Original Article

Study of the Association of Phosphatase and Tensin Homolog and p27 Expressions in Endometrial Hyperplasia and Carcinoma

Ihab Shafek Atta

Department of Pathology, Faculty of Medicine, Assuit, Al-Azhar University, Egypt

Abstract

Introduction: Phosphatase and tensin homolog (PTEN) and p27 are commonly mutated gene in endometrial carcinoma (EC) and their association in development of EC has not been fully understood. **The Aim of the Study:** The aim is to clarify the association of PTEN and p27 in EC and their correlation with the histologic grade. **Material and Methods:** Paraffin-embedded 20 and 50 specimens representing EH and EC were collected, cut into 4 mm thick and stained with H&E stain for histopathological examination. All EC cases were graded according to the percentage of nonsquamous solid pattern into 3 grades. Immunohistochemical (IHC) analyses were done using a rabbit polyclonal anti-PTEN antibody and a rabbit monoclonal anti-p27 antibody. Evaluation of reactivity was categorized: 1+ (weak) = less than 10%, 2+ (moderate) = 11 to 50% and 3+ (strong) = more than 50% tumor. *t*-test, one way ANOVA and chi-square test were used in the statistical analysis. **Results:** Loss of PTEN was seen in 7/20 (35%) and 29/50 (58%) of EH and EC cases with significance (P =0.01824), opposite to 17/20 (85%) and 25/50 (50%) of p27 (P = 0.00334). Both antibodies showed significance in EH cases only (P = 0.00019). No correlation with the histological grade for both antibodies. Four major categories were formulated; PTEN+/p27+ (n = 2, 14, 10%, 28%), PTEN+/p27- (n = 5, 7; 25% and 14%), PTEN-/p27+ (n = 1, 11; 5%, 22%) PTEN-/p27- (n = 12, 18; 60%, 36%) cases of EH and EC, respectively with no significant difference obtained. **Conclusion:** Not all cases of PTEN negative EC showing p27 loss and vice versa. Despite many studies reacted with PTEN and p27 expression in EC, none of them is confirmatory to adjust the correlation between them in EC. So, more studies must be done to correlate between the degree of PTEN loss and p27 comprising all subtypes and grading of EC.

Keywords: Endometrial carcinoma, endometrial hyperplasia, endometrium, p27, phosphatase and tensin homolog

Introduction

Endometrial carcinoma (EC) is reported as the fourth most common cancer of women worldwide and is considered one of the most common malignancies affecting the female genital tract.[1] However, its molecular carcinogenesis has not been fully understood. Phosphatase and tensin homolog (PTEN) is the most commonly mutated gene recognized in EC and is one of a tumor suppressor genes, which mapped on 10q23, and modifications of this gene have been recognized in a wide variety of cancers together with EC.[2,3] PTEN is a lipid phosphatase which acts as dephosphorylating agent on the subsequent messenger of P13K2 through the cascades of phosphatidylinositol 3,4,5-triphosphate.[2,4] The 30%–50% PTEN mutations' prevalence in EC ranks at the top of analyzed tumors.[5] Furthermore, the mutations were discovered in about 20% of cases of endometrial hyperplasia (EH), an antecedent of EC.[6] Therefore, the PTEN inactivation is measured to be the early episode in endometrial carcinogenesis.

Multiple lines of evidence support a role for the PTEN as one such marker for EC. The potential role of PTEN is to inhibit some tumor suppressor genes such as p27, p53, and p21 by producing a second messenger for the AKT pathway.[3] It also regulates proliferation, growth, and apoptosis in a phosphatidylinositol 3-kinase (PI3K)-dependent pathway.[2,7] Finally, to antagonize the action of PI3K activity

Access this article online

Quick Response Code:

Website: http://www.jmau.org/

DOI: 10.4103/JMAU.JMAU_54_18

Address for correspondence: Dr. Ihab Shafek Atta, Department of Pathology, Faculty of Medicine, Assuit, Al-Azhar University, Egypt.
E-mail: Ihab.bassyouny@azhar.edu.eg

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Atta IS. Study of the association of phosphatase and tensin homolog and p27 expressions in endometrial hyperplasia and carcinoma. J Microsc Ultrastruct 2019;7:109-16.

through inhibition of its downstream target; serine/threonine kinase AKt.[8] Phosphorylated-activated Akt adjusts the activity of a diversity of downstream protein cascades that narrate to cell proliferation and survival.[1] The activated Akt phosphorylates followed by inactivation of the proapoptotic factor as Bax suppresses the apoptotic process and hence promotes cell survival and proliferation.[9,10] PTEN inactivation associated with activation of PI3K together will give rise to AKT phosphorylation, as well as accumulation of beta-catenin in nuclei, and hence, activation of gene transcription occurs.[11]

Alternatively, the PTEN overexpression hinders cell growth and encourages a G1 arrest with augmenting the cell cycle kinase inhibitor p27,[12,13] signifying that PTEN inactivation consequences may lead to cell cycle progression via downregulation of p27. Hence, the loss of PTEN function with subsequent activation of PI3K and Akt/protein kinase B signaling pathway plays a considerable role in the pathogenesis of cancer. However, those conclusions are somewhat less obvious, poorly understood and have to be clarified more in EC. Loss of PTEN expression in mice gives rise to both EH and EC.[14,15] Furthermore, mutations in PTEN have been found in patients with Cowden syndrome.[16]

