

# Study of PTEN Expression in the Precursor Lesions of Endometrial Carcinoma

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| Received: 04.04.2019 | Accepted: 13.04.2019 | Published: 30.04.2019

DOI:[10.21276/sjpm.2019.4.4.13](https://doi.org/10.21276/sjpm.2019.4.4.13)

## Abstract

**Background:** Endometrial carcinomas accounts for 4-8% of all gynaecological malignancies. Over the past 30 years, many genes which cause cancer have been identified in endometrial carcinoma and hyperplasias. Recently, many studies have shown that the most frequently variable expression gene in endometrial carcinoma is PTEN (Phosphatase and TENSilon homologue) tumor suppressor gene which is mutated in about 30 - 50% of endometrial carcinomas. Identification of inactivated PTEN gene is a very important step in the early diagnosis of endometrial carcinomas and hyperplasias. This study was carried out to evaluate the expression of PTEN in the precursor lesions of endometrial carcinoma in peri-menopausal women with abnormal uterine bleeding. **Methods:** In this cross sectional study, 3634 samples of endometrial tissue were received in the department during the period of 5 years (Jan 2008 to Dec 2012). Of these formalin fixed and paraffin embedded 100 cases of endometrial hyperplasia from perimenopausal women with abnormal bleeding were retrieved and reviewed. PTEN immunohistochemical staining was done and analyzed. **Results:** Simple hyperplasia without atypia was the most common precursor lesion in perimenopausal women. PTEN expression was significantly higher in immunoreactivity and intensity in simple hyperplasia without atypia. Complex hyperplasia with atypia showed significantly lower immunoreactivity and intensity of PTEN expression. **Conclusion:** Our study recommends PTEN expression by immunohistochemistry, in all endometrial hyperplasias in the biopsy specimens which is a simple and cost effective technique when compared to other molecular studies.

**Keywords:** Endometrial carcinoma, Immunohistochemistry, PTEN expression, Perimenopausal women.

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## INTRODUCTION

In many parts of the world, endometrial cancer is the most common gynaecological cancer which accounts for 4-8% of all Carcinomas [1]. It occupies the fourth position after breast, colon and lung cancers in females and approximately 7400 die due to the disease. It typically occurs in older females, 80% of the patients being postmenopausal at the time of diagnosis. However, it can occur in any age group.

A number of cancer causing genes have been analyzed in endometrial carcinoma. Many studies have shown that the most frequently altered gene in endometrial carcinoma is the PTEN (Phosphatase and TENSilon homologue) tumor suppressor gene, which is located on chromosome 10q23.3 and is mutated in about 30-50% of endometrial cancer cases [2]. This suggests that inactivation of the gene is very important in the early diagnosis of endometrial carcinomas [3]. Therefore, this study was carried out to evaluate the

expression of PTEN in the precursor lesions of endometrial carcinoma in peri-menopausal women with abnormal uterine bleeding.

## METHODOLOGY

### Study Setting

This cross sectional study was carried out on the Formalin fixed paraffin embedded sections of endometrial tissue which were diagnosed as hyperplasias on H & E stained small biopsies and hysterectomy specimens received in the Department of Pathology for five years between 2008 and 2012.

### Study Population

All the endometrial tissue samples received during the study period were included in the study. A total of 3634 samples of endometrial tissue were received.

### Selection Criteria

Samples from peri-menopausal women aged between 40-55 years who presented with abnormal uterine bleeding were included. Samples from women in younger age groups and women with known history of endometrial abnormalities were excluded. A total of 100 formalin fixed and paraffin embedded cases of endometrial hyperplasia were selected for the study.

### Ethical Approval

Approval was obtained from the Institutional Review Board prior to the commencement of the study.

### Data Collection

Immunostaining of PTEN was done using Lyophilized Rabbit Polyclonal PTEN antibody (Abcam) at a dilution of 1:500. Section from endometrium with normal proliferative pattern was included as positive control where distinct cytoplasm staining in glandular epithelial cells was regarded as positive staining [4]. A negative control (without addition of the primary antibody) was included in every batch of immunostaining.

According to Kapucuoglu *et al.*, the immunoreactivity was graded semi-quantitatively by assessing the percentage and intensity of the stain on the whole section [5]. At least 10 representative fields with maximum staining under high power magnification (x400) were chosen and 1000 cells were counted for each case. If the number of cells was less than 1000, then all available cells were counted and the results were expressed as mean percentage.

The scoring criteria of the percentage of positive cells are as follows.

