Endometrial hyperplasia versus carcinoma: does phosphatase and tensin homolog immunohistochemical expression differentiate between them

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Context Phosphatase and tensin homolog (PTEN) is a protein that acts as a tumor suppressor by dephosphorylating the lipid second messenger phosphatidylinositol 3,4,5-trisphosphate. Loss of PTEN function and mutation in *PTEN* gene have been implicated in the pathogenesis of endometrial carcinoma (EC).

Objective The aim was to evaluate the immunohistochemical expression of PTEN in endometrial hyperplasia and EC and to evaluate the relationship between its expression and tumor grade in EC.

Materials and methods Specimens included 16 cases of endometrial hyperplasia without atypia, six cases of atypical endometrial hyperplasia, and 18 EC specimens. Immunohistochemical staining for PTEN was performed using diaminobenzidine detection kit on formalin-fixed and paraffin-embedded tissue samples. Tumor tissue blocks and clinical data were collected from the files of the Pathology Department of Al-Zahraa University Hospital during the period 2010–2014.

Results Immunohistochemistry showed that PTEN was positive for nuclei and cytoplasm of glandular endometrial cells. The PTEN expression was decreased significantly in atypical hyperplasia or EC compared with simple or complex hyperplasia (*P*0.041). In EC, we proved that PTEN expression is downregulated in high-grade tumors.

Conclusion A positive PTEN expression correlates significantly with hyperplasia without atypia and well-differentiated tumors. The downregulation of PTEN indicates a more malignant phenotype.

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Introduction

Noncancerous changes of the endometrium are commonly known as hyperplasia. Endometrial hyperplasia essentially implies an overgrowth of the endometrium. It is almost exclusively associated with a relative excess of endogenous or exogenous estrogen. Simple hyperplasia resembles the normal endometrial tissue growth pattern, whereas complex hyperplasia has a more complex and thus more abnormal architectural growth pattern. Both simple and complex hyperplasias can be associated with cellular atypia, which seems to be the most important predictor of malignant potential [1].

Endometrial cancer (EC) is the fourth common malignancy in women [2]. EC is the most common gynecologic malignancy in developed countries and the second most common in developing countries [3]. There are two basic types of EC: endometrioid (estrogen related with indolent behavior) and non-endometrioid (unrelated to estrogen and aggressive) [1]. Type I is the endometrioid adenocarcinoma, which is mostly a well-differentiated tumor and comprises most ECs. Type I EC is associated with the unopposed estrogen stimulation and is mostly progressed by endometrial hyperplasia [4].

Endometrioid ECs account for three-fourths of ECs and are thought to develop following a continuum of premalignant lesions ranging from endometrial hyperplasia without atypia, to hyperplasia with atypia, and finally to well-differentiated carcinoma [5]. Therefore, new insights into the morphology of biologically defined premalignant lesion of endometrium had a great benefit [6]. Type I EC shows microsatellite instability and mutations in K-ras, phosphatase and tensin homolog (*PTEN*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-α (*PIK3CA*), and catenin β-1 (*CTNNB1*) genes [7]. Moreover, these molecular genetic alternations have been described in atypical endometrial hyperplasia [8]. Currently, PTEN is the most frequently altered gene in endometrioid EC [9].

The tumor suppressor gene named *PTEN*, also called *MMAC1*, is located on chromosome 10q23. It is

somatically mutated in several types of tumors [10]. *PTEN* gene appears to be normally regulated, with greatest physiological endometrial gland expression in an estrogen-rich environment. Thus, the effect of diminished PTEN tumor suppressor function is directly proportional to cancer risk, particularly in high-estrogenic states [11]. The PTEN protein has a crucial role in the control of the PI3K–AKT pathway through dephosphorylation of PIP3 at the cell membrane. The absence of functional PTEN protein leads to unopposed action of PI3K, with resultant uncontrolled PIP3 production. One major effector of the PI3K–AKT pathway is mTOR, which stimulates protein synthesis, initiates entry into G1 phase of the cell cycle, and interacts with proteins that regulate apoptosis [12]. The *PTEN* gene has both lipid and protein phosphate activities, and the combination of the losses of PTEN lipid and protein phosphate activity can cause an aberrant cell growth and an escape from apoptosis, as well as abnormal cell spreading and migration [13]. In spite of the underlying mechanism, immunohistochemistry study of PTEN expression is an effective screening method for EC [14]. Interestingly, studies have also shown the relationship between PTEN and prognosis in several cancers, including EC [8].

