3		
1.	Normal endometrium (n = 12)	12 (100)	0 (0)	0 (0)	0 (0)	3.33	0.060
	Atypical hyperplasia (n = 8)	6 (87.5)	0 (0)	2 (12.5)	0 (0)		
2.	Hyperplasia without atypia (n = 30)	30 (100)	0 (0)	0 (0)	0 (0)	7.91	0.040
	Atypical hyperplasia (n = 8)	6 (87.5)	0 (0)	2 (12.5)	0 (0)		
3.	EC (n = 50)	26 (52.0)	1 (2.0)	6 (12.0)	17 (34.0)	3.91	0.140
	Atypical hyperplasia (n = 8)	6 (87.5)	0 (0)	2 (12.5)	0 (0)		
4.	Type I EC (n = 48)	27 (10.4)	0 (0)	7 (18.7)	15 (37.5)	0.77	0.370
	Type II EC (n = 2)	0 (0)	0 (0)	0 (0)	2 (100)		
5	Grade I (n = 38)	26 (68.4)	0 (0)	5 (10.4)	7 (14.8)		
	Grade II (n = 7)	1 (14.2)	1 (14.2)	0 (0)	5 (71.4)	15.44	0.003
	Grade III (n = 3)	0 (0)	0 (0)	0 (0)	3 (100)		
6	MI < 1/2 (n = 30)	23 (76.6)	0 (0)	2 (6.6)	5 (16.6)	0.77	0.067
	MI > 1/2 (n = 20)	4 (20.0)	0 (0)	4 (20.0)	12 (60.0)		
7	LVI present (n = 10)	0 (0)	0 (0)	0 (0)	10 (100)	7.44	0.059
	LVI absent (n = 40)	27 (67.5)	1 (2.5)	6 (15.0)	7 (17.5)		
8	LNM present (n = 7)	0 (0)	0 (0)	0 (0)	7 (100)	4.32	0.228
	LNM absent (n = 43)	27 (62.7)	0 (0)	6 (13.9)	10 (23.2)		
9	FIGO stage I (n = 14)	13 (92.8)	0 (0)	0 (0)	1 (7.1)		
	FIGO stage II (n = 18)	14 (77.7)	0 (0)	3 (16.6)	1 (5.5)	40.56	< 0.001
	FIGO stage III (n = 16)	0 (0)	0 (0)	3 (18.7)	13 (81.5)		
	FIGO stage IV (n = 2)	0 (0)	0 (0)	0 (0)	2 (100)		

Data are presented as number and percent.
EC: Endometrial carcinoma; MI: Myometrial invasion; LVI: Lymphovascular invasion; LNM: Lymph node metastasis; FIGO: International Federation of Gynecology and Obstetrics

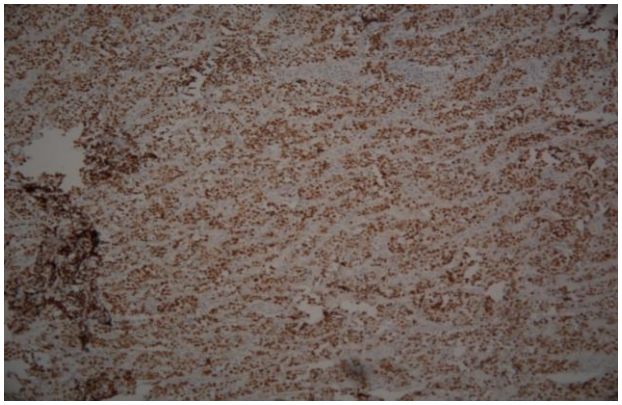

Figure 6. Photomicrograph of poorly-differentiated endometrial carcinoma (EC) showing positive reaction with p53 [Extent: 3 (> 50% of cells expressing p53, 100X)]

Discussion

Overexpression of cyclin D1, a cell cycle regulator, induces excessive cellular proliferation and it is often overexpressed in human cancers. Different studies have reported increased cellular proliferation co-existing with progressive derailment of cyclin D1 leading to progression of EH to EC.[6,8,10] Thus, the present study was conducted to determine expression of cyclin D1 and p53 in EH, EC as well as normal endometrium by IHC and to assess the prognostic significance in case of EC.