### Based on immunoreactivity

- Negative – 0 to 10% of positive cells
- Score +1 – 10 to 50 % of positive cells
- Score +2 – more than 50% of positive cells

### Based on Intensity

- Score 0 – absent in the cytoplasm
- Score +1 – light brown in the cytoplasm
- Score +2 – brown to dark brown in the cytoplasm

## RESULTS

Simple hyperplasia without atypia was the most common precursor lesion among perimenopausal women (Figure-1). The immunoreactivity and intensity of PTEN expression was higher in simple hyperplasia than in complex hyperplasia (Figure 2 & 3).

There was statistically significant correlation between immunoreactivity and intensity of PTEN expression in all precursor lesions. In simple hyperplasia without atypia, the kappa value is 0.726 which suggests that there is good agreement between immunoreactivity and intensity. The observations were statistically significant ( $p < 0.001$ ) (Table-1).

In complex hyperplasia without atypia, the kappa value was 1.000, suggestive of good agreement between immunoreactivity and intensity. The observations were statistically significant ( $p < 0.05$ ). In complex hyperplasia with atypia, the kappa value was 0.718 indicating a good agreement between immunoreactivity and intensity; the findings were statistically significant ( $p < 0.0001$ ). There was statistically significant difference of immunoreactivity between simple and complex hyperplasia ( $p < 0.05$ ) also statistically significant difference of intensity between simple and complex hyperplasia ( $p < 0.05$ ).