The current study was designed to investigate the hyperplastic and neoplastic endometrial glands expression by using PTEN as a marker and differentiate between hyperplastic and malignant endometrial glands, thereby evaluating the role of possibility in early diagnosis of endometrial premalignant lesions.

Materials and methods

Tissue specimens

Formalin-fixed and paraffin-embedded 40 specimens were collected and prepared for this study from Al-Zahraa University Hospital during the period 2010–2014, after obtaining an informed consent and approval of the local ethical committee. Specimens included 16 cases of the endometrial hyperplasia without atypia (10 cases were simple and six cases were complex), six cases of atypical endometrial hyperplasia, and 18 specimens of EC. All patients with EC had undergone surgical intervention (total abdominal hysterectomy and bilateral salpingio-ophrectomy). Endometrial hyperplasia samples were obtained by either curettage or biopsy specimens. Hyperplasia specimens were evaluated according to WHO classification [15]. Regarding EC cases, grading was assessed according to the International Federation of Gynecology and Obstetrics (FIGO) grading system [16,17]: seven cases were grade I, 5 cases were grade II, and six cases were grade III.

Two sections of 5 μm thickness were cut from the paraffin blocks: one was stained by hematoxylin and eosin for histopathological examination, whereas the other section was mounted on a positive charged slide and immunostained with mouse monoclonal antibodies against PTEN.

Immunohistochemistry

For immunohistochemical study, unstained positively charged slides (Biogenix, Atlanta) were prepared from each paraffin block for immunostaining with a prediluted monoclonal primary PTEN antibody (clone 6H2.1; Dako) (recommended dilution 1 : 100). Immunohistochemical reactions were carried out using Labeled Streptavidin-Biotin 2 System-Horseradish Peroxidase (LSAB2 System-HRP). The LSAB2 system-HRP is based on a modified labeled avidin–biotin (LAB) technique in which a biotinylated secondary antibody forms a complex with peroxidase-conjugated streptavidin molecules. The entire antibody complex is made visible by addition of an appropriate substrate chromogen reagent, which is converted by the peroxidase label to brown-colored precipitate at the site of antigen localization in tissue. The chromogen used is diaminobenzidine produced by Dako (USA). Finally, the sections were counterstained with Mayer's hematoxylin.

Positive and negative control

As a negative control for PTEN, a tissue was processed through the aforementioned sequences, but the primary antibody was omitted instead, and phosphate buffer solution was added. For positive external control, a section of breast invasive ductal carcinoma was used as a positive control for PTEN.

Evaluation of immunostaining

Positive staining was indicated as a brown color in the nucleus or cytoplasm of endometrial glandular cells. Quantification of positivity was expressed in percentages. Samples with nuclear staining of at least 10% of tumor cells were considered PTEN positive. Staining of cells was scored as negative if less than 10% [6].

Statistical analysis

Data were analyzed using statistical program for the social sciences (SPSS; SPSS Inc., Chicago, Illinois,

USA) version 18.0. Quantitative data were expressed as mean±SD. Qualitative data were expressed as frequency and percentage. χ^2-Test of significance was used to compare proportions between two qualitative parameters.

Probability (*P* value)

The probability of significance was as follows:

(1) *P* value less than or equal to 0.05 was considered significant.
(2) *P* value less than 0.001 was considered as highly significant.
(3) *P* value greater than 0.05 was considered insignificant.

Results

A total of 40 cases [22 (55%) cases of endometrial hyperplasia and 18 (45%) cases of EC] were enrolled in this study. The cases were distributed as follows: simple hyperplasia (25%), complex hyperplasia (15%), atypical hyperplasia (15%), and EC (45%). According to the International Federation of Gynecology and Obstetrics criteria, the cases were divided into following grades: grade I (38.9%), grade II (27.8%), and grade III (33.3%) lesions (Table 1).