The mean age for EC was 61 years and the majority (34%) of cases were found in sixth decade, while mean age for EH was 46 years with 50% of cases being in late forties. Our findings showed closest resemblance to Sindhu et al.[11] and Liu et al.[2] study who observed that maximum number of EC and EH cases were in the age group of 61-70 and 41-50 years, respectively. Most of the patients with EC (n = 25, 50%) presented with postmenopausal bleeding whereas abnormal uterine bleeding (n = 22, 57.8%) was the predominant symptom associated with EH. Obesity (20.45%) was the commonest risk factor for both EC and EH. Similar findings were noticed by Beavis et al.[12]

Forty-eight (96%) cases were of type I EC and only 2 (4%) cases were of type II. Our findings of type I EC was in agreement with Sangwan et al.[13] We found 38 (79%) cases of WD EC, and 7 (15%) and 3 (6%) cases of moderately and poorly differentiated EC, respectively. Similar observations were also made by Sangwan et al.[13] and Yildirim et al.[14] Similar to the findings of Abdou et al.[15] in study of 67 cases of EC, we noted that more than half-MI, LVI, and LNM were more common in type II EC. However, no significant correlation (P = 0.62) was found between type I and II EC and depth of MI, LVI, and LNM as there were only two cases of type II EC in the present study.

In our study, majority of cases of type I EC were in FIGO stage II. However, Yildirim et al.[14] and Abdou et al.[15] found that most of cases of type I EC were in FIGO stage I. This discrepancy could be mainly because their studies were from developed countries where majority of the patients were diagnosed at an early stage.

Cyclin D1: Cyclins with their respective cyclin-dependent kinases are key components in the cell cycle regulation and altered phenotype of cyclins has been linked with malignant transformation. Cyclin D1 overexpression is one of the several mechanisms involved in endometrial neoplasm, as proliferative endometrial glands and stroma, even when actively mitotic, do not overexpress cyclin D1.[6,7,10]

We found gradual and progressive increase in cyclin D1 expression when results are compared between proliferative endometrium, EH with or without atypia, and malignant endometrium.

All the 12 cases of normal endometrium were negative for cyclin D1 expression; these findings were consistent with Sindhu et al.[11] In contrast, Ozuysal et al. noticed that 30% of proliferating endometrium and 40% of secretory endometrium showed expression of cyclin D1.[10]

Five (16.66%) cases of HwA showed positive expression of cyclin D1 with a score of

1+ and 2+. Seven cases of AH showed positive expression of cyclin D1. The differences in expression of cyclin D1 between normal endometrium and AH (P = 0.001), and AH versus HwA (P = 0.0045) were statistically significant. Similar observations were also made by Sangwan et al.,[13] Ozuysal et al.,[10] and Kundu et al.[16]

Overall positivity of cyclin D1 in EC was 88% which was in agreement with recent studies of Sindhu et al.[11] and Sangwan et al.[13] who reported cyclin D1 positivity of 83.4% and 73.3%, respectively.

However, similar to findings from Kundu et al.[16] and Khabaz et al.,[17] we found no correlation of cyclin D1 expression between AH and EC (P = 0.64). This suggests that deregulation of cyclin D1 is maximum at the level of AH; thus, it acts as a precancerous lesion of EC.

Similar to the observation by Yildirim et al., we noticed inverse relation of cyclin D1 staining with differentiation of the EC (P = 0.023).[14] Additionally, we found the significant association of cyclin D1 expression with depth of MI (P = 0.016) consistent with findings of Sangwan et al.[13] and Khabaz et al.[17] revealing that the cyclin D1 positivity was more when MI was more than half thickness.

No significant correlation was found between cyclin D1 expression and presence of LNM (P = 0.062) and LVI (P = 0.15) which was consistent with findings of Yildirim et al.[14] We observed cyclin D1 positivity more in FIGO stage IV tumor, similar to Shih et al.[18] and Kala et al.[19]

p53: Proapoptotic gene p53 is a tumor suppressor gene and its inactivation provides the neoplastic cells with a higher capacity for division and proliferation, and therefore contributes to malignant change and tumor formation. p53 mutation in serous papillary carcinoma is the most frequent genetic alteration; in endometrioid carcinoma, it is considered as a late event and occurs during progression in about 10-20 percent of these tumors.[20,21]

