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Expression of PTEN in Endometrioid Carcinoma, Histopathological and Immunohistochemical Study

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Abstract: Endometrial endometrioid carcinoma (EECA) has a variety of molecular alterations. Currently the most frequently altered is loss of the PTEN (phosphate and tensin homolog) protein, a tumor suppressor gene. The aim of this study was to evaluate the expression pattern of PTEN gene in normal proliferative endometrium and EECA. This work included sixty paraffin-embedded endometrial tissue samples obtained from surgical pathology files of Pathology Department, Faculty of medicine, Cairo University, Kasr El Aini Hospital and other private laboratories; diagnosed as: 30 within normal proliferative (as a control group) and 30 EECA. Immunoreactivity was graded arbitrarily and semi quantitatively by considering the percentage and intensity of staining. The results indicated that PTEN immunoreactivity was noted in all cases of normal proliferative endometrium. In EECA, 46.7% were PTEN negative, 16.7% showed positive immunoreactivity of 10-50% and 36.7% showed positive immunoreactivity of >50%. The difference in immunoreactivity was highly significant ($P<0.001$). PTEN intensity was significantly higher in normal proliferative endometrium than in EECA ($P<0.001$). It can be concluded that PTEN expression was significantly higher in cyclical endometrium than in endometrioid carcinoma.

Key words: PTEN · Endometrial cancer · Endometrioid carcinoma · Neoplastic endometrium

INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy in developed countries and the second most common in developing countries [1]. There are two major classes of endometrial carcinoma. These are commonly described as Type I (the majority) and Type II cancers, which respectively correspond to endometrioid and non-endometrioid histologic types [2]. Type I endometrial cancers are primarily associated with unopposed estrogen exposure and develop in a background of endometrial hyperplasia [3]. Endometrioid endometrial carcinoma (EECA) is the prototypical endometrial adenocarcinoma. It is 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 [4, 5]. Accurate diagnosis of premalignant lesions in routine endometrial biopsies has a great clinical value in patient management. Unfortunately several studies have shown that cytological atypia which is predominant criterion for diagnosis of premalignant lesions (atypical endometrial hyperplasia), have poor reproducibility [4, 6]. Recent molecular diagnostic methods have provided new ancillary tools for premalignant lesion diagnosis. EECA has a variety of genetic alternations, including microsatellite instability (MI) and mutations of PTEN, k-ras and β-catenin genes [7, 8]. Also, these molecular genetic alternations have been described in atypical endometrial hyperplasia [8]. Currently, PTEN is the most frequently altered gene in EECA which is located on chromosome 10 [9]. The PTEN gene has both lipid and protein phosphate activity 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 [10]. PTEN-null glands (i.e., loss of PTEN expression) are shown in a diffuse pattern in

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EECA but also may be detected in morphologically normal endometrial tissue, in atypical and non-atypical hyperplasias. These findings suggest that PTEN alternation occurs in the earliest phase of endometrial carcinogenesis [8, 11, 12]. The hypothesis that loss of PTEN expression could be assessed by immunohistochemical method has led to the suggestion that PTEN immunostaining may be an effective tool for screening of malignant and premalignant endometrial lesions [5, 13-15].

In the present study we used immunohistochemical method to evaluate PTEN expression in two groups of specimens; normal proliferative endometrium and EECA

MATERIALS AND METHODS

This work included sixty paraffin-embedded endometrial tissue samples diagnosed as: 30 within normal proliferative (as a control group) and 30 EECA. All endometrial tissue samples of the normal proliferative were obtained through dilatation and curettage (D&C). Twenty of the EECA were obtained through D&C and 10 through hysterectomy. The cases were obtained from surgical pathology files of Pathology Department, Faculty of Medicine, Cairo University, Kasr El Aini Hospital and other private laboratories during the period from March, 2011 till December, 2012.

Histopathological Evaluation: A paraffin block for each biopsy was re-cut at 4 microns thickness and stained with hematoxylin and eosin for routine histopathological examination. The diagnosis was confirmed.

The most representative paraffin block for each case was then selected for immunohistochemical analysis.