Immunohistochemical expression of PTEN

PTEN staining was nuclear or cytoplasmic. Of 40 cases, 20 (50%) were positive for PTEN. The positive rate increased significantly in endometrial hyperplasia (68.2%) than EC (27.8%), using χ^2-test (*P*=0.011) (Table 2). There was also a statistically significant difference between types of lesion and PTEN expression of the patient groups (*P*=0.041*); the expression decreased in atypical hyperplasia (50%) and EC (27.8%) than simple hyperplasia (70%) and complex hyperplasia (83.3%) (Figs 1, 2, 3). Regarding the cases of EC, the expression of PTEN decreased in high-grade tumors: 42.9% in grade I, 20% in grade II, and 16.7% in grade III (Fig. 3, Table 3).

Table 1 Type of lesion distribution of the patient groups

Lesions	*n* (%)
Simple hyperplasia	10 (25.0)
Complex hyperplasia	6 (15.0)
Atypical hyperplasia	6 (15.0)
Endometrial carcinoma	18 (45.0)
Grade I	7 (38.9)
Grade II	5 (27.8)
Grade III	6 (33.3)

Discussion

EC is the most prevalent malignancy of the gynecologic tract in the developed world [12]. There are two major classes of EC, commonly described as type I and type II cancers, which respectively correspond to endometrioid and non-endometrioid histologic types [18]. Type I usually arises in the background of endometrial hyperplasia, and type II is unrelated to estrogen [5].

The pathogenesis of EC and its precursor lesion is complex and involves many molecular disturbances including microsatellite instability (MI) and mutations of *PTEN*, *PIK3CA*, k-ras, and β-catenin genes [19]. The most frequently altered gene in endometrial tumors of endometrioid histology showing microsatellite instability is PTEN inactivation [20]. Several studies have found that PTEN inactivation is correlated with clonal growth pattern detected in endometrial hyperplasia and carcinoma [21]. Most of the endometrioid endometrial carcinoma (EECA) had occurred on top of atypical endometrial hyperplasia, so an accurate diagnosis of premalignant lesions in routine endometrial biopsies has a great value in patient management. However, cytological atypia, which is a predominant criterion for the diagnosis of premalignant lesions (atypical endometrial hyperplasia), has poor reproducibility [22]. Evaluation of PTEN loss by immunohistochemistry is highly reproducible and may be an effective tool for screening of malignant and premalignant endometrial lesions [23].

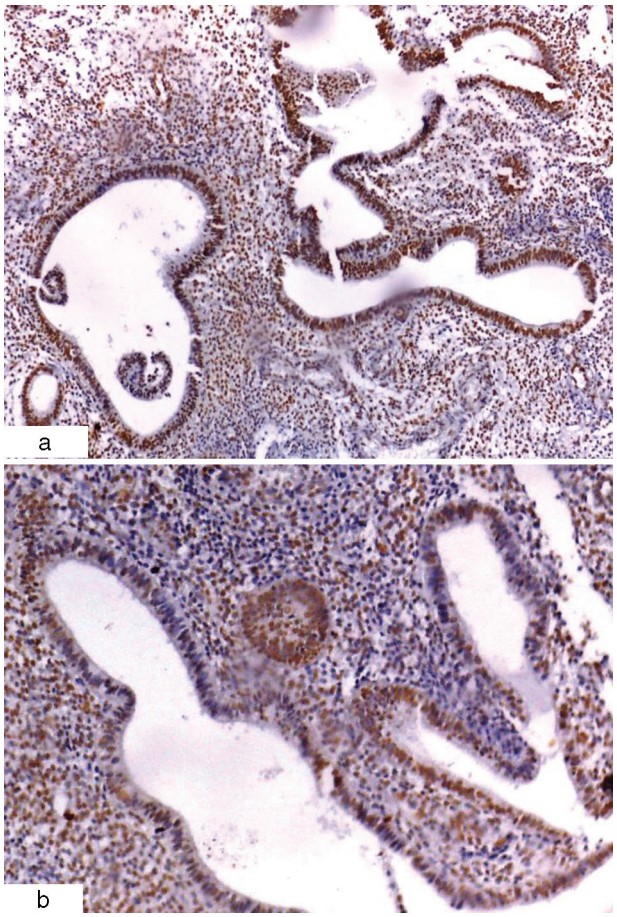
In the current study, PTEN negative immunoreactivity was detected in most EECA and atypical complex hyperplasia (ACH), but positive PTEN expression was noted in typical simple and complex hyperplasia. The expression decreased in atypical hyperplasia (50%) and EC (27.8%) than simple hyperplasia (70%) and complex hyperplasia (83.3%). These results are in agreement with those obtained by Kapucuoglu *et al.* [8], who stated that PTEN expression was significantly higher in cyclical endometrium than in the carcinomas. Moreover, this

