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Immunoreactivity of PTEN in Cyclic Endometrium and Endometrial Hyperplasia

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Abstract: PTEN (phosphate and tensin homolog) protein is a tumor suppressor gene and plays a significant role in inducing cell cycle arrest and programming apoptosis. PTEN inactivation is correlated with clonal growth patterns detected in endometrial hyperplasia and carcinoma, thus PTEN immunostaining may be an effective tool for screening of premalignant endometrial lesions. The aim of this study was to evaluate the expression pattern of PTEN gene in normal proliferative endometrium, simple endometrial hyperplasia and complex atypical hyperplasia. 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 endometrial hyperplasia including: 15 simple hyperplasia (SH) and 15 complex atypical hyperplasia (CAH). 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 normal proliferative endometrium and all SH cases. In CAH, 33.3% showed negative immunoreactivity (<10% of the slide's area staining), 26.7% showed positive immunoreactivity of 10-50% staining and 40% showed positive immunoreactivity of >50%. The difference in immunoreactivity was highly significant (P<0.001). PTEN intensity was significantly higher in normal proliferative endometrium and SH than in CAH (P<0.001). It can be concluded that PTEN expression was significantly higher in cyclical endometrium than in atypical hyperplasia.

Key words: PTEN · Endometrial hyperplasia · Complex atypical hyperplasia

INTRODUCTION

PTEN (phosphate and tensin homolog) is located on chromosome 10q23. PTEN is a tumor suppressor gene and plays a significant role not only in inducing cell cycle arrest and programming apoptosis, but also in other aspects of cell physiology, including the regulation of cell adhesion, migration and differentiation. Somatic deletions or mutations of this gene have been identified in a large fraction of tumors, including glioblastomas and endometrial and advanced prostate cancers, thus placing PTEN among the most commonly mutated genes in human cancer [1]. The most commonly observed PTEN defect in endometrial endometrioid carcinoma (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 [2]. PTEN has lipid and protein phosphatase activity [3]. The lipid phosphatase domain maintains cell-cycle arrest at the G1–S checkpoint, upregulates AKT-dependent proapoptotic pathways and down regulates Bcl-2–dependent antiapoptotic pathways [4]. PTEN inhibits the PI3K/AKT/mTOR pathway, thus controls the levels of phosphorylated AKT by opposing PIK3CA activity. The protein phosphatase domain inhibits focal adhesion formation, cell spreading and migration, as well as the inhibition of growth factor-stimulated MAPK signaling [5]. Thus, loss or altered PTEN expression results in aberrant cell growth and apoptotic escape [6]. PTEN inhibit the PI3K/AKT/mTOR pathway which plays a key role in endometrial carcinoma [7]. There are two major classes of endometrial carcinoma. These are commonly described as Type I (endometrioid) and Type II cancers (non-endometrioid) [8]. EECA 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 [9, 10].

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Adenocarcinoma tissues contain all of the PTEN, microsatellite and X inactivation patterns seen in atypical hyperplastic lesions from the same patient, objective evidence of direct lineage continuity between premalignant and malignant phases of tumor evolution [11]. Comparison of the extent and range of genomic damage between premalignant and malignant phases indicates a higher cumulative mutational load in cancers, a feature that must contribute to their differing morphology and behavior. For example, whereas 55% of atypical hyperplasia have demonstrable PTEN inactivating events (mutation and/or deletion), the proportion rises to 83% in those cancers which follow atypical hyperplasia [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 [9, 13]. Recent molecular diagnostic methods have provided new ancillary tools for premalignant lesion diagnosis. Currently, PTEN is the most frequently altered gene in EECA [11]. 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 suggested that PTEN alternation occurs in the earliest phase of endometrial carcinogenesis [12, 14, 15]. 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 premalignant endometrial lesions [10, 16-18].

In this study we used immunohistochemical method to evaluate PTEN expression in three groups of specimens from normal proliferative endometrium, simple hyperplastic endometrium and complex atypical hyperplasia.

