Immunohistochemical evaluation of PTEN protein in patients with endometrial intraepithelial neoplasia compared to endometrial adenocarcinoma and proliferative phase endometrium

T. Cirpan[1], *M.D.*; M.C. Terek[1], *M.D.*; L. Mgoyi[1], *M.D.*; O. Zekioglu[2], *M.D.*; O. Iscan[1], *M.D.*; A. Ozsaran[1], *M.D.*

[1]Department of Obstetrics and Gynecology, [2]Department of Pathology, Ege University Faculty of Medicine, Izmir (Turkey)

Summary

Objective: The aim of this study was to reclassify endometrial hyperplasia cases and examine PTEN protein immunoreactivity compared to cases with endometrial adenocarcinoma and proliferative endometrium.

Design: Endometrial samples from 37 women with endometrial hyperplasia with atypia were reclassified as endometrial intraepithelial neoplasia (EIN). Eighteen were complex and 19 were simple endometrial hyperplasia. Twenty-our cases of EIN, ten endometrial adenocarcinoma cases and ten proliferative phase endometrium sections were immunostained for PTEN expression. PTEN expression was documented according to the degree of immunoreactivity as complete loss, partial loss and present.

Results: Twenty-four of 37 (64%) women with endometrial hyperplasia were reclassified as EIN. Complete loss of PTEN immunoreactivity was found in only one of the 24 EIN patients (4.2%), partial loss in eight of 24 (33.3%) and present in 15 of 24 (62.5%). There were no difference in PTEN immunoreactivity between EIN, endometrial adenocarcinoma and endometrial proliferation (p = 0.342). PTEN immunoreactivity was partially lost in seven and present in three of the patients with endometrial adenocarcinoma. None of the patients expressed complete loss of PTEN immunoreactivity in this group.

Conclusion: EIN classification may provide a better and more objective assessment of endometrial hyperplasia cases. PTEN expression showed no differences among the cases of EIN, endometrial carcinoma and proliferative phase endometrium.

Key words: PTEN; Endometrial intraepithelial neoplasia.

Introduction

Endometrial hyperplasia is a well known precursor lesion for endometrial adenocarcinoma especially the endometrioid type [1]. To avoid inter- and intraobserver variation, recently, a new classification scheme was presented for the percursor lesions of endometrial adenocarcinoma as 'endometrial intraepithelial neoplasia (EIN) [2]. PTEN protein is a recently identified tumor suppressor gene inactivated in a wide variety of human cancers, including endometrial cancers. Despite the fact that, mutation of the PTEN tumor suppressor gene has been reported in approximately 50-83% of endometrial adenocarcinoma [2] studies on the expression of PTEN protein in endometrial hyperplasia are limited.

The aim of this study was to reclassify the endometrial hyperplasia cases and to examine the expression of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) protein according to the degree of immunoreaction in these cases.

Revised manuscript accepted for publication January 9, 2006

Materials and Methods

A retrospective analysis of the archives of the Department of Pathology Ege University Faculty of Medicine was performed. Paraffin blocks of women who were diagnosed and treated at the Department of Obstetrics and Gynecology, Ege University Faculty of Medicine between 1998 and 2002 were selected for the study and clinical data were obtained from medical records.

Tissue Samples. Endometrial samples of 37 women who were admitted to hospital and diagnosed with endometrial hyperplasia with atypia were taken for histopathologic re-evaluation and reclassification as EIN according to Mutter's criteria [2]. At the initial diagnosis 18 of them were complex and 19 were simple endometrial hyperplasia with atypia. Twenty-four out of 37 cases were identified as EIN and 13 were excluded. Of these 13 cases six were deemed normal and seven were regarded as hyperplastic with no atypia. A total of 44 patients were included in the immunohistochemical staining part of the study, 24 of them had been previously diagnosed with endometrial hyperplasia with atypia, either simple or complex and were reclassified as EIN. Since loss of PTEN protein immunoreactivity has been reported in endometrial adenocarcinoma [3, 4] and in different periods of normal menstrual cycles [5], ten cases of endometrial adenocarcinoma and ten cases of proliferative endometrium were taken for comparison. Table 1 summarizes the characteristics of the endometrial cancer cases. Diagnoses for all cases were made in the Pathology Department.

EIN classification according to Mutter's criteria (2): Monoclonal endometrial precancerous lesions were reclassified as

EIN by using observational morphometrical measures with hematoxylen-eosin stained sections. The criteria for EIN classification as proposed by Mutter (2) were used in this study. These criteria were volume percentage stroma < 55%, cytological findings (changes in nuclear size, contour and nucleolus, nuclear/cytoplasmic ratio, granular chromatin structure and cytoplasmic differantiation), lesion size > 1 mm and absence of cancer, polyp, secretory endometrium and artifact in the evaluated sample. Twenty-four out of 37 cases of endometrial hyperplasia were identified as EIN and 13 were excluded. Of these 13 cases six were deemed normal and seven were regarded as hyperplastic with no atypia. Figure 1 demonstrates an example of an EIN lesion.