On the other hand, the cell cycle regulator and tumor suppressor p27 play a key role in the development of EC. p27 is a member of the cyclin-dependent kinase interacting protein/kinase inhibitory protein (Cip/Kip) family of cyclin-dependent kinase inhibitors (CKIs), which function to negatively regulate cell cycle progression.[17] The cell cycle and phosphorylation-dependent events that control p27 levels and subcellular localization are catalyzed by different kinases that regulate degradation and nuclear-cytoplasmic shuttling. Nuclear p27 is targeted for degradation by Skp2 E3 ligase.[18] Phosphorylation of p27 by several kinases including AKT causes nuclear p27 to be exported to the cytoplasm.[19,20] In the cytoplasm, p27 is targeted for degradation by a different E3-ligase.[21] In addition to targeting p27 for degradation, phosphorylation also can stabilize this CKI and sequester it in the cytoplasm. In the cytoplasm, p27 has been shown to and mediate cell migration and metastasis by blocking Rho stress fiber formation[22-24] and be antiapoptotic.[25] Dysregulation of p27 has been reported in EC, most commonly as a result of the decreased p27 expression.[26-28] During the progression of EC, normal regulation of p27 is lost, with p27 being absent or undetectable in a significant proportion of EH.[29] Alternatively, p27 expression in some EC is expressed in the cytoplasm.[30] In addition to aberrant p27, alterations in the PTEN tumor suppressor and other cell cycle regulators signaling pathway comprise the important mutation group for development of EC.[31,32]

Although many studies reacted with PTEN and p27 expression in EC, none of them is confirmatory to adjust the correlation between them in EC. Hence, the aim is to stand on the accurate expression of both antibodies in EC, to find a correlation with the histological grade, and to detect the extent of their association and correlation in EC cases.

Materials and Methods

Paraffin-embedded 20 and 50 archival specimens representing EH and EC were collected from the Department of Pathology, Al-Azhar University Hospitals, and from some private laboratories after following the ethical guidelines and obtaining the written permission. These specimens were collected from the period between 2010 and 2017. All specimens were cut into 4-mm thick sections and stained with H and E stain for histopathological examination.

All EC cases were classified according to the International Federation of Gynecology and Obstetrics (FIGO)[33] criteria based on the percentage of nonsquamous or nonmorular solid pattern; Grade 1: <5%, Grade II: 6%–50%, and Grade 3: >50%.

For immunohistochemical (IHC) analyses, the tissue sections were cut at 4 μm and mounted on poly-L-lysine-coated slides. IHC for PTEN was done using a rabbit polyclonal anti-PTEN antibody, ab31392 (Abcam, Cambridge, UK) at a concentration of 100 μg at 1 mg/ml. p27 was done using a rabbit monoclonal anti-p27 antibody KIP 1, ab32034 (Abcam, Cambridge, UK) at a concentration of 100 μg at 0.52 mg/ml.

The staining procedure was automated and consisted of incubation for 45 minutes with the primary antibody, then washed by a brief buffer followed by an incubation with biotinylated anti-mouse IgG/IgM (Abcam, Cambridge, UK) for thirty minutes. The slides were subsequently incubated with avidin/biotin (Abcam, Cambridge, UK) for 30 minutes and reacted with diaminobenzidine (DAB) and hydrogen peroxide (H_2O_2). The sections were counterstained with hematoxylin. Evaluation of staining reactivity was done and any degree of brownish nuclear PTEN and nuclear/cytopasmic p27 reactivity were considered as a positive.[19,20] The percentage of positive cells were calculated and the staining category was graded as follows: 1+ (weak) = less than 10% of cells are stained positive, 2+ (moderate) = 11 to 50% of the stained cells are positive, and 3+ (strong) = more than 50% tumor cells stained positive.[34-37]

t-test was used to compare the degree of reactivity between the two lesions (EH and EC) for PTEN and p27 in a separate manner. Also, one way ANOVA test was used to compare the degree of PTEN and p27 reactivity data with the histological FIGO grading. Chi-square test was used to measure the extent of association of the two antibodies in both endometrial hyperplasia and carcinoma cases (positive PTEN/ positivep27, positive PTEN/negative p27, negative PTEN/ and positive 27, and negative PTEN/negative p27). All *P* values were two-sided. $P \leq 0.05$ was considered statistically significant. Computer software Statistical Package for the Social Science (SPSS) version 16 (Statistics for Windows, IBM Corp., Armonk, N.Y., USA) was used in the analysis of the current study.

Results

Staining of the 20 cases of EH with anti-PTEN antibody revealed that 7/20 (35%) showed negative staining, weak in six (30%), moderate in four (20%), and strong in three (15%)

cases [Figures 1 and 2]. Regarding EC, loss of PTEN expression or negative reactivity was found in 29/50 (58%) cases, weak in nine cases (18%), moderate in seven (14%) cases, and strong in five (10%) cases [Figures 3, 4 and Graph 1]. A significant *P* value was obtained between PTEN expression in EH and EC with $P = 0.0184$ [Table 1].

Regarding p27 expression in EH, loss of expression was found in 17/20 (85%) cases and weak in three (15%) cases, and no moderate or strong expression was found [Figures 1and 2]. Concerning EC, loss of p27 expression or negative reactivity was found in 25/50 (50%) cases of EC, weak in 16 (32%) cases, moderate in six cases (12%), and strong in three cases (6%) [Figures 3 and 4]. A significant *P* value was obtained between p27 expression in EH and EC ($P = 0.00334$) [Table 1].

In EH, a significant *P* value was obtained between expressions of PTEN with that of p27 with $P = 0.00019$, while in EC, no significant *P* value was obtained between expressions of PTEN with that of p27 with $P = 0.4591$.