MATERIALS AND METHODS

This work included sixty paraffin-embedded endometrial tissue samples diagnosed as: 30 within normal proliferative (as a control group), endometrial hyperplasia including: 15 simple hyperplasia (SH) and 15 complex atypical hyperplasia (CAH). All endometrial tissue samples of the normal proliferative, SH and CAH were obtained through dilatation and curettage (D&C). 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 following histopathological features were evaluated:

- The diagnosis was confirmed.
- Hyperplasic specimens were evaluated according to the WHO classification [19]. The WHO divided endometrial hyperplasia into four groups according to the presence or absence of cytological atypia and the degree of architectural complexity and crowding, as follows: simple hyperplasia, complex (adenomatous) hyperplasia, simple atypical hyperplasia and complex atypical hyperplasia.

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

Immunohistochemical Study: Sections of 4ìm 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.* [16] 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 [17].

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 3 study groups. Chi-square/Fisher exact test compared proportions among 3 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%) and SH (15/15, 100%) groups. In CAH, 5 cases (33.3%) showed negative immunoreactivity (<10% of the slide's area staining), 4 cases (26.7%) showed positive immunoreactivity of

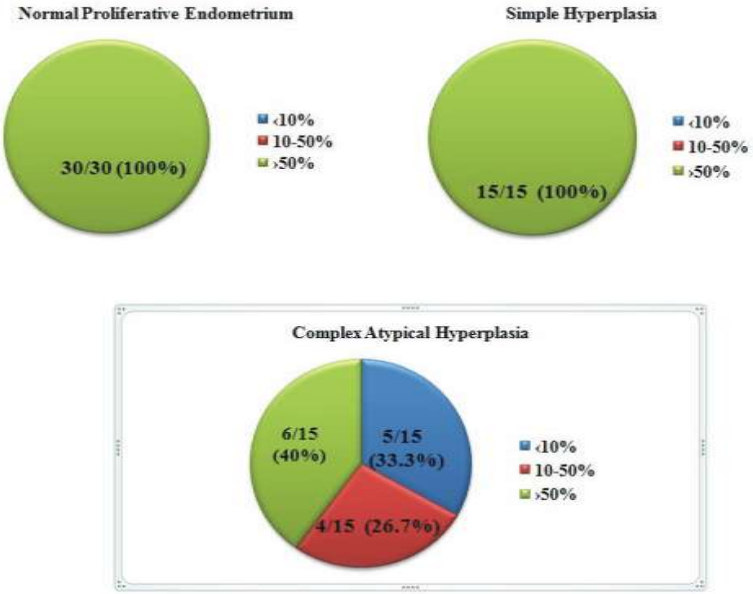
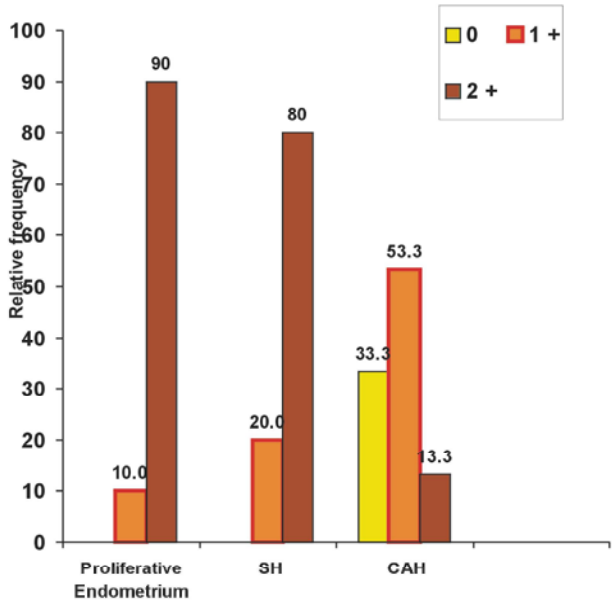