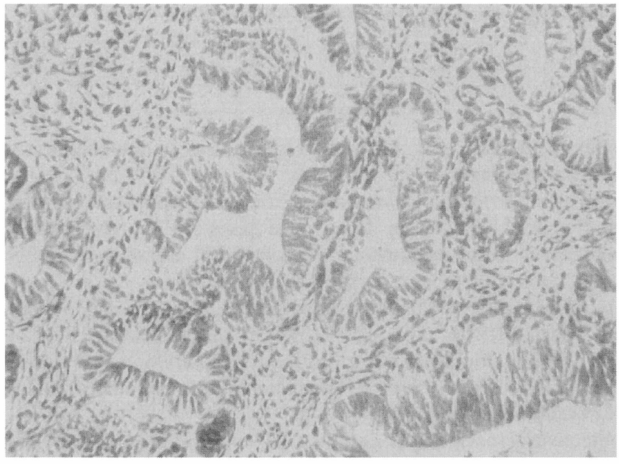

Figure 1. — Crowded endometrial glands with striking nuclear atypia (hematoxylin and eosin x 200).

Immunohistochemistry. New tissue sections were taken for staining from the parrafin blocks. Deparaffinized sections were rehydrated with distillated water. Sections were held in 0.5% hydrogen peroxide/methanol for ten minutes and washed with tap water. Hydrated tissue underwent antigenic retrival for 25 minutes in citrate buffer underpressure in a microwave oven. Sections were treated with primary antibody after having been incubated in normal goat serum for 30 minutes. Mouse monoclonal antibody 28H6 clone (Novocastra, UK) was used for PTEN immunostaining. Slides were then evaluated under light microscope and brown staining in nucleus was accepted as presence of immunoreactivity. The intensity of staining was classified and graded as complete loss (0), partial loss (+) and presence (++) of PTEN immunoreaction.

Statistical analysis. The SPSS 8.0 statistics program was used for data analysis. Data were defined as mean ± standard deviation. The chi-square test was used and $p < 0.05$ was accepted as statistically significant. A nonparametrical analysis test (Kruskal-Wallis test) was used for comparison of PTEN immunoreactivity in EIN, endometrial cancer and proliferative endometrium groups.

Results

Sixty-four percent (24/37) of the patients who were previously diagnosed with endometrial hyperplasia with atypia were reclassified as EIN. Thirteen of 24 were previously diagnosed as having complex endometrial hyperplasia with atypia and 11 of 24 simple endomerial hyperplasia with atypia. Among 13 of 37 patients who did not meet the diagnostic criteria for EIN, five were cases with complex endomerial hyperplasia with atypia in which three were later found to be complex endometrial hyperplasia without atypia and two were found to be proliferative endometrium. Eight patients were dignosed as having simple endomerial hyperplasia with atypia in which four were later found to be endomerial hyperplasia without atypia and the other four were proliferative endometrium.

The mean age of the patients with EIN was 50.5 ± 10.6 (29-80). Out of 24 patients with EIN, complete loss of PTEN immunoreactivity was found in only one patient (4.2%), partial loss in eight patients (33.3%) and the presence of PTEN expression in 15 patients (62.5%). When PTEN immunoreactivity was compared between the groups of proliferative phase endometrium (n = 10), EIN (n = 24) and endometrial cancer (n = 10) groups, there was no significant difference (p = 0.342).

Partial loss of PTEN immunoreactivity was found in seven and present in three of the cases with endometrium adenocarcinoma. None of the patients expressed complete loss of PTEN immunoreactivity in this group. Table 1 shows the histopathologic findings of the endometrial adenocarcinoma group. Whereas, in cases with proliferative endometrium, two patients showed complete loss, two showed partial loss and six showed presence of PTEN immunoreactivity.

Table 1. — *Histopathological findings of the endometrial adenocarcinoma group.*

No.	Size of the lesion (cm)	Grade	Myometrial invasion	Istmus invasion	ER (%)	PR (%)	p-53 (%)	Ki-67 (%)	CErbB2 (%)	PTEN
1	4.5	II	1/2 outer	(+)	(-)	95	1	20	(+)	++
2	4.0	II	1/2 inner	(-)	5	50	(-)	5	(-)	+
3	5.0	II	1/2 inner	(-)	100	40	25	50	50	+
4	7.0	II	Serosal	(+)	40	(-)	45	50	80	+
5	8.5	II	1/2 inner	(-)	5	2	(-)	5	(-)	++
6	2.5	I	1/2 inner	(-)	70	50	5	20	(-)	+
7	3.0	II	1/2 inner	(-)	40	(-)	(-)	20	(-)	++
8	0.5	I	Absent	(-)	20	40	5	40	30	+
9	6.0	II	1/2 inner	(+)	40	(-)	(-)	20	100	+
10	7.0	II	Serosal	(+)	40	(-)	2	90	30	+

ER: estrogen receptor, PR: progesterone receptor.

Discussion

Endometrial cancer is the second most common gynecologic malignancy in the Western world and mostly regarded as a cancer with good prognosis depending on the histologic type and stage of the disease. As the methods of molecular diagnosis on endometrial cancer have become popular, the majoriy of cases of endometrial cancer are thought to develop in a background of endometrial hyperplasia [1]. The genetic events which are responsible for the development of endometrial cancer have been studied for years. Molecular genetics in oncology tries to clarify the basis for management of malignant disease.