Table 2 Phosphatase and tensin homolog expression in endometrial hyperplasia versus carcinoma

	PTEN [*n* (%)]		
Lesions	Positive		Negative
Hyperplasia	15 (68.2)		7 (31.8)
Endometrial carcinoma	5 (27.8)		13 (72.2)
Total	20 (50.0)		20 (50.0)
χ^2		6.465	
P value		0.011	

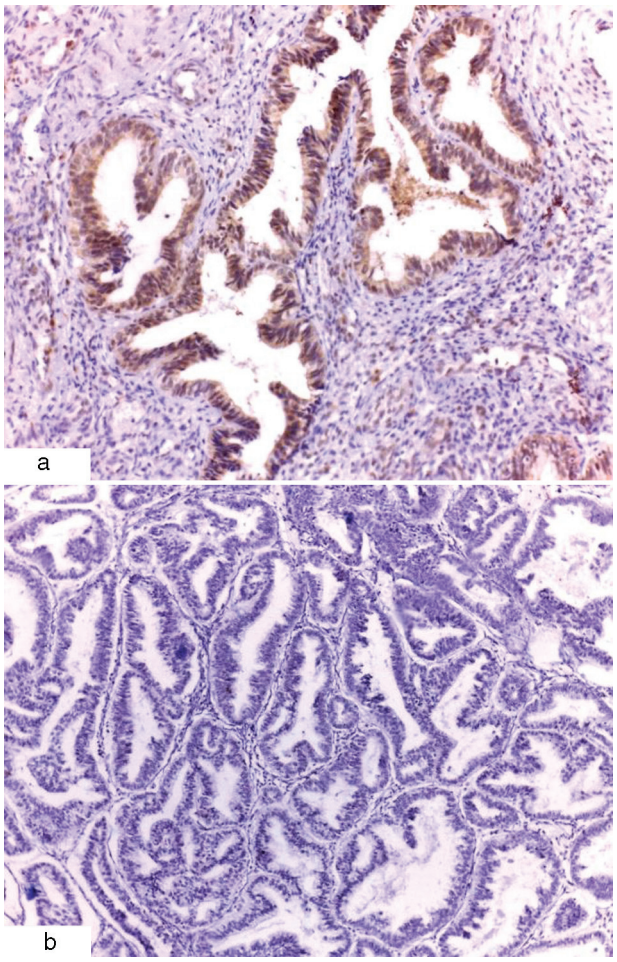
PTEN, phosphatase and tensin homolog.

Fig. 1



Immunohistochemical staining using phosphatase and tensin homolog antibody, showing positive nuclear reactivity of simple hyperplasia of endometrium (a and b) (phosphatase and tensin homolog, ×100) (phosphatase and tensin homolog, ×200).

Fig. 2



Immunohistochemical staining using phosphatase and tensin homolog antibody showing (a) positive phosphatase and tensin homolog nuclear expression in complex hyperplasia and (b) complete negative reaction in atypical complex hyperplasia of endometrium. (phosphatase and tensin homolog, ×100).