In EC cases, the cases were divided into Grade I ($n = 30$, 60%), II ($n = 16$, 32%), and III ($n = 4$, 8%). The differential reactivity of each FIGO grade is summarized in Table 2. Using one-way ANOVA for comparison between the results of the reactivity among three FIGO grading groups, no significant differences were obtained among all groups either for PTEN or p27 with $P = 0.0534$ and 0.9126, respectively [Table 2].

On the comparison between reactivity of PTEN in Grade I, II, and III with their counterparts in p27, using independent *t*-test, no significant difference was obtained between the three groups with $P = 0.2907$, 0.4269, and 0.0640 for FIGO I, II, and III, respectively [Graph 2 and Table 3].

The result of analysis of reactivity of EH and EC cases for both antibodies are categorized into four major categories; PTEN+/p27+ ($n = 2$, 14; 10%, 28%), PTEN+/p27− ($n = 5$, 7; 25%, 14%), PTEN−/p27+ ($n = 1$, 11; 5%, 22%), and PTEN−/p27− ($n = 12$, 18; 60%, 36%) cases of EH and EC, respectively. There is no significant *P* value among these four categories using either Chi-square test, $P = 0.06122$, or the independent *t*-test for the subcategory, $P = 0.0808$.

Further analyses of the four categories into subcategories to illustrate the degree of reactivity are summarized in Table 4.

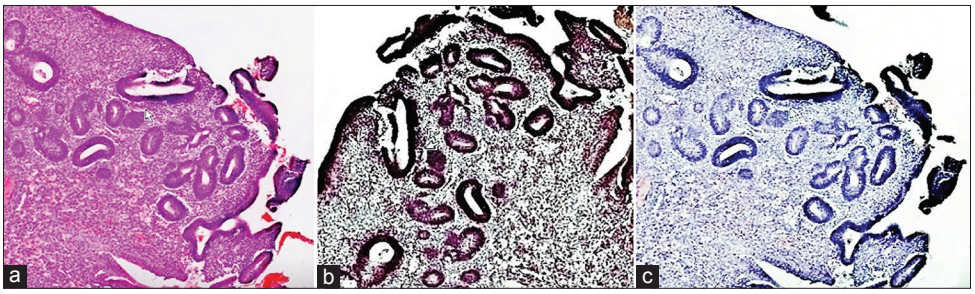


Figure 1: (a) A case of endometrial hyperplasia shows rounded glands, some of these glands show slight angulations (H and E, ×200). (b) Strong positivity for phosphatase and tensin homolog in both epithelial glands and stroma (DAB, ×200). (c) Negative reactivity for p27 in contrast to its strong positivity for phosphatase and tensin homolog (DAB, ×200)

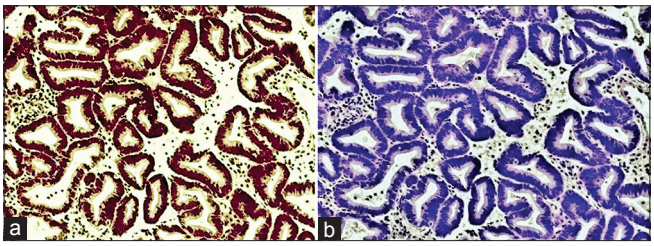


Figure 2: (a) Another case of endometrial hyperplasia shows complex angulated glands with strong reactivity for phosphatase and tensin homolog (DAB, ×200). (b) Weak and patchy reactivity of gland and stroma for p27 (DAB, ×200)

Table 1: Phosphatase and tensin homolog expression in both endometrial hyperplasia and endometrial carcinoma

Antibody	Lesion	*n*	Degree of expression (%)				Independent *t*-test
			Negative (−)	Weak (+)	Moderate (++)	Strong (+++)	
Anti-PTEN	EH	20	7 (35)	6 (30)	4 (20)	3 (15)	*P=0.01824
	EC	50	29 (58)	9 (18)	7 (14)	5 (10)	
p27 antibody	EH	20	17 (85)	3 (15)	0 (0)	0 (0)	*P=0.00304
	EC	50	25 (50)	16 (32)	6 (12)	3 (6)	

*P value is significant at $P \leq 0.05$. PTEN: Phosphatase and tensin homolog, EH: Endometrial hyperplasia, EC: Endometrial carcinoma

Discussion

The majority of the genetic defect in EC comes from PTEN gene inactivation through inactivation of the two alleles by large mutation and deletion. The tumor suppressor p27 is one of the Cip/Kip family members of CKIs, which plays a key role in the development of EC through negative regulation of the cell cycle progression; the normal regulation of p27 is being lost during progression of EC and undetectable or absent in some cases of EH.[38] Alternatively, the expression of p27 has been retained for a number of EC cases with cytoplasm mislocalization.[30] Consequently, both PTEN and p27 tumor suppressor genes alterations comprise an important group for developing EC.

In the present study, loss of PTEN expression was found in 7/20 (35%) and 29/50 (58%) cases of EH and EC, weak in 6/20 (30%) and 9/50 (18%), moderate in 4/20 (20%) and 7/50 (14%), and strong in 3 (15%) and 5 (10%) cases of EH and EC, respectively. A significant *P* value was obtained between PTEN expression in EH and EC ($P = 0.01824$).

