Immunohistochemical expression of phosphatase and tensin homolog in endometrial hyperplasia and carcinoma

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Introduction **The phosphatase and tensin homolog (PTEN) gene is localized on chromosome 10q23. It is a tumor suppressor gene that inhibits cell proliferation by regulating intracellular signaling pathways, and this activity can be abolished by mutations of the PTEN gene. PTEN is frequently mutated in a wide range of human tumors.**

Aim of the study **In this study, we evaluated the use of PTEN gene as a diagnostic marker to differentiate between endometrioid adenocarcinoma and premalignant lesions of the endometrium.**

Materials and methods **We used an immunohistochemical technique to evaluate the alteration of PTEN in 53 biopsy cases of normal, hyperplastic, and neoplastic endometrial tissue.**

Results **We found that PTEN expression was decreasing from hyperplasia (seven of 12 cases) to atypical hyperplasia (three of six cases), to endometrioid carcinoma (six of 21 cases), with statistically significant relationships.**

Conclusion **PTEN alteration of expression is one of the earliest changes in the process of endometrial tumorigenesis from hyperplasia to the carcinoma stage. *Egypt J Pathol* 31:2–5 © Egyptian Journal of Pathology**

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Introduction

Endometrioid endometrial adenocarcinoma accounts for three fourths of the endometrial cancers and are thought to develop after a continuum of premalignant lesions ranging from endometrial hyperplasia without atypia to hyperplasia with atypia and finally to well-differentiated carcinoma (Boruban *et al.*, 2008).

Accurate diagnosis of premalignant lesions in routine endometrial biopsies has a great clinical value in their management. Unfortunately, several recent studies have shown that cytological atypia, which is an important criterion for diagnosis of premalignant lesions (atypical hyperplasia), has poor reproducibility. Therefore, new insight into the morphology of biologically defined precancerous lesions of the endometrium is needed (Mutter, 2002).

Endometrioid endometrial adenocarcinoma has a variety of genetic alterations, including microsatellite instability, and mutations of phosphatase and tensin homolog (PTEN), K-ras, and B-catenin genes. These molecular genetic alterations have been described in atypical endometrial hyperplasia. PTEN is the most frequently altered gene in endometrioid carcinoma. Up to 50% of all endometrioid adenocarcinomas and 83% of tumors with adjacent premalignant lesions show altered PTEN that is characterized by loss of expression (Liu, 2007; Kapucuoglu *et al.*, 2007).

Mutations of PTEN are frequently detected in several cancers such as ovary (Geyer *et al.*, 2009), prostate (Visakorpi, 2003), breast (Bose *et al.*, 2002), glial tumors (Kimura *et al.*, 2004), and endometrium (An *et al.*, 2002). Loss of PTEN expression is detected in a diffuse pattern in endometrial carcinoma, but also may be detected focally in morphologically normal endometrial tissue. This suggests that PTEN alteration occurs in the earliest phase of endometrial carcinogenesis (Lacey *et al.*, 2008).

The hypothesis that loss of PTEN expression could be assessed by immunohistochemical method has led to the use of PTEN immunostaining for the diagnosis of malignant and premalignant lesions (Pallares *et al.*, 2005). In this study, we used an immunohistochemical method to evaluate PTEN expression in normal, hyperplasia, and carcinoma of the endometrium.

Materials and methods

Fifty three samples were retrieved from the Pathology lab of the Sohag University Hospital. They were previously diagnosed as: simple endometrial hyperplasia (8), Complex hyperplasia without atypia (12), complex hyperplasia with atypia (6) and endometrioid adenocarcinoma (21). All Grades of endometrial carcinoma were represented (G1-12) (G2-3) and (G3-6). They were all selected from Surgical Pathology files of the Department of pathology, Sohag University Hospital during the period from January 2007 to December 2008.

The samples included 32 curettage and 21 excisional biopsies. Hematoxylin and eosin-stained sections from each case were reviewed by two pathologists to confirm the histological diagnosis according to WHO histological classification. Specimens with any evidences of

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endometritis or hormone-induced changes were excluded. The protocol of the research was approved by the medical ethics committee in Sohag Faculty of Medicine.