PTEN protein is a recently defined tumor suppressor gene acting on the pathogenesis of endometrial carci-

noma [11, 12] as well as endometrioid carcinomas of the ovary [13]. The PTEN tumor suppressor gene, also known as MMAC1 (mutated in multiple advanced cancers) or TEP1 (TGF-beta-regulated and epithelial cell-enriched phosphatase), is located on chromosome 10q23, a genomic region that suffers from loss of heterozygosity (LOH) in many human cancers [14]. Tumor-suppressive function of PTEN is dependent on its lipid phosphatase activity [15, 16].

The PTEN tumor suppressor gene is found to be mutated in 50-80% of endometroid endometrial adenocarcinomas [2, 17, 18] and approximately 50% of premalignant endometrial lesions-like atypical endometrial hyperplasias [2, 11, 12, 19]. Other genetic abnormalities found in endometrioid adenocarcinomas of the endometrium include microsatellite instability, and mutations in the k-RAS and beta-catenin genes. Whereas nonendometrioid carcinomas of the endometrium often have p53 mutations and loss of heterozygosity on several chromosomes [3, 4, 20, 21]. In our study PTEN immunoreactivity was detected in all cases with endometrial cancer in which partial loss was demonstrated in more than a half of the cases.

The PTEN gene is one of the most frequently mutated genes in most human cancer formations. Somatic deletions or mutations are detected in glioblastoma multiforme, prostate cancer, melanoma as well as endometrial carcinoma. Mutations in the PTEN gene also cause three uncommon autosomal dominant diseases such as Cowden disease, Bannayan-Zonana Syndrome, and Lhermitte-Duclose disease [8].

In this study, cases of endometrial hyperplasia with atypia were reclassified as EIN based on Mutter's criteria [2]. We detected only 64% (24/37) of patients as having EIN in the groups classified as simple or complex endometrial hyperplasia with atypia by the criteria of World Health Organization. Out of 37 cases with an initial diagnosis of endometrial hyperplasia with atypia, six (16%) were found to be normal and seven with no evidence of atypia (19%). Therefore a total of 35% of patients in the study seem to have undergone unnecessary interventions.

PTEN gene expression differs in different phases of the normal mestrual cycle indicating that it is regulated by ovarian steroids [5, 23]. PTEN gene expression in normal endometrium was found to decrease in an estrogenic environment and to increase in the presence of progesterone where it shows cell-type specific changes [5]. In our study, an alteration in PTEN immunoreactivity was detected in four patients with proliferate endometrium where complete and partial loss was found in two cases each. These findings led us to support the sugestion that, loss of PTEN gene expression in an unopposed estrogen environment might be among the initial events of endometrioid type endometrial carcinoma formation [22, 23]. PTEN gene mutations were reported in 22-27% of cases of complex endometrial hyperplasia with atypia [24].

In a review of the literature, some studies reported that the tumor suppressor function of PTEN protein is effective not only during the initial phase of tumor formation but also in progressive and metastatic stages [24]. In contrast, we detected a partial loss of PTEN protein in two cases of endometriosis carcinoma with serosal invasion.

Thirteen of 37 patients who were diagnosed with endometrial hyperplasia with atypical according to WHO criteria, did not meet the diagnostic criteria for EIN as suggested by Mutter. Five of them were cases with complex endomerial hyperplasia with atypia in which three were later found to be complex endometrial hyperplasia without atypia and two were found to be proliferative endometrium. Eight cases were simple endomerial hyperplasia with atypia in which four were later found to be endomerial hyperplasia without atypia and the other four were proliferative endometrium. Although, loss of PTEN immunoreactivity in EIN, a precursor lesion for endometrial carcinoma, was not found to be as high as reported in the literature, we were able to demonstrate complete loss of PTEN immunoreactivity in 4% and partial loss in 33% of cases with EIN.

Terkawa *et al.* [25] examined the expression of PTEN protein in patients with endometrial cancer. Of 103 endometrial cancers, 37 (36%) showed negative immunohistochemical staining for PTEN. In order to investigate the relationship between PTEN expression and prognosis in endometrial cancer, Terkawa *et al.* [25] enrolled 98 patients with advanced endometrial cancer. The survival rate for PTEN-positive patients was significantly higher than that for PTEN-negative or -heterogeneous staining patients. Of the 98 patients, 25 underwent radiation therapy, 62 received chemotherapy after surgery, and the remaining 11 did not have any postoperative treatment. When patients underwent chemotherapy, the survival rate for PTEN-positive cases was clearly higher than that for PTEN-negative or -heterogeneous cases (62.4 vs 11.8%).

In conlusion, EIN classification may provide a better and objective assessment of endometrial hyperplasia cases. PTEN expression showed no differences among the cases of EIN, endometrial carcinoma and proliferative phase endometrium.

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Address reprint requests to:
T. CIRPAN, M.D.
Department of Obstetrics
and Gynecology
Ege University Faculty of Medicine
Bornova, Izmir 35100 (Turkey)