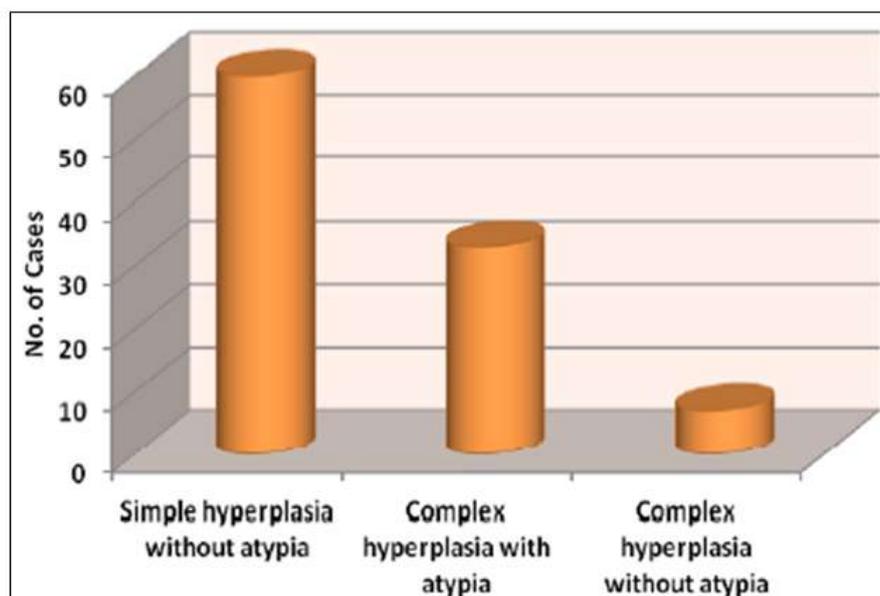


Fig1: Distribution of precursor lesions

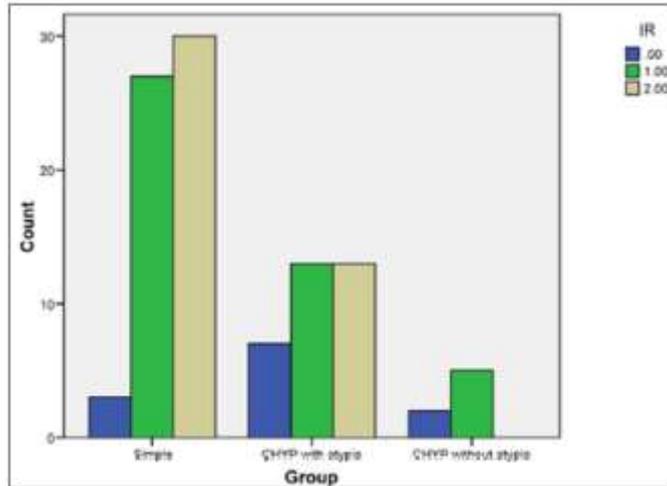


Fig-2: Comparison of PTEN immunoreactivity among various precursor lesions

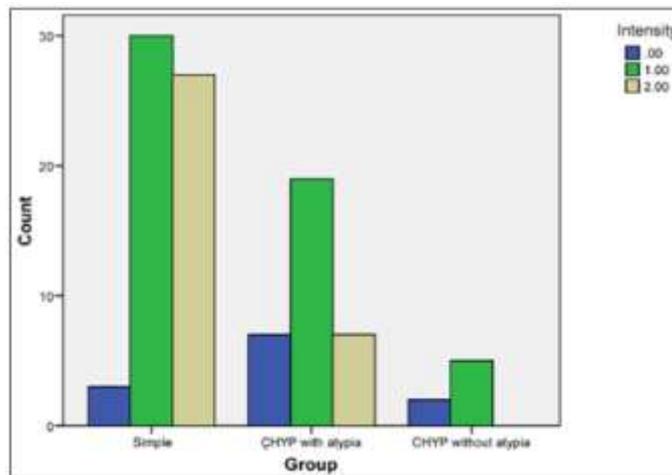
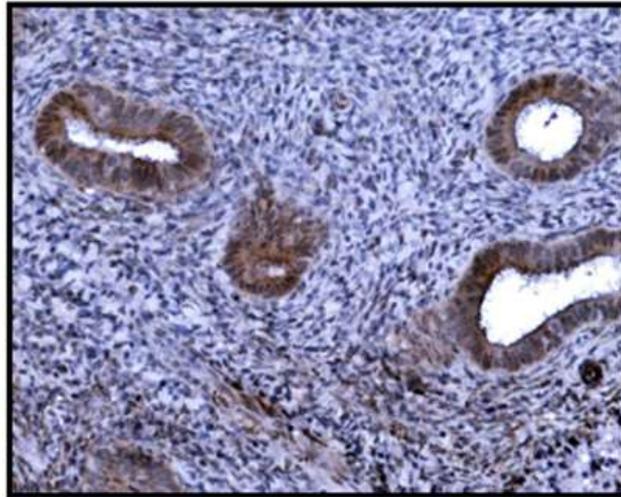


Fig-3: Comparison of PTEN intensity among various precursor lesions

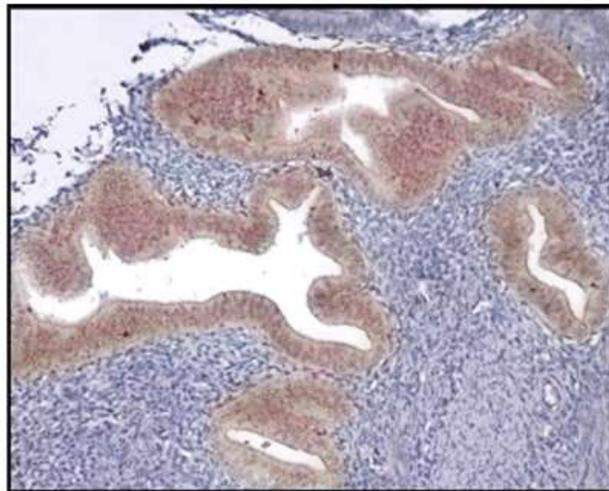
Table-1: Association between immunoreactivity and tumor expression

S. No	Characteristics	Total	Immunoreactivity Score			Chi Sq	P value
			0	1	2		
1	<b>Intensity (simple hyperplasia without atypia)*60</b>						
	0	3	3 (100.0)	0 (0.0)	0(0.0)	0.726	0.0005*
	1	30	0 (0.0)	24(80.0)	6 (20.0)		
2	27	0 (0.0)	3(11.1)	24 (88.9)			
2	<b>Complex hyperplasia without atypia</b>						
	Intensity Score 1	2	0	2	0	1.000	0.048*
Intensity score 2	5	0	0	5			
3	<b>Complex hyperplasia with atypia</b>						
	0	7	7 (100.0)	0 (0.0)	0 (0.0)	0.718	0.0001*
	1	19	0 (0.0)	13(68.4)	6 (31.6)		
2	7	0 (0.0)	0 (0.0)	7 (100.0)			
4	<b>Immunoreactivity*100</b>						
	Simple hyperplasia without atypia	60	3 (3.0)	27 (27.0)	30 (30.0)		0.022*
	Complex hyperplasia with atypia	33	7 (7.0)	13 (13.0)	13 (13.0)		
Complex hyperplasia without atypia	7	2 (2.0)	5 (5.0)	0 (0.0)			
5	<b>Intensity *100</b>						
	Simple hyperplasia without atypia	60	3 (3.0)	30(30.0)	27 (27.0)		0.011*
	Complex hyperplasia with atypia	33	7 (7.0)	19 (19.0)	7 (7.0)		
Complex hyperplasia without atypia	7	2 (2.0)	5 (5.0)	0 (0.0)			