Immunohistochemical staining technique

Sections of four micron thickness from the most representative paraffin blocks for each case were deparaffinized in xylene and rehydrated through a series of graded alcohols. Antigen retrieval was achieved by heating, in a microwave oven, in citrate buffer (pH 6) for 20 min. Endogenous peroxidase activity was blocked by incubating slides in hydrogen peroxide (0.6%) for 10 min. The reaction was visualized using diaminobenzidine chromogen, and then the sections were counterstained with Mayer's hematoxylin. Immunohistochemically stained slides were evaluated by two pathologists under light microscopy. Immunoreactivity was graded semiquantitatively by calculating the intensity and percentage of staining on the whole section. The intensity of PTEN staining was scored from 0 = absent, + 1 = light brown, + 2 = dark brown. The percentage was scored as negative if less than 10%, + 1 if 10–50%, and + 2 if more than 50% of the slide areas was stained (Kapucuoglu *et al.*, 2007). Statistical analysis of the results data was evaluated by SPSS, version 11 (Vijay Gupta, Georgetown University, US) and the χ^2 test with P value of less than 0.05.

Results

Fifty-three patients were included in this study, with age range of 35–80 years and the mean age was 52.1 years. PTEN immunoreactivity was detected in one of four cases of normal proliferative endometrium both in the glandular and stromal tissues, three of eight cases of simple endometrial hyperplasia, seven of 12 complex hyperplasia without atypia, three of six cases of atypical endometrial hyperplasia, and in six of 21 cases of endometrioid adenocarcinoma. The differences of immunoreactivity between groups was statistically significant $P = 0.011$.

PTEN was expressed in six of 21 cases of endometrioid adenocarcinoma with weak immunostaining, as the tumor grading increases from grade 1 (G1) (four of 12 cases) to G2 (one of three cases), and G3 (one of six cases) (Fig. 1) (Table 1).

Discussion

A progression model of endometrial carcinoma type I resembling Vogelstein progression model for colorectal carcinoma has been proposed. This hypothesis is supported by the evidence that: (i) some of the genetic alterations found in endometrioid adenocarcinomas is already present in atypical hyperplasia. (ii) Increased genetic alterations are found in well-differentiated endometrioid carcinoma compared with atypical hyperplasia. (iii) The number of genetic alterations increases according to higher histological grade. (iv) More chromosomal imbalance is identified in endometrial carcinoma compared with atypical hyperplasia using comparative genomic hybridization. Most cases of simple hyperplasia and a subset of complex hyperplasia are polyclonal and are considered to be reactive processes due to hyperestrogenism, which may regress through progesterone therapy. In contrast, most cases of atypical hyperplasia are monoclonal. In addition, the number of chromosomal aberrations in complex hyperplasia is significantly higher than simple hyperplasia and is close to the number found in atypical hyperplasia. Most of the genetic alterations identified in endometrioid carcinoma seem to occur very early in the development of carcinoma (Liu, 2007).

Endometrial precancers are monoclonal lesions that share a common genetic lineage with invasive endometrial carcinoma, including PTEN mutations. Mutations of the PTEN tumor suppressor gene have been identified in histologically normal appearing endometrium exposed to estrogen, 18–55% of endometrial precancers, and in 26–80% of endometrial carcinoma. PTEN has been shown to play several roles in tumor suppression, including cell cycle arrest and promotion of apoptosis. Loss of PTEN function predisposes endometrial cells to neoplastic transformation particularly in high estrogenic states (Latta and Chapman, 2002).

In a more recent study using immunohistochemical analysis, 29 endometrial carcinomas, 38 endometrial hyperplasias, and 10 proliferative endometria were stained with PTEN, and showed that its expression decreased in hyperplasia and carcinoma cases with respect to proliferative endometrium. The main survival was statistically significantly higher in PTEN-positive cases (Erkanli *et al.*, 2006).