All the 30 cases of HwA were negative for p53 expression, and only 2 (25%) cases of AH showed p53 expression with score of 2+. Our findings were consistent with Ahmed and Isaac who found p53 positive staining in 20% of atypical complex hyperplasia.[22] Similar to Sahin et al.,[23] we found no statistical correlation of p53 overexpression in normal endometrium versus AH (P = 0.06) and AH versus EC (P = 0.14). Sherman et al. also concluded that p53 mutation was unrelated to the development of EC from atypical EH.[24]

Twenty-one (43.8%) cases of type I EC and two (100%) cases of type II EC were p53 positive. Overexpression of p53 (3+) was mostly associated with type II histology and poorly-differentiated EC but it was not statistically significant (P = 0.37) due to small sample size and very few (2/50) cases of type II EC. Our findings were consistent with Stavropoulos et al.[20] and Sahin et al.[23] p53 overexpression in type II EC and high-grade tumors, thus, supports the role of p53 in carcinogenetic model of type II EC and also is suggestive of tumor aggressiveness.

A significant correlation of p53 overexpression was noted with high-grade tumor (P = 0.003), LVI (P = 0.059), and advanced FIGO stage (P = 0.0001). These results were in agreement with Akiyama et al.[25] and Hutt et al.[26] However, Sahin et al. found no significant relationship between tumor grade, FIGO stage, and p53 expression which can be explained by lower number of grade III tumor or heterogeneity of p53 mutations in the EC.[23]

Main limitation of the present study is the small sample size due to financial constraints. Besides, selection of cases was done from single institution.

Further studies on cyclin D1 and p53 IHC expression in endometrium with large sample size and standardized methodology are

needed to determine prognostic and therapeutic implications of these biomarkers.

Conclusion

Cyclin D1 overexpression can be used as a biomarker to recognize subsets of endometrial lesions that may be precancerous or precursor lesion for EC. On the other hand, p53 mutation was unrelated to the development of endometrioid carcinoma from atypical EH but is a marker of tumor aggressiveness. Thus, findings of co-abnormal expression of cyclin D1 and p53 in endometrium may be helpful in planning surgical treatment, chemotherapy, and at the same time, excluding the lesions that may be responsive to hormonal manipulations.

Conflict of Interests

Authors have no conflict of interests.

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References

1. American Cancer Society. Cancer facts and figures 2022 [Online]. American Cancer Society; 2022. Available from: URL: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2022/2022-cancer-facts-and-figures.pdf.
2. Liu L, Habeshian TS, Zhang J, Peeri NC, Du M, De Vivo I, et al. Differential trends in rising endometrial cancer incidence by age, race, and ethnicity. JNCI Cancer Spectr. 2023; 7(1): pkad001.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022; 72(1): 7-33.
4. Sanderson PA, Critchley HO, Williams AR, Arends MJ, Saunders PT. New concepts for an old problem: the diagnosis of endometrial hyperplasia. Hum Reprod Update. 2017; 23(2): 232-54.
5. Mahdy H, Casey MJ, Vadakekut ES, Crotzer D. Endometrial cancer [Online]. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2024. Available from: URL: https://www.ncbi.nlm.nih.gov/books/NBK525981/.
6. Thukral S, Bhat S, Bashir N. Study of expression of PTEN and cyclin D1 in endometrium at a tertiary care centre. Int J Adv Med. 2019; 6(2): 495-501.
7. Liang S, Mu K, Wang Y, Zhou Z, Zhang J, Sheng Y, et al. Cyclin D1, a prominent prognostic marker for endometrial diseases. Diagn Pathol. 2013; 8: 138.
8. Fadare O, Parkash V. P53 aberrations in low grade endometrioid carcinoma of the endometrium with nodal metastases: possible insights on pathogenesis discerned from immunohistochemistry. Diagn Pathol. 2017; 12(1): 81.
9. Ruhul Quddus M, Latkovich P, Castellani WJ, James Sung C, Steinhoff MM, Briggs RC, et al. Expression of cyclin D1 in normal, metaplastic, hyperplastic endometrium and endometrioid carcinoma suggests a role in endometrial carcinogenesis. Arch Pathol Lab Med. 2002; 126(4): 459-63.
10. Ozuysal S, Oztürk 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(2): 123-6.
11. Sindhu A, Kaur S, Kundu PR, Agarwal R, Singh Y, Mahajan P. Evaluation of expression of cyclin D1 in endometrial hyperplasia and endometrial carcinoma. Eur J Pharm Med Res. 2019; 6(1): 344-53.
12. Beavis AL, Najjar O, Cheskin LJ, Mangal R, Rositch AF, Langham G, et al. Prevalence of endometrial cancer symptoms among overweight and obese women presenting to a multidisciplinary weight management center. Gynecol Oncol Rep. 2020; 34: 100643.
13. Sangwan K, Garg M, Pathak N, Bharti L. Expression of cyclin D1 in Hyperplasia and carcinoma of endometrium and its correlation with histologic grade, tumor type, and clinicopathological features. J Lab Physicians. 2020; 12(3): 165-70.
14. Yildirim HT, Nergiz D, Sadullahoglu C, Akgunduz Z, Yildirim S, Dogan S, et al. The extent of cyclin D1 expression in endometrial pathologies and relevance of cyclin D1 with the clinicopathological features of endometrioid endometrial carcinoma. Indian J Pathol Microbiol. 2020; 63(3): 412-7.
15. Abdou AG, Al-Sharaky DR, Kasem HA, Abd El-Wahed MM. Clinicopathological differences between type I and type II endometrial carcinoma. Menoufia Med J. 2017; 30(3): 946-51.
16. Kundu PR, Sindhu A, Kaur S, Kulhria A, Hooda R. Expression of cyclin D1 in normal and hyperplastic

