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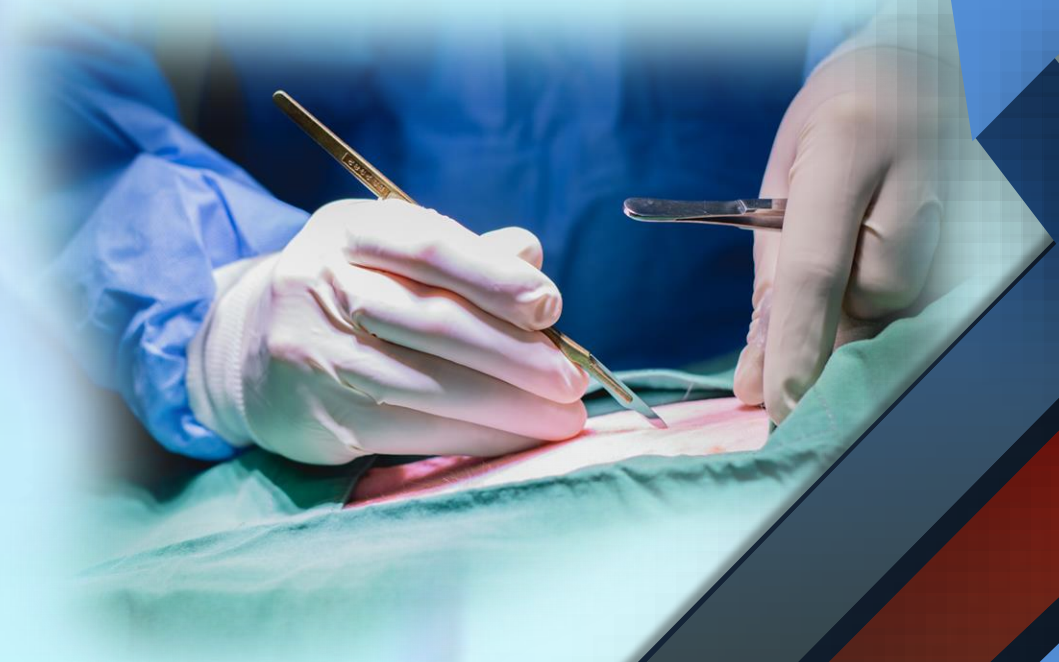

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Original article

Immunohistochemical Expression of Cyclin D1 and PIN1 in Endometrial Carcinoma

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ABSTRACT

Background: Cyclin D1, a positive regular of the cell cycle, may lead to uncontrolled cell proliferation. The overexpression of Cyclin D1 has been associated with numerous tumors' diagnosis and prognosis. PIN1 binds and isomerizes the phosphorylated serine/threonine–proline motif, which leads to alteration in the structure and function of proteins. The altered phosphorylated proteins by PIN1 are closely linked to cancer development. PIN1 is strongly expressed in most tumors, suggesting it promotes tumorigenesis and is negatively associated with the clinical prognosis.

Objectives: To assess Cyclin D1 & PIN1 expression and correlation in endometrial adenocarcinoma. Also, to assess the relationship between Cyclin D1 & PIN1 expression and clinicopathological variables of cases with endometrial carcinoma.

Materials and Methods: The study included 30 cases of endometrial adenocarcinoma specimens. Immunohistochemical staining was performed for both Cyclin D1 and PIN1. Blocks of tumor tissue and clinical data were gathered from Pathology Department of Al-Zahraa University Hospital files between July 2017 and October 2019.

Results: Cyclin D1 positive expression and PIN1 high expression were increased significantly with age, high clinical-stage, high pathological grade, and more myometrium invasion depth. Cyclin D1 expression was positively associated with PIN1 expression (P-value = 0.004).

Conclusions: Cyclin D1 and PIN1 expression are associated with age, stage, grade, and depth of myometrial wall invasion in patients with endometrial carcinoma. The overexpression of Cyclin D1 & PIN1 seems to indicate a more malignant phenotype of endometrial carcinoma.

Keywords: Endometrial carcinoma; Immunohistochemistry; Cyclin D1; PIN1; Prognostic markers.

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* Main subject and any subcategories have been classified according to the research topic

INTRODUCTION

Endometrial carcinoma is the fourth most common cancer that develops in women after breast, bowel & lung cancers [1]. Endometrial carcinomas have been categorized into two types. The most common is Type I, usually endometrioid carcinomas, which are estrogen-dependent with a good prognosis. On the other side, Type II tumors, serous carcinomas, that are not estrogen-dependent with a poor prognosis and affect older age [2].

The presence of many histological subtypes of endometrial carcinoma indicates various tumorigenesis pathways for endometrium cancer development and progression[3], including stepwise attainment of numerous genetic variations in tumor suppressor genes and oncogenes [4].