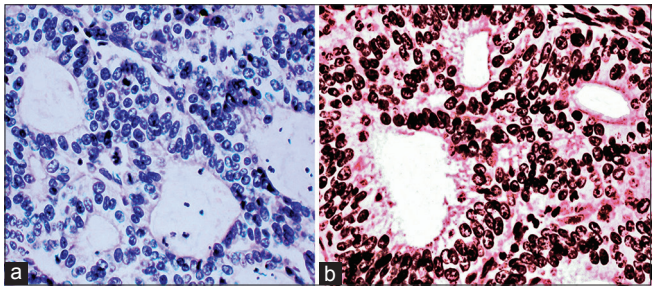


Figure 3: A case of endometrial carcinoma. (a) Negative staining for p27 (DAB, 400). (b) Strong staining for phosphate and tensin homolog (DAB,400)

These results are in agreement with the study of Mutter *et al*.,[39] who studied the PTEN expression in 33 EC cases and found a complete absence of PTEN expression in 20/33, 61% of EC cases. An *et al*.[38] studied the PTEN expression in EH and EC. They found that 4/13, 30% of EH and 40/61, 66% of cases of EC demonstrated a complete loss of PTEN expression. Orbo *et al*.[34] and Salvesen *et al*.[40] reported loss of PTEN protein expressions in 55% and 54% of EC cases, respectively. Allison *et al*.[41] showed that the PTEN expression in combination with particular histological features was of great importance to make a clear difference between EH and EC. Sarmadi *et al*.[35] detected loss of PTEN expression in 25% of EH and 52% of EC. In contrast to the above studies, the results of EH are somewhat far from the study of Kapucuoglu *et al*.,[36] who found a complete loss of PTEN in 20% of EH cases.

In the present study, a strong expression of PTEN was found in 5/50, 10% of EC cases which was distributed as follows: 2/19, 10.5% of cases of FIGO I; 1/10, 10% of cases in FIGO II; and 2/4, 50% of cases of FIGO III. Although the high percentage of the strong reactivity is seen in high FIGO grade (50%), there is no significant difference obtained between the histological grade and the degree of reactivity with $P = 0.0534$.

This may be attributed to the small number of cases, especially in FIGO III, which is considered as the major limitation of the current study. This finding was in agreement with An *et al*.,[38] who found no significant correlation with the histologic grade. Furthermore, it is in concordance with the study of Kimura *et al*.,[42] who studied PTEN expression in 117 EC cases and 20 cases of EH and found that PTEN staining scores of EC were

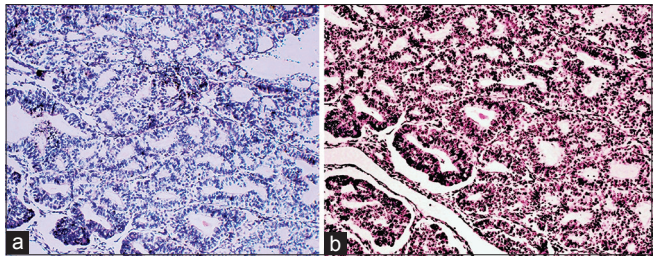


Figure 4: A case of endometrial carcinoma. (a) Weak focal staining for phosphate and tensin homolog (DAB, 100). (b) Strong staining for p27 (DAB,100)

Table 2: Distribution of phosphatase and tensin homolog and p27 expressions according to the International Federation of Gynecology and Obstetrics grading system in the 50 studied endometrial carcinoma cases

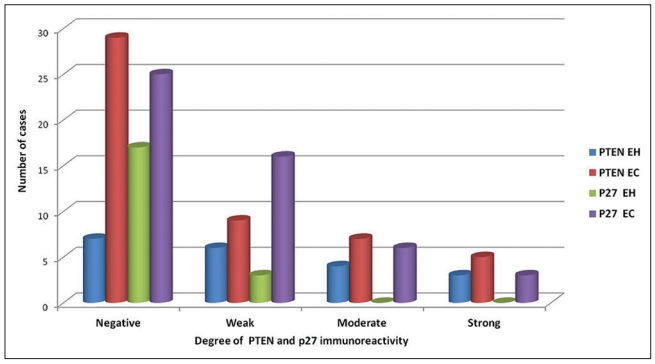
Antibody	FIGO grading	*n*	Negative (−)	Weak (+)	Moderate (++)	Strong (+++)	One-way ANOVA statistics
Anti-PTEN (%)	I	30	19 (63.3)	5 (16.6)	4 (13.3)	2 (6.6)	*P=0.0534
	II	16	10 (62.5)	3 (18.7)	2 (12.5)	1 (6.2)	
	III	4	0 (0)	1 (25)	1 (25)	2 (50)	
p27 (%)	I	30	14 (46.6)	11 (36.6)	3 (10)	2 (6.6)	P=0.9126
	II	16	9 (56.2)	4 (25)	2 (12.5)	1 (6.2)	
	III	4	2 (50)	1 (25)	1 (25)	0 (0)	

*P value is significant at $P \leq 0.05$. FIGO: International Federation of Gynecology and Obstetrics, PTEN: Phosphatase and tensin homolog, ANOVA: Analysis of variance

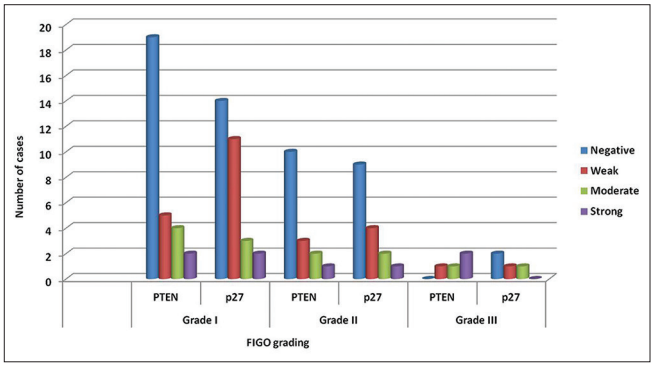
Table 3: Comparison between phosphatase and tensin homolog and p27 expressions according to the International Federation of Gynecology and Obstetrics grading system in the 50 studied endometrial carcinoma cases