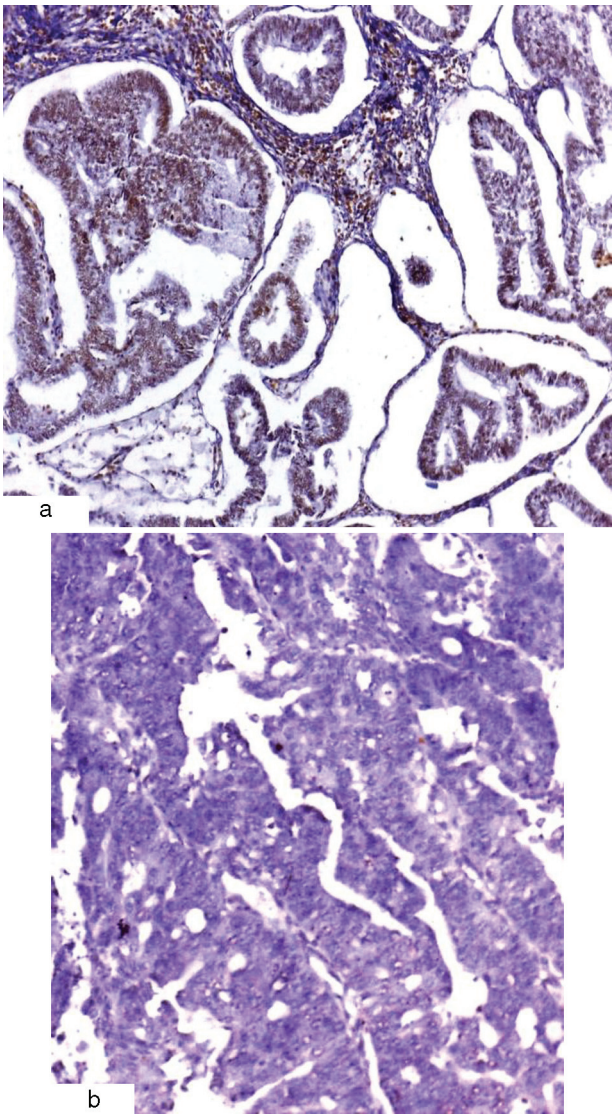
study was in line with another study done by Boruban *et al.* [5], which stated that mutation of PTEN with absent or at least diminished expression is present in 83% of EECA cases. Similar to the result of this study, Elwy *et al.* [24] found that PTEN immunoreactivity was noted in all normal proliferative and hyperplastic endometrium, whereas 80% of EECA showed complete absence or diminution of PTEN expression. Hecht and Mutter [25] explained this by stating that the most commonly observed PTEN defect in EECA is inactivation of both alleles to generate a complete loss of PTEN protein, and even a PTEN hemizygous inactivation leads to a protein deficiency, rather than a null state, which is functionally significant when combined with abnormalities of other genes that converge on its downstream pathway. Garg *et al.* [23] stated that the evaluation of PTEN by immunohistochemistry is highly reproducible, provided the application of standard immunohistochemical techniques and simple scoring criteria.

Furthermore, the present study exhibited decreased PTEN expression in higher grade of EECA than in lower grades, with 42.9% in grade I, 20% in grade II, and 16.7% in grade III. This is consistent with another study by Mao *et al.* [26] which revealed PTEN loss in higher grade endometrioid carcinoma. These findings suggest that PTEN alternation occurs in the earliest phase of endometrial carcinogenesis. PTEN inactivation initiated in precancers from a normal background state, and then additional PTEN damage accumulates in the transition from premalignant to malignant disease.

Conclusion

Decreased PTEN expression is associated with malignant endometrium, with a statistically significant difference of PTEN immunoreactivity between hyperplastic endometrium and carcinoma. Data from this study revealed that a loss of PTEN expression is partly associated with the ECs passing through a premalignant phase. The PI3K–AKT

Fig. 3



Immunohistochemical staining using phosphatase and tensin homolog antibody showing (a) positive expression in grade I endometrioid endometrial carcinoma. (phosphatase and tensin homolog, ×100) and (b) complete lack of reaction in grade III endometrioid endometrial carcinoma (phosphatase and tensin homolog, ×200).

Table 3 Relation between type phosphatase and tensin homolog expression and types of lesion

	PTEN [*n* (%)]	
Lesions	Positive	Negative
Simple hyperplasia	7 (70.0)	3 (30.0)
Complex hyperplasia	5 (83.3)	1 (16.7)
Atypical hyperplasia	3 (50.0)	3 (50.0)
Endometrial carcinoma		
Grade I	3 (42.9)	4 (57.1)
Grade II	1 (20.0)	4 (80.0)
Grade III	1 (16.7)	5 (83.3)
χ^2	8.876	
P value	0.041	

PTEN, phosphatase and tensin homolog.

pathway is frequently activated in EC, often in part owing to functional PTEN loss. Therefore, accurate detection of this aberration is an important component of patient selection to reach optimal targeted therapeutic agents.

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Conflicts of interest

There are no conflicts of interest.

References

1 Gbelcova H, Bakes P, Priscáková P, Šišovský V, Hojsíková I, Straka L. PTEN sequence analysis in endometrial hyperplasia and endometrial carcinoma in slovak women. *Anal Cell Pathol* 2015; **5**:1–7.