The transformed cell's proliferation and differentiation processes are controlled by several fostering and preventing elements of the cell cycle[5]. Cyclins are key elements in regulating the cell cycle in combination with their respective cyclin-dependent kinases CDK4 and CDK6, which phosphorylate retinoblastoma protein contribute to release of proteins E2F. These proteins can motivate genes mandatory for the cell's progression to the S-phase in the G1-phase [4].

In eukaryotic cells, being a cyclin family member, Cyclin D1 is a regulator of the cell cycle [6]. Cyclin D1 is a protein encoded by the CCND1 gene located on chromosome 11q13[7]. Mutations, amplification, and over-expression of the CCND1 gene can alter cell cycle progression and cause tumorigenesis [8]. Cyclin D1 is thought to be a possible oncogene. Its gene alteration or amplification is repeatedly realized in many tumors as a clonal pathology [9].

In many cancers, including lung, breast, pancreatic, esophageal, and colorectal cancers, several studies have documented Cyclin D1 overexpression [10-13]. **Nikaido et al.** recorded that 40% of endometrial tumors overexpressed Cyclin D1, indicated that Cyclin D1 might have a role in carcinogenesis of the endometrium [14]. Many studies have also shown that Cyclin D1 expression correlates with histologic grade, stage, and other clinicopathological variables in the endometrial carcinoma cases [15,16].

Peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 [PIN1] binds and isomerizes the phosphorylated proteins serine/threonine–proline motif, which consequently leads to the structural and functional alterations of these proteins. The altered phosphorylated proteins by PIN1 are strictly linked to the development of cancer [17]. PIN1 was initially recognized as a regulator of mitosis, and several studies found that it promotes multiple proliferation promoting pathways in cancer [18]. PIN1 is also recognized as an essential regulator of the cell cycle [19]. Abnormalities in PIN1 expression has been known to be included in many physiological and pathological conditions like; immune response, apoptosis and, different types of cancer [20].

Chromosomal instability and tumorigenesis are caused by PIN1 overexpression [21]. Its over-expression in cancer is associated with a specific malignant phenotype [22]. Also, high PIN1 expression in cancer patients is associated with a poor clinical outcome and metastasis of the lymph nodes [23]. PIN1 over-expression can stimulate the expression of downstream genes, including Cyclin D1 through Ras, Wnt/β-catenin, and C-Jun/AP-1 pathways, leading to abnormality in cell cycle, abnormal cell metabolism, excessive proliferation and even tumorigenesis [24].

PIN1 inhibitors have recently been developed elsewhere, using structure-based drug designs and natural compounds that inhibit cancer activity. Certainly, PIN1 may be a desirable target for cancer management and treatment [17].

Although PIN1 inhibitors and PIN1-targeted gene therapy have received considerable attention, PIN1 expression and its association with Cyclin D1 in endometrial cancer have not been clarified well [25].

AIM OF THE WORK

In this study, we detected the expression of Cyclin D1 & PIN1 in different cases of endometrial adenocarcinoma by immunohistochemistry to explore the possibility of Cyclin D1 and PIN1 as diagnostic and prognostic markers in endometrial cancer. Also analyzed their association with clinicopathological parameters of cases like age, histopathological grade, clinical stage & depth of myometrium invasion. The study may elucidate the

role of Cyclin D1 & PIN1 in the pathogenesis of endometrial cancer.

MATERIAL AND METHODS

Tissue Specimens:

Formalin-fixed paraffin-embedded 30 endometrial adenocarcinoma blocks from the Pathology Department of Al-Zahraa University Hospital files were collected and prepared for this retrospective study, between July 2017 and October 2019, after obtaining informed consent and approval from the local ethics committee. Clinicopathological information was extracted from medical charts. All patients underwent surgical intervention [Total abdominal hysterectomy and bilateral salpingio-ophrectomy]. The staging was assessed according to the International Federation of Gynecology and Obstetrics system [FIGO 2009 Staging System]. Grading was assessed according to International Federation of Gynecology & Obstetrics criteria [FIGO histologic classification].

Three sections of 5-micron thickness were split from the paraffin blocks; one section was stained with hematoxylin & eosin to reassess the diagnosis and determine the histopathological grading and staging of tumors; the other two sections were mounted on positively charged slides and immune-stained by Cyclin D1 & PIN1.

Immunohistochemistry:

For the immunohistochemical study, positively charged slides [Biogenix] were prepared from each paraffin block and immunostained with primary antibodies: mouse monoclonal antibody against Cyclin D1 [San Francisco, USA, diluted 1:200] and rabbit polyclonal antibody against PIN1 [San Diego, CA, USA, diluted 1:100]. Immunohisto-chemical reactions were performed using Labeled Streptavidin-Biotin2 System- Horseradish Peroxidase [LSAB2 System-HRP], based on a modified labeling technique called Avidin-Biotin [LAB], in which a secondary biotinylated antibody forms a complex with peroxidase-conjugated streptavidin molecules.