FIGO grading	Antibody	*n*	Negative (−)	Weak (+)	Moderate (++)	Strong (+++)	One-way ANOVA statistics
Grade I (%)	PTEN	30	19 (63.3)	5 (16.6)	4 (13.3)	2 (6.6)	*P=0.2907
	p27		14 (46.6)	11 (36.6)	3 (10)	2 (6.6)	
Grade II (%)	PTEN	16	10 (62.5)	3 (18.7)	2 (12.5)	1 (6.2)	P=0.4269
	p27		9 (56.2)	4 (25)	2 (12.5)	1 (6.2)	
Grade III (%)	PTEN	4	0 (0)	1 (25)	1 (25)	2 (50)	P=0.0640
	p27		2 (50)	1 (25)	1 (25)	0 (0)	

*P value is significant at $P \leq 0.05$. ANOVA: Analysis of variance, PTEN: Phosphatase and tensin homolog, FIGO: International Federation of Gynecology and Obstetrics



Graph 1: Immunoreactivity of phosphatase and tensin homolog and p27 in the studied cases



Graph 2: The distribution of cases according to the International Federation of Gynecology and Obstetrics grading and the degree of reactivity

increasing from FIGO Grade I–III, suggesting that the disturbed PTEN expression occurs in an early phase of tumorigenesis of well-differentiated EC and concluded that PTEN expression was decreased in well-differentiated EC associated with high levels of estrogen and progesterone receptors. On the other hand, the finding is in contrast to Salvesen *et al.*'s study[40] which revealed significant correlations with young age, low FIGO grading, and endometrioid subtype. Lacey *et al.*[43] compared the endometrial PTEN expression from patients with EH who developed to EC versus patients with EH who did not develop. They found that loss of PTEN expression was not linked with the progression to EC.

In the present study, PTEN expression was detected in seven cases of EH, three of seven cases of were diagnosed as atypical EH, and this finding was in agreement with the study of Lax,[44] which stated that in atypical EH, alterations of PTEN were present in approximately 50% of the cases.

In the current study, loss of p27 expression was found in 17/20 (85%) and 25/50 (50%) cases of EH and EC, weak in 3/20 (15%) and 16/50 (32%), moderate in 0/20 (0%) and 6/50 (12%), and strong in 0 (0%) and 3 (6%) cases of EH and EC, respectively. A significant P value was obtained between p27 expression in EH and EC ($P = 0.00304$).

These findings are in proximity to the study of Nycum *et al.*[45] and Lahav-Baratz *et al.*[46] Nycum *et al.*[45] studied the p27 expression in different stages of EC and found that expression of p27 was decreased in endometrial cancers compared with normal endometrial cells; furthermore, they found a p27-positive stain (>50% of cells) in 32.1%, 23.1%, 35.7%, and 36.4% for Stage 1–4, respectively, with no significant correlation among the grades; further, no association of p27 staining with age or histology was found. Alternatively, a trend in increasing staining with an increase in grade was found. Lahav-Baratz *et al.*[46] found that the p27 expression was seen in 41% of EC and found that there is no difference in expression with that seen in the proliferative phase of the endometrium.

Alternatively, these findings are in midline between the two extremities' groups of the studies; one showing high level of p27 above 80%[29,38,47-49] and one showing successive reductions in the p27 expression in a range from normal to endometrial carcinoma.[50-55] These variable observations may be attributed to many factors as the number of specimens, different histologic types, and grades or the likelihood that the p27 has a complex role in the process of cell cycle regulation, which may clarify the incoherent observations.[54]

Bamberger *et al.*[52] analyzed 41 EC stained with p27 and found the negativity in 56.0% – low (29.3%), moderate (14.7%), and high (0.0%). Further, they revealed that all the p27-positive tumors were of grade G1. Schmitz *et al.*[53] studied the p27 expression in uterine serous papillary carcinoma and pointed to the presence of p27 expression alteration in 63% of cases displaying reduced p27 expression, suggesting that p27 abnormalities play an important role in the development of this disease. An *et al.*[38] found that decreased reactivity of p27 was found in 48/61 (79%) of 61 EC cases. In addition, they found no significant relationship between

Table 4: Distribution of anti-phosphatase and tensin homolog and p27 expressions in the endometrial hyperplasia and endometrial carcinoma cases

Category	Subcategory	EH	EC	*P*
Positive PTEN/p27, EH=2, EC=14	PTEN+/p27+	1	6	1. Chi-square test for comparison of the 4 categories, *P*=0.06122 2. Using the independent *t*-test for the subcategory, *P*=0.0808
	PTEN++/p27+	1	2	
	PTEN+++/p27+	0	1	
	PTEN+/p27++	0	1	
	PTEN+/p27+++	0	1	
	PTEN++/p27++	0	2	
	PTEN++/p27+++	0	1	
Positive PTEN and negative p27, EH=5, EC=7	PTEN+/p27−	3	1	
	PTEN++/p27−	1	2	
	PTEN+++/p27−	1	4	
Negative PTEN and positive p27, EH=1, EC=11	PTEN−/p27+	1	7	
	PTEN−/p27++	0	3	
	PTEN−/p27+++	0	1	
Negative PTEN and p27	PTEN−/p27−	12	18	
Total		20	50	

P value is significant at $P \leq 0.05$. PTEN: Phosphatase and tensin homolog, EH: Endometrial hyperplasia, EC: Endometrial carcinoma

the degree of p27reactivity with the histological grade. Watanabe *et al*.[47] proved the positivity of p27 staining in the nuclei of 124/127, 97.6% EC cases; however, they found a correlation with the higher grade. Masciullo *et al*.[30] revealed a significant loss of p27 expression from proliferative endometrium (33%) through EH (50%) to EC (71%; $P \leq 0.001$). Furthermore, they noted cytoplasmic localization of p27 193; 91% of 217 cases examined.