2 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**:69–90.

3 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. DFT-comparison of anti-cancer effect of ibuprofen drug anions and breast cancer treatment by ethanolic solution of nitrobenzaldehyde in two hours. *CA A Cancer J Clin* 2015; **65**:87–108.

4 National Cancer Institute. *Genetics of breast and gynecologic cancers: health professional version*. Bethesda: PDQ Cancer Genetics Editorial Board; 2002.

5 Boruban MC, Altundag K, Kilic GS, Blankstein J. From endometrial hyperplasia to endometrial cancer: insight into the biology and possible medical preventive measures. *Eur J Cancer Prev* 2008; **17**:133–138.

6 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; **25**,4:41.

7 Matias-Guiu X, Prat J. Molecular pathology of endometrial carcinoma' *Histopathology* 2013; **62**:111–123.

8 Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N, Çiriş F. M: 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; **28**;203:153–162.

9 Liu F. Molecular carcinogensis of endometrial cancer. *Taiwanese J Obstet Gynecol* 2007; **64**:26–32.

10 Chow LM, Baker SJ. PTEN function in normal and neoplastic growth. *Cancer Lett* 2006; **28**;241:184–196.

11 Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. *Cancer Res* 1997; **1**;57:4736–4738.

12 Djordjevic B, Hennessy BT, Li J, Barkoh BA, Luthra R, Mills GB, Broaddus RR. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. *Modern Pathol* 2012; **25**:699–708.

13 Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer* 2011; **11**:289–301.

14 Terakawa N, Kanamori Y, Yoshida S. Loss of PTEN expression followed by Akt phosphorylation is a poor prognostic factor for patients with endometrial cancer. *Endocrine Relat Cancer* 2003; **1**;10:203–208.

15 Silverberg SG, Kurman RJ, Nogales F, Mutter GL, Kubik-Huch RA, Tavassoli FA. *World Health Organization Classification of Tumors. Pathology and genetics of tumors of the breast and female genital organs: Epithelial tumours and related lesions'* Lyon: IARC Press, 2003.

16 Mikuta JJ. International Federation of Gynecology and Obstetrics staging of endometrial cancer 1988. *Cancer* 1993; **71**:1460–1463.

17 Zaino RJ, Kurman RJ, Diana KL, Morrow CP. The utility of the revised International Federation of Gynecology and Obstetrics histologic grading of endometrial adenocarcinoma using a defined nuclear grading system. *Cancer* 1995; **75**:81–86.

18 Doll A, Abal M, Rigau M. Novel molecular profiles of endometrial cancer-new light through old windows. *J Steroid Biochem Mol Biol* 2008; **108**:221–229.

19 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**:1412–1418.

20 Hayes MP, Wang H, Espinal-Witter R, Douglas W, Solomon GJ, Baker SJ. PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. *Clin Cancer Res* 2006; **12**:5932–5935.

21 Athanassiadou P, Athanassiades P, Grapsa D, Gonidi M, Athanassiadou AM, Stamati PN. The prognostic value of PTEN, p53, and beta-catenin in endometrial carcinoma: a prospective immunocytochemical study. *Int J Gynecol Cancer* 2007; **17**:697–704.

22 Mutter GL. Diagnosis of premalignant endometrial disease. *J Clin Pathol* 2002; **55**:326–333.

23 Garg K, Broaddus RR, Soslow RA, Urbauer DL, Levine DA, Djordjevic B. Pathologic scoring of PTEN immunohistochemistry in endometrial carcinoma is highly reproducible. *Int J Gynecol Pathol* 2012; **31**:48–56.

24 Elwy DA, Abd Al-Aziz AM, El Sheikh SA, Darweesh MF, Dina F. Elyasergy: expression of PTEN in endometrioid carcinoma, istopathological and immunohistochemical study. *World J Med Sci* 2016; **13**:118–125.

25 Hecht JL, Mutter GL. Molecular and pathologic aspects of endometrial carcinogenesis. *J Clin Oncol* 2006; **24**:4783–4791.

26 Mao TL, Ayhan A, Kuo KT, Lin MC, Tseng LH, Ogawa H. Immunohistochemical study of endometrial high-grade endometrioid carcinoma with or without a concurrent low-grade component: Implications for pathogenetic and survival differences. *Histopathology* 2015; **67**:474–482.