The entire antibody complex is rendered noticeable with the addition of an effective substrate chromogen reagent, which is transformed by the peroxidase label to brown-colored precipitate at the site of antigen in the tissue. Diaminobenzidine [DAB] developed by Dako [USA], is the chromogen used.

Positive and Negative Control:

Tissue was processed by phosphate buffer solution instead of the primary antibody, which was used as a negative control. A positive external control, represented by breast carcinoma sections for both markers.

Evaluation of Immunostaining:

For both markers, positive staining was indicated as brown color in the cells' nucleus. Scoring was done by taking into account both the intensity of staining and the extent [ratio of positive cells].

Regarding Cyclin D1, the intensity of the staining was categorized as: no staining [0], weak [+1], moderate [+2], or strong [+3]. The extent was semi-quantitatively estimated; a score of [0] was assigned when <10% of cells were positive, while when 11% to 30% cell positivity was documented a score of [+1] was recorded. When 31% to 60% positivity were documented, a score of [+2] had been assigned, and > 60% positive cells were scored as [+3]. If the sum of the two scores was more than 1, the case was considered cyclin D1-positive. A cases with a score of ≤1 was considered negative expression [8].

Regarding PIN1, the stain intensity was categorized as: no staining [0], weak [+1], moderate [+2] or strong [+3]. Positive staining rate of 0%-9% was scored as [0], 10%-33% was scored as [1], 34%-66% was scored as [2], and 67% or higher was scored as [3]. If the sum of the average intensity score and average stinging rate score was above 3, the case was considered high PIN1 expression. Otherwise, the case was considered low PIN1 expression [25].

Statistical analysis: Recorded data were analyzed using the statistical package for social sciences, version 20.0 [SPSS Inc., Chicago, Illinois, USA]. Quantitative data were expressed as mean± standard deviation [SD]. Qualitative data were expressed as frequency and percentage. The following tests were done: Chi-square [x^2] test of significance was used in order to compare proportions between qualitative parameters, Pearson's correlation coefficient [r] test was used

to calculate the degree of association between two sets of variables. The confidence interval was set to 95%, and the margin of error accepted was set to 5%. So, the P-value was considered significant as the following: P-value ≤ 0.05 was considered significant, P-value ≤ 0.001 was considered as highly significant, P-value > 0.05 was considered insignificant.

RESULTS

Thirty cases of endometrial adenocarcinoma were enrolled in this study. The age of the patients ranged from 39 to 71 with a mean [53.63], 11 cases were less than 50 years [36.7%], while 19 cases were older than or equal 50 years [63.3%] **[Table 1].**

The staging was evaluated based on the International Federation of Gynecology & Obstetrics system [FIGO 2009 staging system], and it was divided into 17 cases were in stage I [56.6%], 8 in stage II [26.7%], 5 in stage III/IV patients [16.7%]. Grading was assessed according to International Federation of Gynecology & Obstetrics criteria [FIGO histologic classification], and it was divided into 10 cases well-differentiated Grade I [33.3%], 13 moderately-differentiated Grade II [43.4%], and 7 poorly-differentiated Grade III [23.3%] adenocarcinoma **[Table 1].**

According to the depth of myometrium invasion, 11 cases had the depth of myometrium invasion less than 1/2 [36.7%], and the remaining 19 cases had the depth equal or more than 1/2 [63.3%] **[Table 1].**

Immunohistochemical expression of Cyclin D1 and PIN1: For both markers, positive staining was indicated as brown color in the cells' nucleus. Regarding Cyclin D1 expression, 13 cases showed positive expression [43.3%], and the remaining 17 cases showed negative expression [56.7%]. In contrast, PIN1 expression showed 19 cases with high expression [63.3%] and 11 cases with low expression [36.7%] **[Table 1].**

Correlation of Cyclin D1 & PIN1 expression with age groups: Regarding age groups, the ratio of positive Cyclin D1 expression in the age group ≥ 50 years [84.6%] was significantly higher than that in age group < 50 years [15.4%] with a [P-value = 0.034]. Whereas the ratio of high PIN1 expression in age group ≥ 50 years [89.5%] was highly significantly more than that in age group < 50 years [10.5%] with a [P-*value* < 0.001] **[Table 2].**