endometrium. Int J Reprod Contracept Obstet Gynecol. 2019; 8(11): 4474-8.
17. Khabaz MN, Abdelrahman AS, Butt NS, Al-Maghrabi B, Al-Maghrabi J. Cyclin D1 is significantly associated with stage of tumor and predicts poor survival in endometrial carcinoma patients. Ann Diagn Pathol. 2017; 30: 47-51.
18. Shih H-C, Shiozawa T, Kato K, Imai T, Miyamoto T, Uchikawa J, et al. Immunohistochemical expression of cyclins, cyclin-dependent kinases, tumor-suppressor gene products, Ki-67, and sex steroid receptors in endometrial carcinoma: positive staining for cyclin A as a poor prognostic indicator. Hum Pathol. 2003; 34(5): 471-8.
19. Kala M, Gill M, Gupta V, Srivastava D, Tanwar P, Goyal V. Cyclin D1 expression in hyperplasia and carcinoma of the endometrium and its correlation with histologic grade and tumor type. J Gynecol Surg. 2014; 30(1): 1-4.
20. Stavropoulos A, Varras M, Vasilakaki T, Varra VK, Tsavari A, Varra FN, et al. Expression of p53 and PTEN in human primary endometrial carcinomas: Clinicopathological and immunohistochemical analysis and study of their concomitant expression. Oncol Lett. 2019; 17(5): 4575-89.
21. Ray S, Jha A, Islam AA, Sengupta M. Study of prognostic and diagnostic significance of P53 and PTEN mutation in proliferative lesions of endometrium. J Curr Med Res Opin. 2020; 3(08): 563-9.
22. Ahmed NY, Isaac RH. P53 expression in endometrial hyperplasia and endometrial carcinoma. Zanco J Med Sci. 2010; 14(2): 28_34.
23. Sahin N, Bezmi, Akatli A, Akpolat N, Yilmaz E, Inci Coskun E, et al. The significance of p53 and estrogen expression in endometrial hyperplasias and endometrioid carcinomas: How should we evaluate immunohistochemistry? Clin Oncol Res. 2018; 1(1): 2-4.
24. Sherman ME, Bur ME, Kurman RJ. P53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorigenesis. Hum Pathol. 1995; 26(11): 1268-74.
25. Akiyama A, Minaguchi T, Fujieda K, Hosokawa Y, Nishida K, Shikama A, et al. Abnormal accumulation of p53 predicts radioresistance leading to poor survival in patients with endometrial carcinoma. Oncol Lett. 2019; 18(6): 5952-8.
26. Hutt S, Tailor A, Ellis P, Michael A, Butler-Manuel S, Chatterjee J. The role of biomarkers in endometrial cancer and hyperplasia: a literature review. Acta Oncol. 2019; 58(3): 342-52.