Fig. 1: PTEN expression based on the slide's area staining. P value<0.001



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

Fig. 2: PTEN expression based on intensity of color reaction

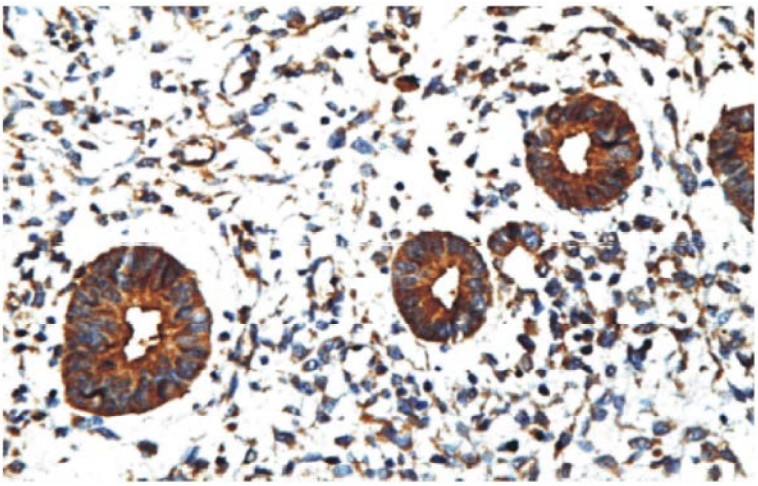


Fig. 3: Immunohistochemical staining using PTEN antibody showing strong (dark brown) and diffuse positivity (> 50% slide? area) in glandular epithelium of the normal proliferative endometrium (PTEN x 400)

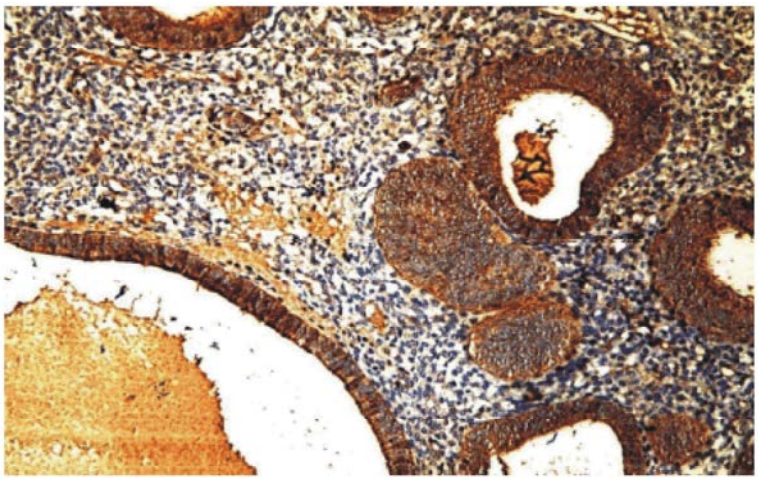


Fig. 4: Immunohistochemical staining using PTEN antibody showing diffuse and strong reactivity of simple hyperplasia of endometrium (PTEN ×400)

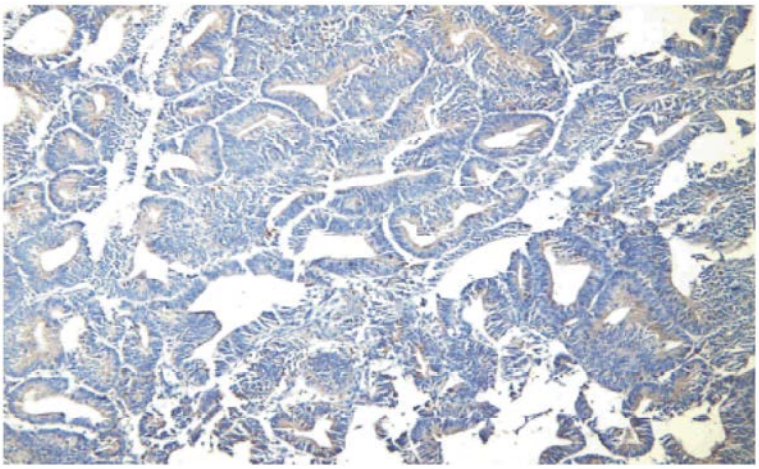


Fig. 5: Complex atypical hyperplasia showing diffuse and weak (light brown) immunohistochemical staining using PTEN antibody (PTEN x 100)