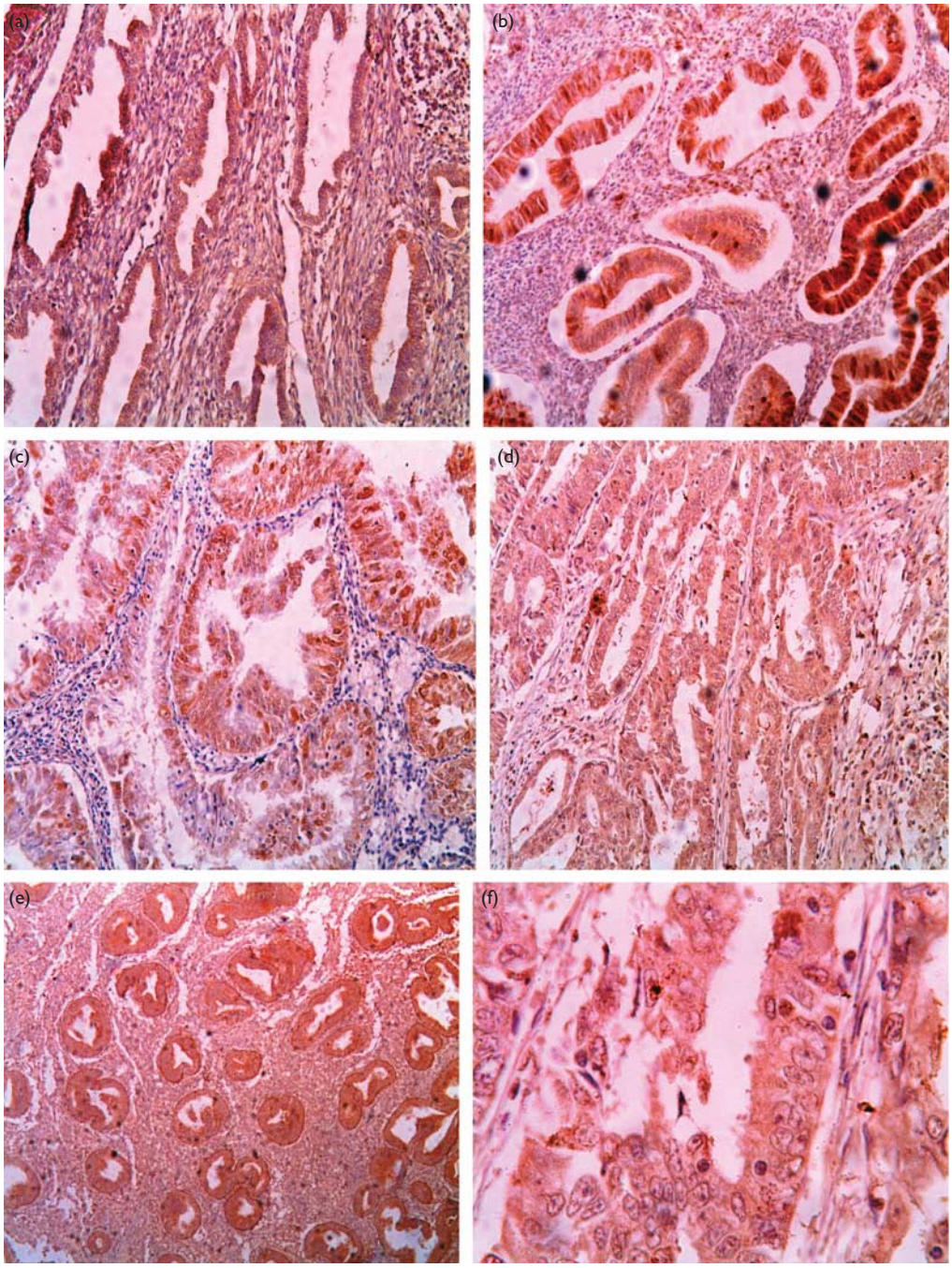
Loss or decreased expression of PTEN was observed in 66% (41 of 60) of cases of uterine endometrioid carcinoma, whereas most uterine serous carcinoma (four of five cases) showed intense PTEN expression. However, alteration of PTEN expression was not correlated with the histological grade or stage (An *et al.*, 2002).

In this study, expression of PTEN was correlated with grading of the endometrioid carcinoma. PTEN expression decreased as the histological grade increased. PTEN expression was positive in 6/21 cases with (G1-4 cases), (G2-one case) and (G3-one case). In G1 positive cases, PTEN expression was of moderate intensity, while it was of weak intensity in both G2 and G3 positive cases, with a statistically insignificant relationship.

This finding was in agreement with Salvesen's population-based study, who detected PTEN mutation in 54% of endometrial carcinomas and that the presence of PTEN mutation significantly correlated with young age, low FIGO stage, endometrioid subtype, low grade, microsatellite instability, and favorable prognosis. They suggested that PTEN mutations are frequent in sporadic endometrial carcinoma and define a prognostically favorable subgroup (Salvesen *et al.*, 2004).