Horrée *et al*.[29] revealed that p27 expression was found in 67% of the tumor cells and conclude that, in EC carcinoma, p27 re-expression by hypoxia is HIF-1 alpha-dependent and leads to cell cycle arrest. Seeber *et al*.[48] found that expression of p27 was found in 86/93 (93%) of EC with no correlation between percentage p27-positive cells and grade. Gezginc *et al*.[54] reported that p27 reactivity decreased progressively as lesions progress from proliferative benign endometrium to frank carcinoma. Felix *et al*.[49] studied 281 EC and clarified the positivity in 80% of EC cases. In addition, the results are in somewhat close to the results of Al-Maghrabi *et al*.,[55] which revealed that p27 reactivity was 26.7% in carcinomas compared with 43% of the noncancerous endometrium.

In the present study, no significant difference was obtained between the p27 expression with the histological grade ($P = 0.9126$). This coincides with Ahn *et al*.[51] and Bamberger *et al*.,[52] who confirmed the correlation of decreased p27 expression with the higher grade and inconsistent with the study of Watanabe *et al*.,[47] who stated that the high expression of p27 in endometrial EC was correlated with a higher grade. This alteration in p27 expression may be attributed to the disorder of p27 degradation mechanism.[50]

In the present study, analysis of 11 cases expressing positivity for both antibodies revealed that 6/11 cases were of FIGO I Grade; among these cases, the strongest PTEN expression was found in three cases (one case in FIGO I, two cases for FIGO II); alternatively, two cases showed strong expression for p27 and distributed as one case for FIGO I and FIGO II. This denotes that the positive cases are mainly confined with the low and intermediate grading with weak expression among high grade. This observation coincides with An *et al*.,[38] who found that decreased p27 expression was found in 48 cases (79%) of 61 EC, and 76% (36 cases) of these cases also revealed decrease or even loss of PTEN reactivity.

On the other hand, positive expression of p27 associated with weak or loss of PTEN expression is found in 12 cases (one case showed weak PTEN expression) of EC; this means that the development of EC is independent on PTEN mutation alone, and there is an alternative pathway involved in upregulation of p27 independent on PTEN. This hypothesis is supported by findings of Akiyama-Abe *et al*.,[37] who found that not all EC showed bimodal loss of expression for PTEN and p27 and revealed that loss of PTEN expression was found in 56 cases (25%), while 165 (75% of cases) showed PTEN expression and nuclear p27 expression was found in 78 (35%) cases and lost in 143 (65%).

An *et al*.[38] found that 36/48 (76%) cases of positive PTEN revealed decrease or even loss of p27 reactivity. Furthermore, they found that four of five uterine serous carcinomas revealed strong p27 expression, which in turn showed intense PTEN expression. Also, they found a positive correlation between PTEN and p27 expression was statistically significant which in turn coincides with the results of the current study. Erkanli *et al*.[56] found that PTEN and p27 expressions were decreased in hyperplasia and carcinoma cases with significant differences obtained. In addition, a positive correlation was found between PTEN expression and p27. Neither PTEN nor p27 showed correlations with the grade although a higher mean survival in PTEN-positive cases was noticed.

The limitation of the current study is attributed to the small number of FIGO III cases. This may be due to health education and adventure of imaging techniques which aided to a large extent in the early detection. This view is supported by the limited number of cases towards the moderate and high grade. On the other hand, the main aim of the study is to find the extent of association of the PTEN with p27 in EC cases and not to find primarily the association of both separately with the histological grade.