Immunohistochemical Study: Sections of 4µm in thickness were deparaffinized in xylene and rehydrated through a series of graded alcohols. Antigen retrieval was achieved by heat treatment at 98 centigrade's in PT module buffer 1 (citrate buffer, pH = 6.0) for 20 minutes. Endogenous peroxidase activity was blocked by incubating slides in serum blocking solution. The sections were incubated with anti-PTEN polyclonal antibody and diluted 1:100 in phosphate buffer, for 60 minutes respectively and then incubated in enzyme conjugate for 10 minutes. The reaction was visualized with the Zymed immunohistochemical detection kit using diaminobenzidine chromogene as substrate. Finally, the sections were counterstained with Mayer's hematoxylin. Immunohistochemical slides were evaluated under light microscope and uniform criteria were used. Immunoreactivity was regarded as positive when brown staining was localized in the nuclei or cytoplasm of normal endometrial glandular cell (an internal positive control) or tumoral cell. According to Kapucuoglu *et al.* [8] and An *et al.* [17] the immunoreactivity was graded arbitrarily and semi quantitatively by considering the percentage and intensity of staining. Staining of cells was scored as negative if <10%, + 1 if 10%-50% and +2 if >50% of slide's area was stained positive. The intensity of PTEN staining was scored from 0 = absent, +1 = light brown, +2 = brown to dark brown in the nucleus or cytoplasm of glandular cells for each specimen [18].

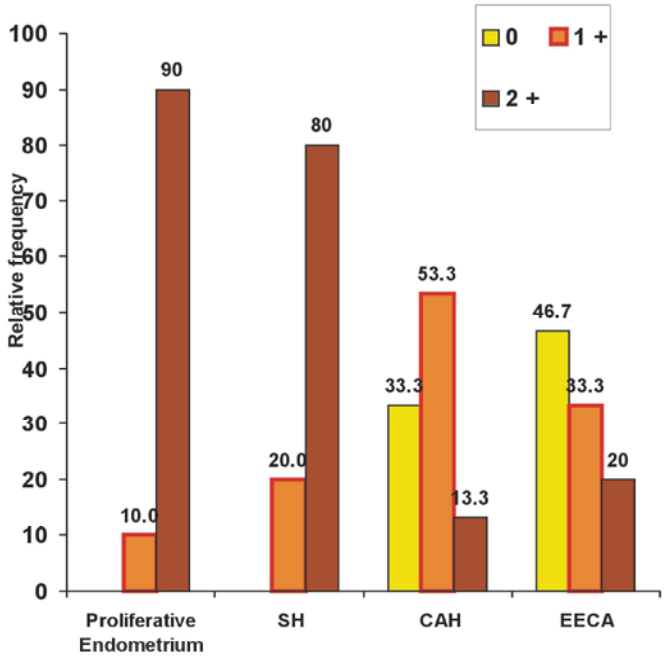
Statistical Analysis: SPSS (statistical package for social sciences) was used for data management. Mean and standard deviation described quantitative variable (age) and ANOVA test compared means among 2 study groups. Chi-square/Fisher exact test compared proportions among 2 groups. Pearson correlation analysis tested association between numerical data (as grade and PTEN). P value is significant if ≤ 0.05.

RESULTS

As regard PTEN expression based on the slide's area staining, PTEN immunoreactivity was noted in all normal proliferative endometrium (30/30, 100%) group. In EECA, 14 cases (46.7%) were PTEN negative, 5 cases (16.7%) showed positive immunoreactivity of 10-50% and 11 cases (36.7%) showed positive immunoreactivity of >50%. The difference in immunoreactivity was highly significant (P<0.001) (Table 1). PTEN intensity was significantly higher in normal proliferative endometrium than in EECA (P<0.001) (Fig. 1). As regard PTEN expression based on intensity of color reaction, 90% of the normal proliferative endometrium (27/30) (Fig. 2 and 3) showed intense cytoplasmic and/or nuclear staining in the glandular epithelial cells (+2), while only 20% of EECA (6/30) showed intense staining (+2). On the other hand, 33.3% of EECA (10/30) showed light staining (+1) (Fig. 4), while 3 cases only of the normal proliferative (3/30, 10%) showed light staining. PTEN was negative in 46.7% of EECA (14 cases) (Fig. 5, 6 and 7).