In the study by Kimura *et al.* (2004), it was concluded that PTEN expression was decreased in well-differentiated and less growth aggressive endometrial carcinoma with high levels of estrogen receptors and progesteron receptors. In 117 cases of endometrioid adenocarcinoma and 20 cases of endometrial hyperplasia, they found that PTEN staining scores of endometrioid adenocarcinoma was increasing from G1 to G3. They suggested that disturbed PTEN expression occurs in an early phase of tumor genesis of well-differentiated endometrioid adenocarcinoma.

Fig. 1



Phosphatase and tensin homolog (PTEN) immunoreactivity in different endometrial lesions. (a) simple endometrial hyperplasia (× 40). (b) Complex endometrial hyperplasia without atypia with strong PTEN expression (× 40). (c) Complex endometrial hyperplasia with atypia (× 60). (d) Endometrial adenocarcinoma grade 1 (× 40). (e) Proliferative endometrium showed strong PTEN expression in both stroma and glands (× 40). (f) Endometrial adenocarcinoma showed weak PTEN expression (× 100).

Cirpan *et al.* (2006) observed that complete loss of PTEN immunoreactivity was found in 4.2% of cases of endometrial intraepithelial neoplasia, with partial loss in 33.3% of cases and expression in 62.5% of cases. They concluded that PTEN expression showed no differences among cases of intraepithelial neoplasia, endometrial carcinoma, and proliferative endometrium.

In this study, PTEN expression was detected in three of six cases of atypical endometrial hyperplasia, and this finding was in agreement with a recent study by Lax (2004), who reported that in atypical hyperplasia, alterations of PTEN are present in approximately 50% of the cases. However, he suggested that PTEN and K-ras mutations occurred earlier, as they were found in simple hyperplasia and were partially associated with monoclonality.

Lacey *et al.* (2008) compared PTEN expression by immunohistochemistry in endometrial biopsy specimen from patients with endometrial hyperplasia who progressed to carcinomas versus patients with endometrial hyperplasia who did not clinically progress. They

Table 1 Phosphatase and tensin homolog immunoreactivity in different cases of the study group

Diagnosis	Number of cases	PTEN +	PTEN + +	Percentage
Proliferative	4	1		
Secretory	2	–		
Simple hyperplasia	8	3		
Complex hyperplasia	12	2	5	58
Complex hyperplasia with atypia	6	1	2	
Endometrioid adenocarcinoma	21	6		28
Total	53	20		

Showed that PTEN expression was detected in endometrial hyperplasia and lost in most of the cases of endometrioid adenocarcinoma with statistically significant relationship.
PTEN, phosphatase and tensin homolog.

concluded that loss of PTEN expression in endometrial biopsies was neither associated with, nor sensitive or specific marker to subsequent progression to endometrial carcinoma.

Mutter *et al.* (2000) determined the earliest stage of endometrial neoplasia, in which PTEN mutation occurred, by examining 30 hysterectomy specimens containing endometrioid adenocarcinoma and premalignant and benign endometria for the presence of PTEN mutations, using denaturing gradient gel electrophoresis and immunohistochemistry in two separate groups. They found that somatic PTEN mutations were detected in 83% of endometrial carcinoma and in 55% in premalignant lesions, and the difference was statistically significant ($P = 0.025$). They concluded that loss of PTEN function by mutation is an early event in endometrial carcinogenesis that may occur in response to known endocrine risk factors, and offers an informative immunohistochemical biomarker to known premalignant diseases. Individual PTEN-negative glands in estrogen-exposed endometria are the earliest recognizable stage of endometrial tissue carcinogenesis followed by proliferation into dense clusters that form discrete premalignant lesions.

PTEN expression level was significantly higher in nonatypical hyperplasia than in endometrioid adenocarcinoma in the study by Kapucuoglu *et al.* (2007). They presumed that PTEN was involved in the early phase of endometrial tumorigenesis, and it can be speculated that decreased PTEN expression with loss of differentiation in carcinoma can contribute to the emergence of tumors with a more aggressive phenotype.

A similar study, Tantbirojn *et al.* (2008) found that PTEN protein was detected in 60% of nonatypical endometrial hyperplasia and 24% of atypical endometrial hyperplasia. In the majority of nonatypical hyperplasia, PTEN was strongly expressed (70% of simple hyperplasia and 47% of complex hyperplasia), with statistically significant difference ($P = 0.004$).

Conclusion

We concluded that alteration in PTEN immunohistochemical expression is an early event in the process of carcinogenesis of endometrial hyperplasia to carcinoma. Expression of PTEN can be used to differentiate between atypical hyperplasia and endometrioid adenocarcinoma.

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