Conclusion

Not all cases of PTEN negative EC showing a p27 loss and vice versa. In addition, much debate about PTEN and p27 expression in EC is strongly present regarding the accurate percentage of their expressions and correlations with the histological grade more studies must be done to correlate between the degree of PTEN loss and p27 comprising all subtypes and grading of EC especially those of high FIGO grade.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000;100:387-90.
2. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 1998;273:13375-8.
3. Testa JR, Bellacosa A. AKT plays a central role in tumorigenesis. Proc Natl Acad Sci U S A 2001;98:10983-5.
4. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2002;2:489-501.
5. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 1997;57:4736-8.
6. Levine RL, Cargile CB, Blazes MS, van Rees B, Kurman RJ, Ellenson LH, *et al.* PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. Cancer Res 1998;58:3254-8.
7. Sansal I, Sellers WR. The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol 2004;22:2954-63.
8. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, *et al.* Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 1998;95:29-39.
9. Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997;91:231-41.
10. del Peso L, González-García M, Page C, Herrera R, Nuñez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science 1997;278:687-9.
11. Dinulescu DM, Ince TA, Quade BJ, Shafer SA, Crowley D, Jacks T, *et al.* Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. Nat Med 2005;11:63-70.
12. Li DM, Sun H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. Proc Natl Acad Sci U S A 1998;95:15406-11.
13. Ramaswamy S, Nakamura N, Vazquez F, Batt DB, Perera S, Roberts TM, *et al.* Regulation of G1 progression by the PTEN tumor suppressor protein is linked to inhibition of the phosphatidylinositol 3-kinase/Akt pathway. Proc Natl Acad Sci U S A 1999;96:2110-5.
14. Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW, *et al.* High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/- mice. Cancer Res 2000;60:3605-11.
15. Wang H, Douglas W, Lia M, Edelmann W, Kucherlapati R, Podsypanina K, *et al.* DNA mismatch repair deficiency accelerates endometrial tumorigenesis in PTEN heterozygous mice. Am J Pathol 2002;160:1481-6.
16. Zhou XP, Marsh DJ, Morrison CD, Chaudhury AR, Maxwell M, Reifenberger G, *et al.* Germline inactivation of PTEN and dysregulation of the phosphoinositol-3-kinase/Akt pathway cause human Lhermitte-Duclos disease in adults. Am J Hum Genet 2003;73:1191-8.
17. Bloom J, Pagano M. Deregulated degradation of the CDK inhibitor p27 and malignant transformation. Semin Cancer Biol 2003;13:41-7.
18. Fujita N, Sato S, Katayama K, Tsuruo T. Akt-dependent phosphorylation of p27Kip1 promotes binding to 14-3-3 and cytoplasmic localization. J Biol Chem 2002;277:28706-13.
19. Rodier G, Montagnoli A, Di Marcotullio L, Coulombe P, Draetta GF, Pagano M, *et al.* P27 cytoplasmic localization is regulated by phosphorylation on ser10 and is not a prerequisite for its proteolysis. EMBO J 2001;20:6672-82.
20. Ishida N, Kitagawa M, Hatakeyama S, Nakayama K. Phosphorylation at serine 10, a major phosphorylation site of p27(Kip1), increases its protein stability. J Biol Chem 2000;275:25146-54.
21. Kamura T, Hara T, Matsumoto M, Ishida N, Okumura F, Hatakeyama S, *et al.* Cytoplasmic ubiquitin ligase KPC regulates proteolysis of p27(Kip1) at G1 phase. Nat Cell Biol 2004;6:1229-35.
22. Shin I, Yakes FM, Rojo F, Shin NY, Bakin AV, Baselga J, *et al.* PKB/Akt mediates cell-cycle progression by phosphorylation of p27(Kip1) at threonine 157 and modulation of its cellular localization. Nat Med 2002;8:1145-52.
23. Viglietto G, Motti ML, Bruni P, Melillo RM, D'Alessio A, Califano D, *et al.* Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27(Kip1) by PKB/Akt-mediated phosphorylation in breast cancer. Nat Med 2002;8:1136-44.
24. Denicourt C, Dowdy SF. Cip/Kip proteins: More than just CDKs inhibitors. Genes Dev 2004;18:851-5.
25. Liang J, Shao SH, Xu ZX, Hennessy B, Ding Z, Larrea M, *et al.* The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. Nat Cell Biol 2007;9:218-24.
26. Oshita T, Shigemasa K, Nagai N, Ohama K. P27, cyclin E, and CDK2 expression in normal and cancerous endometrium. Int J Oncol 2002;21:737-43.
27. Abargel A, Avinoach I, Kravtsov V, Boaz M, Glezerman M, Menczer J, *et al.* Expression of p27 and p53: Comparative analysis of uterine carcinosarcoma and endometrial carcinoma. Int J Gynecol Cancer 2004;14:354-9.
28. Pavlides SC, Huang KT, Reid DA, Wu L, Blank SV, Mittal K, *et al.* Inhibitors of SCF-skp2/Cks1 E3 ligase block estrogen-induced growth stimulation and degradation of nuclear p27kip1: Therapeutic potential for endometrial cancer. Endocrinology 2013;154:4030-45.
29. Horrée N, van Diest PJ, van der Groep P, Sie-Go DM, Heintz AP. Progressive derailment of cell cycle regulators in endometrial carcinogenesis. J Clin Pathol 2008;61:36-42.
30. Masciullo V, Susini T, Zamparelli A, Bovicelli A, Minimo C, Massi D, *et al.* Frequent loss of expression of the cyclin-dependent kinase inhibitor p27(Kip1) in estrogen-related endometrial adenocarcinomas. Clin Cancer Res 2003;9:5332-8.
31. McCampbell AS, Harris HA, Crabtree JS, Winneker RC, Walker CL, Broaddus RR, *et al.* Loss of inhibitory insulin receptor substrate-1 phosphorylation is an early event in mammalian target of rapamycin-dependent endometrial hyperplasia and carcinoma. Cancer Prev Res (Phila) 2010;3:290-300.
32. Tanwar PS, Kaneko-Tarui T, Zhang L, Tanaka Y, Crum CP, Teixeira JM, *et al.* Stromal liver kinase B1 [STK11] signaling loss induces oviductal adenomas and endometrial cancer by activating mammalian target of rapamycin complex 1. PLoS Genet 2012;8:e1002906.
33. Amant F, Mirza MR, Koskas M, Creutzberg CL. Cancer of the corpus uteri. Int J Gynaecol Obstet 2015;131 Suppl 2:S96-104.
34. Orbo A, Nilsen MN, Arnes MS, Pettersen I, Larsen K. Loss of expression of MLH1, MSH2, MSH6, and PTEN related to endometrial cancer in 68 patients with endometrial hyperplasia. Int J Gynecol Pathol 2003;22:141-8.
35. Sarmadi S, Izadi-Mood N, Sotoudeh K, Tavangar SM. Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. Diagn Pathol 2009;4:41.
36. Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N, Ciriş M, *et al.* Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, Bcl-2, bax, and apoptotic index. Pathol Res Pract 2007;203:153-62.
37. Akiyama-Abe A, Minaguchi T, Nakamura Y, Michikami H, Shikama A, Nakao S, *et al.* Loss of PTEN expression is an independent predictor of favourable survival in endometrial carcinomas. Br J Cancer 2013;109:1703-10.
38. An HJ, Lee YH, Cho NH, Shim JY, Kim JY, Lee C, *et al.* Alteration of PTEN expression in endometrial carcinoma is associated with down-regulation of cyclin-dependent kinase inhibitor, p27. Histopathology 2002;41:437-45.
39. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, *et al.* Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000;92:924-30.
40. Salvesen HB, Stefansson I, Kretzschmar EI, Gruber P, MacDonald ND, Ryan A, *et al.* Significance of PTEN alterations in endometrial carcinoma: A population-based study of mutations, promoter methylation and PTEN protein expression. Int J Oncol 2004;25:1615-23.
41. Allison KH, Tenpenny E, Reed SD, Swisher EM, Garica RL. Immunohistochemical markers in endometrial hyperplasia: Is there a