Correlation of Cyclin D1 expression with clinic-pathological parameters*:* According to the grade of the tumor, the ratio of positive Cyclin D1 expression in Grade III [85.7%] was significantly higher than that in Grade II [38.5%] and Grade I [20.0%] with a [P-value = 0.024] [Figure 1]. According to the stage of the tumor, the ratio of positive Cyclin D1 expression in Stage III/IV [80.0%] was significantly higher than that in Stage II [62.5%] and Stage I [23.5%] with a [P-value = 0.036]. Regarding to depth of myometrium invasion, the ratio of positive Cyclin D1 expression in patients with the depth of myometrium invasion ≥ 1/2 [57.9%] was significantly higher than that in patients with the depth of myometrium invasion < 1/2 [18.2%] with a [P-value = 0.034] **[Table 3].**

Correlation of PIN1 expression with the clinico-pathological parameters: According to the grade of the tumor, the ratio of high PIN1 expression in Grade III [100.0%] was significantly higher than that in Grade II [69.2%] and Grade I [30.0%] with a [P-value = 0.011] [Figure 2]. According to the stage of the tumor, the ratio of high PIN1 expression in Stage III/IV [100.0%] was significantly higher than that in Stage II [75.0%] and Stage I [47.1%] with a [P-value = 0.046]. Regarding to depth of myometrium invasion, the ratio of high PIN1 expression in patients with the depth of myometrium invasion ≥ 1/2 [84.2%] was significantly higher than that in patients with the depth of myometrium invasion < 1/2 [27.3%] with a [P-value = 0.002] **[Table 4].**

Correlation between Cyclin D1 & PIN1 expression: Out of 19 cases with high PIN1 expression, 12 cases showed positive Cyclin D1 expression [63.2%] compared to only 7 cases that showed negative Cyclin D1 expression [36.8%]. Whereas Out of 11 cases with low PIN1 expression, there were 10 cases showed negative Cyclin D1 expression [90.9%] compared to only one case that showed positive Cyclin D1 expression [9.1%]. There was a higher positive Cyclin D1 expression in cases with high PIN1 expression [63.2%] compared to cases with low PIN1 expression [9.1%]. So, we have a statistically significant correlation between Cyclin D1 & PIN1 expression in our cases with a [P-value = 0.004] **[Table 5].**

Table [1]: Distribution of patients according to their Age group, Grade, Stage, Depth of myometrium invasion, Cyclin D1 and PIN1 expression

	Parameters	No.	%
Age	<50 years	11	36.7%
	≥50 years	19	63.3%
	Range [Mean±SD]	39-71 [53.63±9.71]	
Grade	Grade I	10	33.3%
	Grade II	13	43.4%
	Grade III	7	23.3%
Stage	Stage I	17	56.6%
	Stage II	8	26.7%
	Stage III/IV	5	16.7%
Depth of Myometrium invasion	< ½	11	36.7%
	≥ ½	19	63.3%
Cyclin D1 expression	Positive expression [>1]	13	43.3%
	Negative expression [≤1]	17	56.7%
PIN1 expression	High expression [>3]	19	63.3 %
	Low expression [≤3]	11	36.7%

Table [2]: Correlation between age group with Cyclin D1 and PIN1

		< 50 years [n=11]		≥ 50 years [n=19]		Total [n=30]	Pearson's R	x2	P-value
		No.	%	No.	%				
Cyclin D1	Positive expression	2	15.4%	11	84.6%	13	0.386	4.474	0.034*
	Negative expression	9	52.9%	8	47.1%	17			
PIN1	Low expression	9	81.8%	2	18.2%	11	0.713	15.248	<0.001**
	High expression	2	10.5%	17	89.5%	19			

x^2: Chi-square test; R-Pearson Correlation Coefficient; *P-value <0.05 Significant; **P-value <0.001 highly significant

Table [3]: Correlation between Cyclin D1 with grade, stage and myometrium invasion

		Positive expression [n=13]		Negative expression [n=17]		Total [n=30]	Pearson's R	x2	P-value
		No.	%	No.	%				
Grade	Grade I	2	20.0%	8	80.0%	10	0.478	7.463	0.024*
	Grade II	5	38.5%	8	61.5%	13			
	Grade III	6	85.7%	1	14.3%	7			
Stage	Stage I	4	23.5%	13	76.5%	17	0.462	6.650	0.036*
	Stage II	5	62.5%	3	37.5%	8			
	Stage III/IV	4	80.0%	1	20.0%	5			
Myometrium invasion	<1/2	2	18.2%	9	81.8%	11	0.386	4.474	0.034*
	≥1/2	11	57.9%	8	42.1%	19			

x^2: Chi-square test; R-Pearson Correlation Coefficient; *P-value < 0.05 Significant