Table 1: PTEN expression based on the slide's area staining

		Group								
		Normal proliferative endometrium		SH		CAH		EECA		Total
Item		No	%	No	%	No	%	No	%	p value
PTEN area	<10%	--	--	--	--	5	33.3	14	46.7	P<0.001
	10-50%	--	--	--	--	4	26.7	5	16.7	
	>50%	30	100.0	15	100.0	6	40.0	11	36.7	
Total	--	30	100.0	15	100.0	15	100.0	30	100.0	



*0=absent, +1= light brown, +2= dark brown

Fig. 1: PTEN expression based on intensity of color reaction

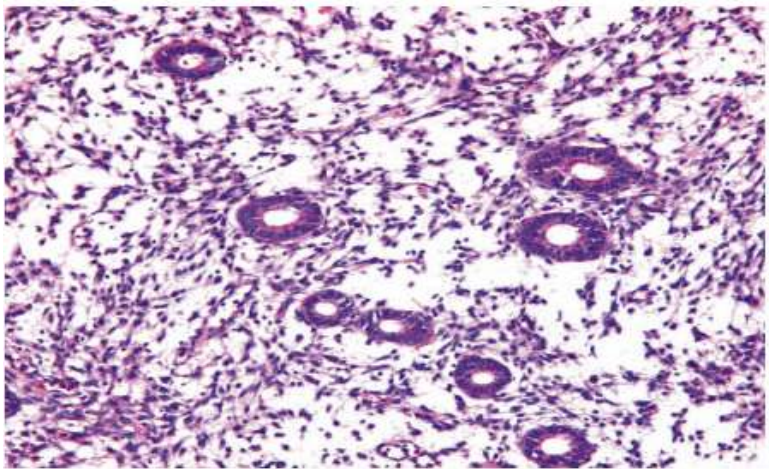


Fig. 2: Normal proliferative endometrium (H&E x 200)

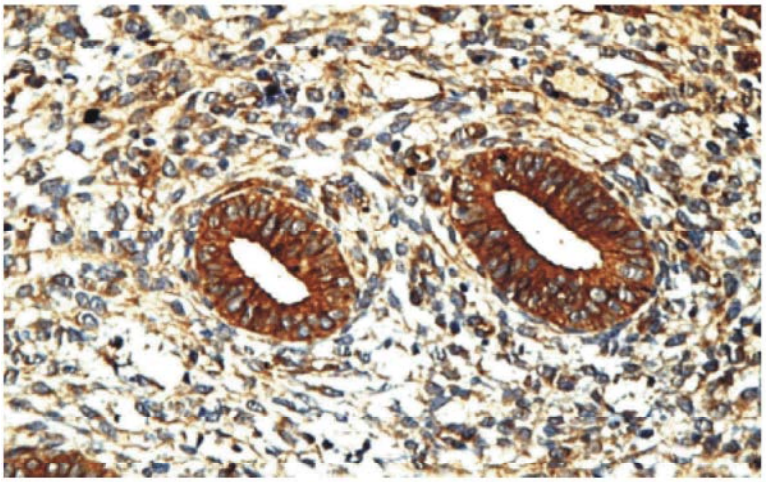


Fig. 3: Immunohistochemical staining using PTEN antibody showing strong (dark brown) and diffuse positivity (> 50% slide? area) in glandular epithelium of the normal proliferative endometrium (PTEN x 400)

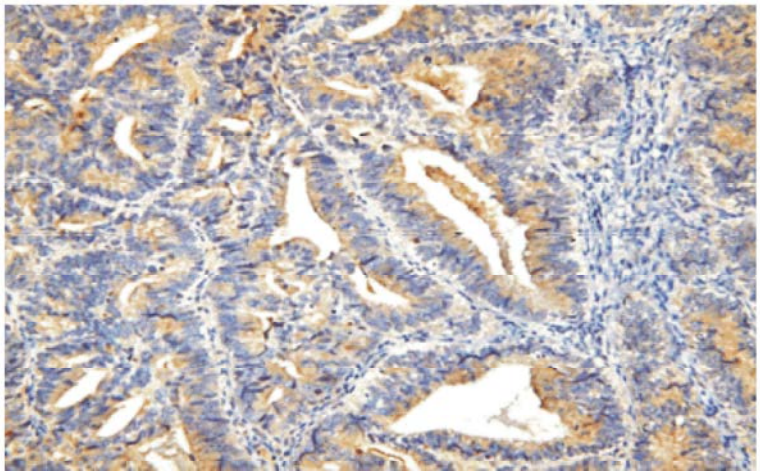


Fig. 4: Endometrioid carcinoma showing weak immunohistochemical staining (light brown) using PTEN antibody (PTEN x 200)