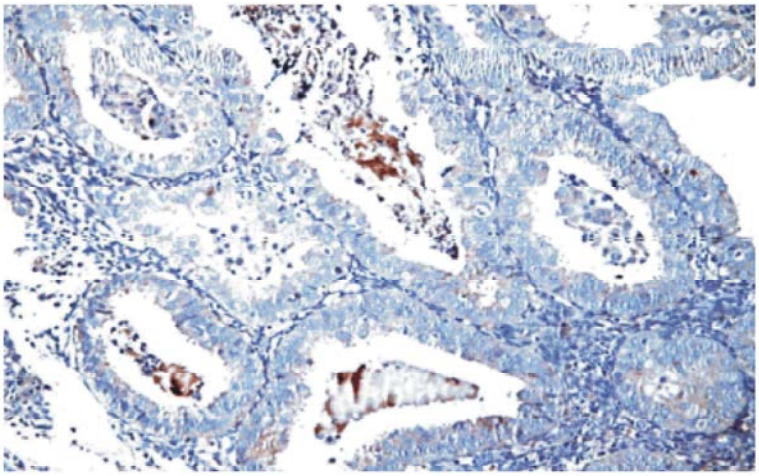


Fig. 6: Complex atypical hyperplasia. Immunohistochemical staining using PTEN antibody. The glandular epithelium showed negative immunostaining (PTEN x 400)

10-50% staining and 6 cases (40%) showed positive immunoreactivity of >50%. The difference in immunoreactivity was highly significant (P<0.001) (Fig. 1). PTEN intensity was significantly higher in normal proliferative endometrium and SH than in CAH (P<0.001) (Fig. 2). As regard PTEN expression based on intensity of color reaction, 90% of the normal proliferative endometrium (27/30) (Fig. 3), 80 % of SH (12/15) (Fig. 4) showed intense cytoplasmic and/or nuclear staining in the glandular epithelial cells (+2), while only 13.3% of CAH (2/15) showed intense staining (+2). On the other hand, 53.3% of CAH (8/15) (Fig. 5) showed light staining (+1), while 3 cases only of the normal proliferative (3/30, 10%) and 3 of the SH (3/15, 20%) showed light staining. PTEN was negative in 33.3% of CAH (5 cases) (Fig. 6).

DISCUSSION

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 (EH), endometrial intraepithelial neoplasm (EIN) and endometrial carcinoma [20, 21]. EECA has a variety of genetic alternations, including microsatellite instability (MI) and mutations of PTEN, PIK3CA, k-ras and â-catenin genes. PTEN is the most frequently altered gene in EECA [2, 22] and several studies have found that PTEN inactivation is correlated with clonal growth patterns detected in endometrial hyperplasia and carcinoma [23]. 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 [14, 15]. 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 [9, 13]. 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 [9, 10, 18, 19]. In the current study, PTEN immunoreactivity was noted in all normal proliferative endometrium and SH groups, 66.7% of CAH (10 cases). As regard the intensity of staining, 90% of the normal proliferative endometrium, 80% of the SH groups showed intense PTEN staining. Among the PTEN-positive cases of CAH (66.7%, 10 cases), 2 cases only of them (20%) showed strong staining and 8 cases (80%) showed light staining. Therefore, PTEN intensity was significantly higher in normal proliferative endometrium and SH than in CAH. These results are in agreement with those obtained by Kapucuoglu *et al*. [15], who stated that PTEN expression was significantly higher in cyclical endometrium and non-atypical hyperplasias than in atypical hyperplasia but there were no differences between SH and cyclical endometrium. Also in agreement with the current study, Tantbirojn *et al*. [24] found a

significant statistical difference of PTEN immunoreactivity among proliferative endometrium and EH on one side and atypical hyperplasia group. They reported that complete loss of PTEN expression was most commonly found in hyperplasia with cytologic atypia and EECA. In their study, they compared PTEN expression among groups of normal proliferative endometrium, EH and EECA. In their study, they found complete absence of PTEN expression in 60% of CAH which is higher percentage of PTEN negativity than in our study which revealed negative expression in 33.3% of CAH. This may be attributed to the use of monoclonal antibody in their research and the use of polyclonal antibody in our research. As regard the intensity, similar to our study, the majority of EH without atypia group, revealed moderate to strong PTEN expression. In accordance to our results, Sarmadi *et al*. [25] performed a study to evaluate the expression pattern of PTEN gene in normal and hyperplastic endometrium and neoplastic endometrium. PTEN immunoreactivity was present in all normal proliferative endometrium, all SH and 75% of CAH and the intensity of PTEN reaction were significantly higher in group with proliferative endometrium than hyperplastic endometrium. Also, the present results are in agreement with those obtained by Feng *et al.* [26] they reported that the presence of PTEN protein was significantly decreased, as lesions progressed from normal endometrium to atypical hyperplasia. Garg *et al*. [18] 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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