Table [4]: Correlation between PIN1 with grade, stage and myometrium invasion

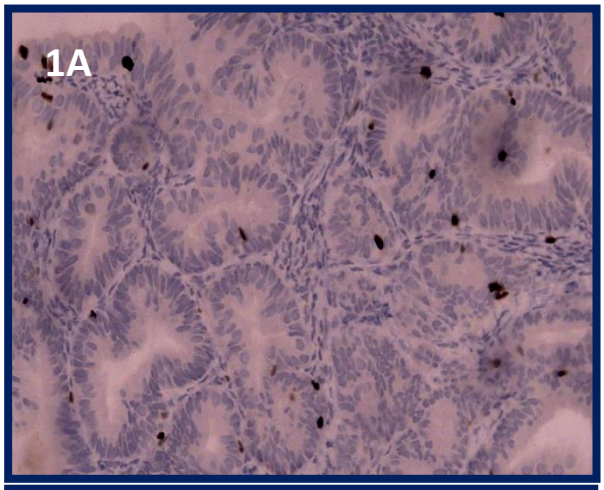
		Low expression [n=11]		High expression [n=19]		Total [n=30]	Pearson's R	x2	P-value
		No.	%	No.	%				
Grade	Grade I	7	70.0%	3	30.0%	10	0.547	9.032	0.011*
	Grade II	4	30.8%	9	69.2%	13			
	Grade III	0	0.0%	7	100.0%	7			
Stage	Stage I	9	52.9%	8	47.1%	17	0.420	5.303	0.046*
	Stage II	2	25.0%	6	75.0%	8			
	Stage III/IV	0	0.0%	5	100.0%	5			
Myometrium invasion	<1/2	8	72.7%	3	27.3%	11	0.569	9.726	0.002*
	≥1/2	3	15.8%	16	84.2%	19			

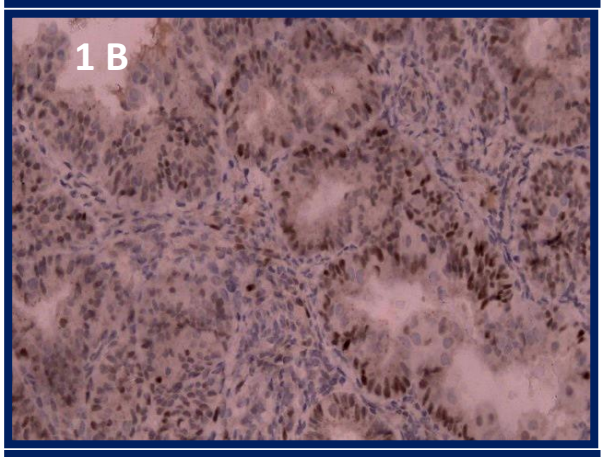
x^2: Chi-square test; R-Pearson Correlation Coefficient; *P-value < 0.05 Significant

Table [5]: Correlation between Cyclin D1 and PIN1

	Positive expression [n=13]		Negative expression [n=17]		Total [n=30]	Pearson's R	x2	P-value
	No.	%	No.	%				
Low expression	1	9.1%	10	90.9%	11	0.526	8.294	0.004*
High expression	12	63.2%	7	36.8%	19			

x^2: Chi-square test; R-Pearson Correlation Coefficient; *P-value <0.05 Significant





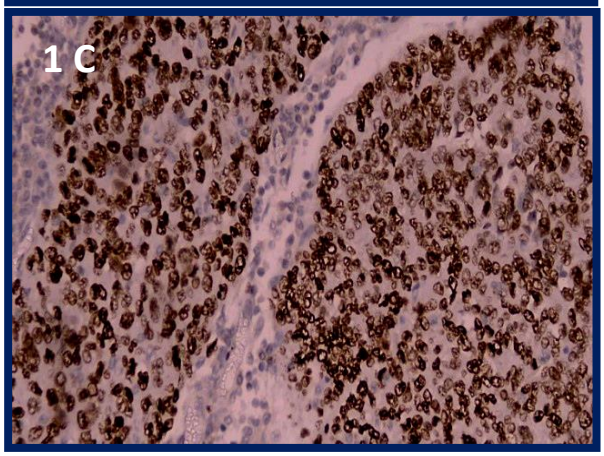
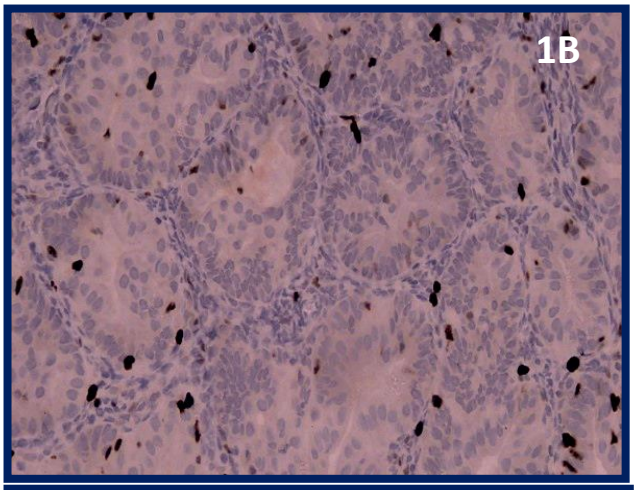
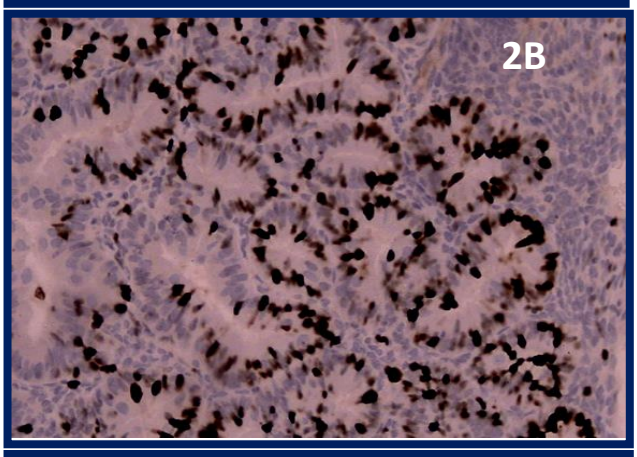