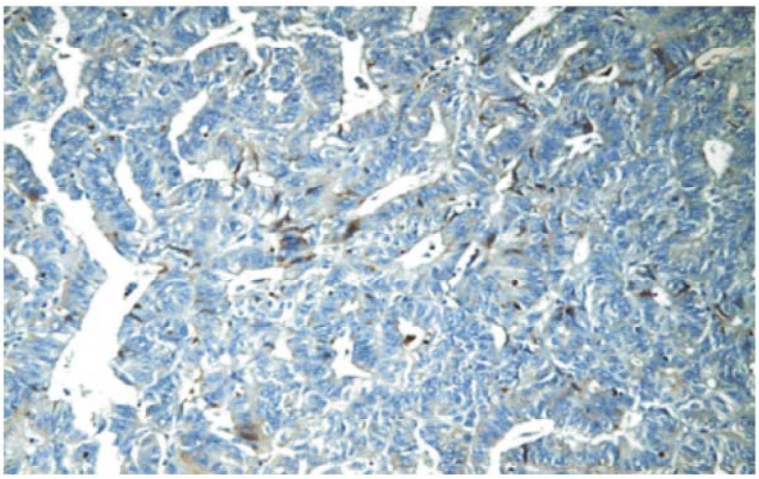


Fig. 5: Endometrioid carcinoma showing negative immunohistochemical staining using PTEN antibody. The glandulular epithelium showed < 10% slide? area light brown (weak) staining (PTEN x200).

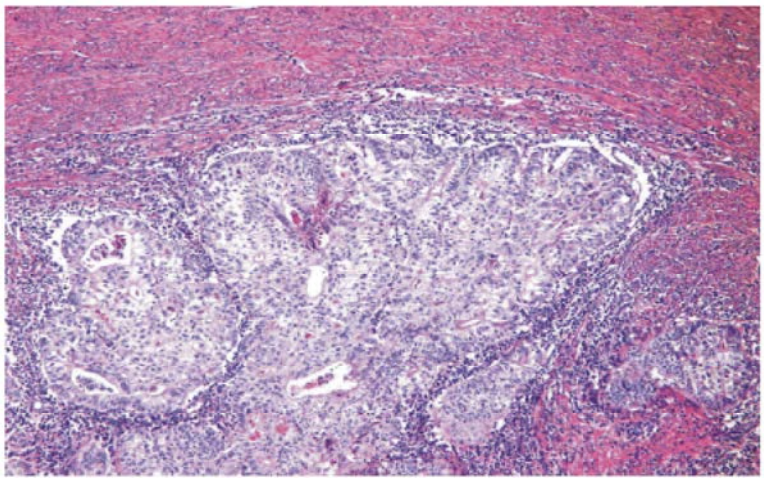


Fig. 6: Endometriod carcinoma (H&E x 200)

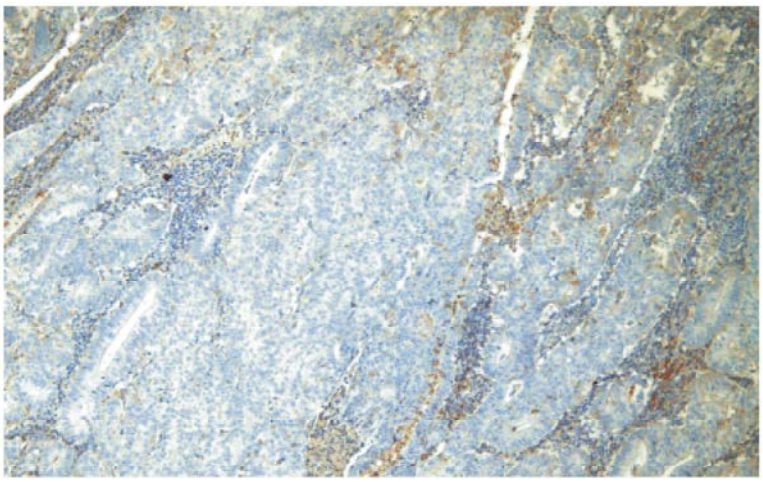


Fig. 7: Endometriod carcinoma, illustrated in the previous figure shows negative PTEN immunoreactivity (PTEN x 100)