panel with promise? A review. Appl Immunohistochem Mol Morphol 2008;16:329-43.
42. Kimura F, Watanabe J, Hata H, Fujisawa T, Kamata Y, Nishimura Y. PTEN immunohistochemical expression is suppressed in G1 endometrioid adenocarcinoma of the uterine corpus. J Cancer Res Clin Oncol 2004;130:161-8.
43. Lacey JV Jr., Mutter GL, Ronnett BM, Ioffe OB, Duggan MA, Rush BB, *et al.* PTEN expression in endometrial biopsies as a marker of progression to endometrial carcinoma. Cancer Res 2008;68:6014-20.
44. Lax SF. Molecular genetic pathway in various types of endometrial carcinoma: From a phenotypical to a molecular-based classification. Virchows Arch 2004;444:213-23.
45. Nycum LR, Smith LM, Farley JH, Kost ER, Method MW, Birrer MJ, *et al.* The role of p27 in endometrial carcinoma. Gynecol Oncol 2001;81:242-6.
46. Lahav-Baratz S, Ben-Izhak O, Sabo E, Ben-Eliezer S, Lavie O, Ishai D, *et al.* Decreased level of the cell cycle regulator p27 and increased level of its ubiquitin ligase Skp2 in endometrial carcinoma but not in normal secretory or in hyperstimulated endometrium. Mol Hum Reprod 2004;10:567-72.
47. Watanabe J, Sato H, Kanai T, Kamata Y, Jobo T, Hata H, *et al.* Paradoxical expression of cell cycle inhibitor p27 in endometrioid adenocarcinoma of the uterine corpus-correlation with proliferation and clinicopathological parameters. Br J Cancer 2002;87:81-5.
48. Seeber LM, Horrée N, van der Groep P, van der Wall E, Verheijen RH, van Diest PJ, *et al.* Necrosis related HIF-1alpha expression predicts prognosis in patients with endometrioid endometrial carcinoma. BMC Cancer 2010;10:307.
49. Felix AS, Sherman ME, Hewitt SM, Gunja MZ, Yang HP, Cora RL, *et al.* Cell-cycle protein expression in a population-based study of ovarian and endometrial cancers. Front Oncol 2015;5:25.
50. Watanabe J, Kamata Y, Seo N, Okayasu I, Kuramoto H. Stimulatory effect of estrogen on the growth of endometrial cancer cells is regulated by cell-cycle regulators. J Steroid Biochem Mol Biol 2007;107:163-71.
51. Ahn HJ, Kwon WK, Choi Y, Cho NH. Expression of cyclin E and cyclin dependent kinase inhibitor, p27KIP in uterine endometrial carcinoma relationship with p53 status. Int J Surg Pathol 1998;6:205-12.
52. Bamberger AM, Riethdorf L, Milde-Langosch K, Bamberger CM, Thuneke I, Erdmann I, *et al.* Strongly reduced expression of the cell cycle inhibitor p27 in endometrial neoplasia. Virchows Arch 1999;434:423-8.
53. Schmitz MJ, Hendricks DT, Farley J, Taylor RR, Geradts J, Rose GS, *et al.* P27 and cyclin D1 abnormalities in uterine papillary serous carcinoma. Gynecol Oncol 2000;77:439-45.
54. Gezginc ST, Celik C, Dogan NU, Toy H, Tazegul A, Colakoglu MC, *et al.* Expression of cyclin A, cyclin E and p27 in normal, hyperplastic and frankly malignant endometrial samples. J Obstet Gynaecol 2013;33:508-11.
55. Al-Maghrabi J, Abdelrahman AS, Al-Maghrabi B, Buhmeida A, Abuzenadah A, Al-Qahtani M, *et al*. Loss of p27 expression in endometrial carcinoma patients with recurrent tumor is significantly associated with poor survival. Eur J Gynaecol Oncol 2018;39:119-123. [doi: 10.12892/ejgo3784].
56. Erkanli S, Kayaselcuk F, Kuscu E, Bagis T, Bolat F, Haberal A, *et al.* Expression of survivin, PTEN and p27 in normal, hyperplastic, and carcinomatous endometrium. Int J Gynecol Cancer 2006;16:1412-8.