Figure 1: [A] Endometrial carcinoma GI showing negative expression of Cyclin D1 [× 200]; [B] Endometrial carcinoma GII showing positive nuclear expression of Cyclin D1 [× 200]; [C] Endometrial carcinoma GIII showing positive nuclear expression of Cyclin D1 [× 200].





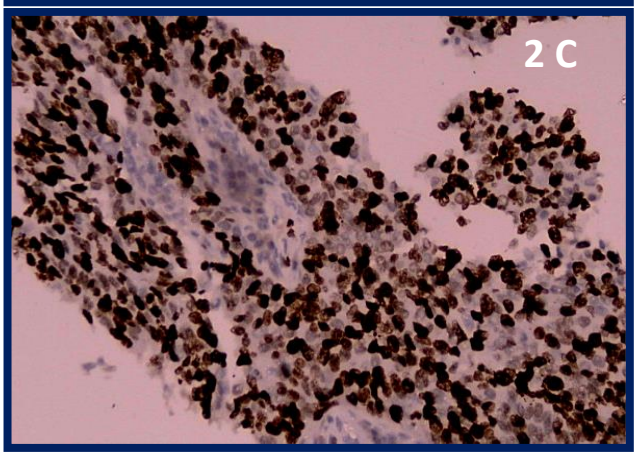


Figure 2: (A) Endometrial carcinoma GI showing low nuclear expression of PIN1 (× 200); (B) Endometrial carcinoma GII showing high nuclear expression of PIN1 (× 200); (C) Endometrial carcinoma GIII showing high nuclear expression of PIN1 (× 200).

DISCUSSION

Endometrial cancer is the most common malignancy of the female genital system. Endometrial carcinoma development involves the stepwise acquisition of several genetic alterations involving oncogenes and tumor suppressor genes [4]. Various prognostic factors have been widely researched to improve the patients' management, treatment, and follow-up.

Cyclin D1 is a core cell cycle protein that promotes cellular proliferation by activating CDK4/6 kinases and the G1/S phase of the cell cycle. CCND1 gene acts as an oncogene and is frequently overexpressed in many types of cancer, including endometrial carcinoma, often via gene amplification or gene rearrangement [26]. Thus it is an essential sensor and activator of initiation and progression of the cell cycle [7].

PIN1 plays an essential part in the transition from G1/S to G2/M [27]. It controls protein function via conformational modifications of the target protein, and it is associated with the oncogenic pathway activation by regulating tumor suppressors and oncogenes. In cancer tissues & cancer stem cells [CSCs], PIN1 is overexpressed and associated with poor prognosis in different patients with cancer [17].

It has been known that the Cyclin D1 gene is one of the specific downstream targets of PIN1. PIN1 can stimulate overexpression of Cyclin D1[28].

PIN1 and Cyclin D1 overexpression can cause an imbalance in cell metabolism, disrupt the cell cycle and eventually promote normal cells' transformation into cancer cells [24].

Our study cases were subdivided according to tumor grade, tumor stage, and invasion depth to the myometrial wall.

Regarding the grade, most cases in this study were Grade II [43.4% of cases]; these findings are in agreement with **Yan *et al.*** [25], who reported that [42.3%] of their patients with endometrial cancer were Grade II.

In contrast, this result differs from that reported by **Nishimura *et al.*** [29] who found that the most cases in their study were Grade I [55.3%]. Also, **Khabaz *et al.*** [9] reported that about [58%] of their patients with endometrial cancer were Grade I.