DISCUSSION

Endometrial adenocarcinoma is the most common invasive malignant neoplasm of the female genital tract [19, 20]. There are two major classes of endometrial carcinoma. These are commonly described as Type I (the majority) and Type II cancers, which respectively correspond to endometrioid and non-endometrioid histologic types [2]. Type I endometrial cancers are primarily associated with unopposed estrogen exposure and develop in a background of endometrial hyperplasia [3]. In contrast, type II is unrelated to estrogen exposure [21]. Endometrial hyperplasia is classified by the WHO into four groups, namely simple hyperplasia, simple hyperplasia with atypia, complex hyperplasia and complex hyperplasia with atypia. Currently, there is a lack of criteria that could accurately predict the disease outcome and there is need for a new classification composed of three groups: endometrial hyperplasia, endometrial intraepithelial neoplasm (EIN) and endometrial carcinoma [22, 23]. EECA has a variety of genetic alternations, including microsatellite instability (MI) and mutations of PTEN, PIK3CA, k-ras and β-catenin genes. PTEN is the most frequently altered gene in EECA [24, 25] and several studies have found that PTEN inactivation is correlated with clonal growth patterns detected in endometrial hyperplasia and carcinoma [26]. PTEN-null glands (i.e., loss of PTEN expression) are shown in a diffuse pattern in EECA but also may be detected in morphologically normal endometrial tissue, in atypical and non-atypical hyperplasias. These findings suggest that PTEN alternation occurs in the earliest phase of endometrial carcinogenesis [8, 11, 12]. Accurate diagnosis of premalignant lesions in routine endometrial biopsies

has a great clinical value in patient management. Unfortunately several studies have shown that cytological atypia which is predominant criterion for diagnosis of premalignant lesions (atypical endometrial hyperplasia), have poor reproducibility [4, 6]. Evaluation of PTEN loss by immunohistochemistry is highly reproducible. This has led to the suggestion that PTEN immunostaining may be an effective tool for screening of malignant and premalignant endometrial lesions [5, 13-15].

In the current study, PTEN immunoreactivity was noted in all normal proliferative endometrium and 53.3% of EECA (16 cases). As regard the intensity of staining, 90% of the normal proliferative endometrium, showed intense PTEN staining. Among the PTEN-positive cases of EECA (53.3%, 16 cases), 10 cases (62.5%) showed light staining and only 6 cases (37.5%) showed intense staining. Therefore, PTEN intensity was significantly higher in normal proliferative endometrium than in EECA. 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 and the PTEN expression level was significantly higher in cyclic endometrium than in EECA. In our study, 80% of EECA showed complete absence or diminution of PTEN expression. In accordance to this, Boruban *et al.* [5] said that mutation of PTEN with absent or at least diminished expression is present in 83% of EECA cases. Hecht and Mutter [24] explained this, they said 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 deficient, rather than null state, is functionally significant when combined with abnormalities of other genes which converge on its downstream pathway. Also in agreement with the current study, Tantbirojn *et al*. [27] found a significant statistical difference of PTEN immunoreactivity among proliferative endometrium and endometrial carcinoma group. They said that complete loss of PTEN expression was most commonly found in EECA. In their study, they compared PTEN expression among groups of normal proliferative endometrium and EECA. In their study, they found complete absence of PTEN expression in 60% of EECA which is higher percentage of PTEN negativity than in our study which revealed negative expression in 46.7% of EECA. This may be attributed to the use of monoclonal antibody in their research and the use of polyclonal antibody in our study.

In accordance to our results, Sarmadi *et al*. [28] performed a study to evaluate the expression pattern of PTEN gene in normal and neoplastic endometrium. PTEN immunoreactivity was present in all normal proliferative endometrium and in 48% of EECA and the intensity of PTEN reaction was significantly higher in group with proliferative endometrium than EECA. Also, the present results are in agreement with those obtained by Feng *et al.* [29] they reported that the presence of PTEN protein was significantly decreased, as lesions progressed from normal endometrium to carcinoma. Also in a study performed by Peterson *et al.* [30] 55% of EECA were PTEN-negative, making it the most common abnormality in their study. Garg *et al*. [15] stated that evaluation of PTEN by immunohistochemistry is highly reproducible provided that application of standard immunohistochemical techniques and simple scoring criteria.

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