Regarding the stage, most cases in this study were stage I [56.6% of cases]; this is in accordance with the results of **Yan *et al.*** [25], who reported that [44.2%] of their endometrial carcinoma cases were stage I.

Also, our finding is in line with **Nishimura *et al.*** [29] who reported that the most cases in their study were stage I [64.5%]. Also, this is nearly in agreement with **Khabaz *et al.*** [9] who reported that [69.6%] of their endometrial carcinoma staged cases were stage I.

According to the depth of myometrium invasion, most cases in our study had depth $\geq$1/2 [63.3%]. This is in accordance with the results of **Yan *et al.*** [25] who reported that [63.5%] of their cases had the depth of myometrium invasion $\geq$ 1/2.

In contrast, our result differs from that reported by **Liang *et al.*** [7], who found that [70.3%] of their endometrial carcinoma cases had depth <1/2.

In this current study, the patients' age ranged from 39 to 71 with a mean [53.63]; this is consistent with the study of **Khabaz *et al.*** [9] who reported that their patients' average age was fifty-five years [ranging 26–86 years.]

In our study, most cases were $\geq$ 50 years [63.3%]; this is also consistent with **Yan *et al.*** [25] who reported that [59.6%] of their patients were aged 50 or older.

Regarding Cyclin D1 expression, [43.3%] of our cases showed positive expression. These findings are almost comparable, with slight differences, to the data reported by different studies like **Nikaido *et al.*** [14] who reported Cyclin-D1 positive expression in 40% of their cases compared with [68%] detected in **Quddus *et al.*** [30].

Nishimura *et al.* [29] reported [46.1%] of their

patients with endometrioid adenocarcinomas were positive for Cyclin D1.

Ozuysal *et al.*[31] detected Cyclin D1 positive expression in [26.6%] of their cases.

Liang *et al.* [7] detected expression of Cyclin D1 in [52%]. **Khabaze *et al.*[9]** reported that [56.3%] of their cases showed positive expression of Cyclin D1.

Yan *et al.*[25] demonstrated that [42.3%] of their cases exhibited positive expression of Cyclin D1. While **Suri *et al.* [32] & Thukral *et al.*[4]** detected that [85.71%] of carcinoma endometrium were positive for Cyclin D1.

Regarding PIN1 expression, [63.3%] of our cases showed high expression. This is in accordance with the results of **Tian *et al.*[33]** who reported that PIN1 was overexpressed in [66%] of their endometrial carcinoma cases.

These results are also partially agree with **Yan *et al.*[25]** who reported that [53.9%] of endometrial cancer cases exhibited high PIN1 expression.

According to age groups, the ratio of positive Cyclin D1 and high PIN1 expression was significantly higher in age group ≥ 50 years [84.6%, P-value = 0.034 & 89.5%, P-value < 0.001 respectively]. These findings are not in agreement with **Liang *et al.* [7]** who reported that expression of Cyclin D1 was not correlated with age [P-value > 0.05]. Also, **Yan *et al.*[25]** demonstrated no association between PIN1 & Cyclin D1 expression and age [P = 0.457, 0.483 respectively]. Otherwise, **Khabaze *et al.*[9]** reported that positive cyclin D1 staining has been significantly related to patient age [P = 0.0001], but more positive expression was noted in females who are <40 years of age.

In our current study, the ratio of positive expression of Cyclin D1 showed a statistically significant stepwise increase from Grade I [20.0%] to Grade II [38.5%] to Grade III [85.7%] with a [P-value = 0.024]. These findings are in line with **Yan *et al.* [25]** who reported that the ratio of positive Cyclin D1 in GIII [90.0%] was significantly increased than that in GII and I [36.4% & 25.0% respectively, P = 0.020].

Also. **Nishimura *et al.* [29]** reported that the positive rate for cyclin D1 in GI, GII and GIII was [39.7%**,** 41.2% & 69.0%] respectively, elucidated that Cyclin D1 expression increased in higher histological grade [P-value = 0.0071].

Nikaido *et al.* [14], Shih *et al.* [16], Ozuysal *et al.* [31] & **Wu *et al.* [15]** also detected that cyclin D1 was positively correlated with histopathological grade. While **Khabaze *et al.* [9]** detected no significant association of Cyclin D1 staining with grade [P-value = 0.239], in line with many studies **De Jong *et al.* [34] Kala *et al.* [35].**

The ratio of positive expression of Cyclin D1 in Stage III/IV [80.0%] was significantly higher than that in Stage II [62.5%] and Stage I [23.5%] with a [P-value = 0.036]. This finding is in line with **Yan *et al.* [25],** who reported that the ratio of positive Cyclin D1 in stage III/IV [81.8%] was significantly increased when compared to stage II & stage I [55.6% & 13.0% respectively, P = 0.000].

Also, **Nikaido *et al.* 14] & Shih *et al.*[16]** reported that Cyclin D1 was positively correlated with the stage. **Khabaze *et al.*[9]** detected that Cyclin D1 expression was significantly associated with stage [P-value = 0.029], but the negative expression is more in stage III and IV [66.7%]. While **Nishimura *et al.*[29]** reported no statistically significant correlation between Cyclin D1 expression and FIGO stage.

Regarding to depth of myometrium invasion, the ratio of positive Cyclin D1 expression in patients with depth ≥1/2 [57.9%] was significantly higher than that in patients with depth <1/2 [18.2%] with a [P-value = 0.034]. These findings are in accordance with the results of **Yan *et al.*[25],** who reported that the ratio of high Cyclin D1 expression in patients with the depth of myometrium invasion ≥ 1/2 [60.6%] was significantly higher than that in other patients with depth < 1/2 [10.5%], [P = 0.000]. Also, **Nikaido *et al.* [14], Shih *et al.* [16], Ozuysal *et al.* [31] & Wu *et al.*[15]** reported a

significant correlation between Cyclin D1 immunoreactivity and degree of invasion to deep myometrium. In contrast, **Nishimura *et al.*[29]** reported that there was no statistically significant correlation between Cyclin D1 expression and myometrium invasion. Also, **Liang *et al.* [7]** reported that Cyclin D1 expression was 60% in the non-invasion group, 48% in <1/2 invasion group and 58% in ≥1/2 invasion group [P-value > 0.05], which meant that Cyclin D1 had no relationship with tumor invasion.

This study also demonstrates that ratio of high PIN1 expression showed statistically significant stepwise increase from Grade I [30.0%] to Grade II [69.2%] to Grade III [100.0%] with a [P-value = 0.011]. This is in accordance with the result of **Yan *et al.* [25]** who reported that the ratio of high PIN1 expression in G3 [100%] was significantly higher than that in G2 and 1 [50.0% & 35.0% respectively, P = 0.020]. On the other side, **Saegusa *et al.* [36],** reported that immunoreactivity for PIN1 showed stepwise decreases from G1 to G2 to G3 tumors. Also, **Tian *et al.*[33],** detected that expression of PIN1 was significantly decreased with tumor differentiation [P < 0.05].

According to the stage of the tumor, the ratio of high PIN1 expression in Stage III/IV [100.0%] was significantly higher than that in Stage II [75.0%] and Stage I [47.1%] with a [P-value = 0.046]. This result is consistent with the result of **Yan *et al.* [25],** who reported that the ratio of high PIN1 expression in stage III/IV patients [90.9%] was significantly higher compared with stage II and I patients [61.1% & 30.4% respectively, P = 0.030]. In contrast, **Saegusa *et al.* [36]** detected that high expression for PIN1 was correlated significantly with an early stage.

Regarding to depth of myometrium invasion, the ratio of high PIN1 expression in patients with depth ≥1/2 [84.2%] was significantly higher than that in patients with depth <1/2 [27.3%] with a [P-value = 0.002]. This result is in line with the result of **Yan *et al.* [25]** who reported that the ratio of high PIN1 expression in patients with the depth of myometrium invasion ≥1/2 [75.8%] was significantly higher than that in patients with depth <1/2 [15.8%], [P = 0.000]. In comparison, **Saegusa *et al.* [36]** reported that PIN1 expression showed no significant correlation with the degree of myometrium invasion. Whereas **Tian *et al.*[33]** detected that expression of PIN1 was negatively associated with depth of myometrium invasion.

In our study, there was a higher positive Cyclin D1 expression in cases with high PIN1 expression [63.2%] compared to cases with low PIN1 expression [9.1%]. So, we have a statistically significant positive correlation between Cyclin D1 & PIN1 expression in our cases with a [P-value = 0.004]. These findings are in line with **Yan *et al.*[25]** who reported that among their cases with high PIN1 expression, [64.3%] were cyclin D1-positive, suggesting a higher positive cyclin D1 expression rate in endometrial cancer patients with high PIN1 expression [P = 0.001].

This finding indicates a close association between Cyclin D1 and PIN1. But we had 7 cases with high PIN1 expression showed negative Cyclin D1 expression [36.8%], indicating that PIN1 may affect the incidence and progress of endometrial carcinoma without involving the Cyclin D1 pathway.

In summary, our results suggested a significant association between positive Cyclin D1 & high PIN1 expression with older age, higher clinical stage, higher pathological grade, and more depth of myometrium invasion. More future studies on the role of Cyclin D1 & PIN1 in endometrial carcinogenesis are needed.

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None

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