\*p value statistically significant



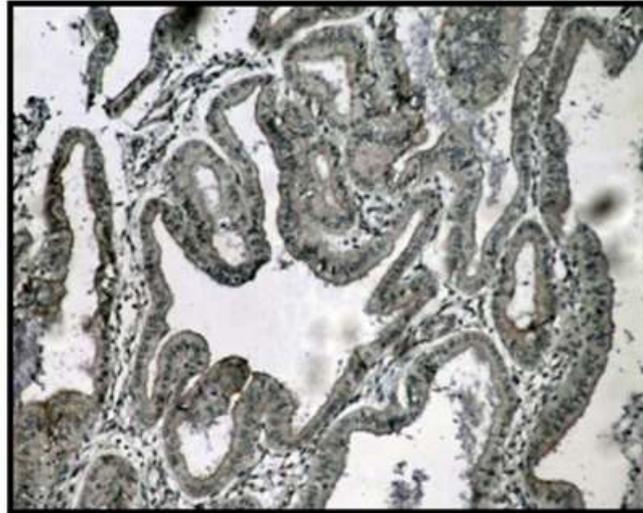
**Fig-4: Positive Control. Normal proliferative endometrium showing distinct cytoplasm PTEN staining of glandular epithelial cells, PTEN x200**



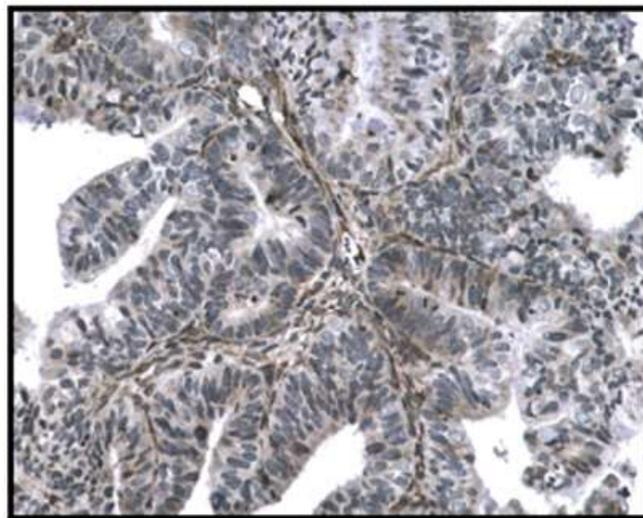
**Fig-5: Simple hyperplasia without atypia. PTEN stain showing light brown in the cytoplasm. Intensity score of 1+. PTEN x100**



**Fig-6: Simple hyperplasia without atypia. PTEN stain showing brown to dark brown in the cytoplasm. Intensity score of 2+. PTEN x100**



**Fig-7: Complex hyperplasia without atypia - PTEN stain showing light brown in the cytoplasm. Intensity score of 1+. PTEN x100**



**Fig-8: Complex hyperplasia with atypia showing no PTEN stain in the cytoplasm. Intensity score of 0. PTEN x200**



**Fig-9: Complex hyperplasia with atypia. PTEN stain showing light brown in the cytoplasm. Intensity score of 1+. PTEN x200**

## DISCUSSION

Endometrial carcinoma is one of the most common malignancies of the female genital tract [6]. Risk of endometrial carcinomas is increased in patients with stimulation of the endometrium by unopposed endogenous or exogenous estrogen and in patients with precancerous lesions [7, 8]. Endometrial hyperplasia is a precancerous lesion and is commonly seen in perimenopausal women. Although, less than 10% of hyperplasias without atypia progress to invasive cancer, the risk of malignant transformation is as high as 20-30% in the presence of atypia [9]. PTEN is a tumor suppressor gene and alterations of this gene have been identified in many endometrial carcinomas [10]. This necessitated the need to evaluate the role of PTEN gene expression in the precursor lesions of endometrial carcinoma by immunohistochemistry.

PTEN is the most commonly mutated gene identified in endometrial carcinoma [11]. PTEN is located on chromosome 10q23.3 and encodes a dual specificity phosphatase. The primary target is the lipid molecule Phosphatidyl Inositol-3, 4, 5-triPhosphate (PIP3) that plays a role in the signal transduction pathway which regulates cell growth and apoptosis. The dephosphorylation of PIP3 counteracts the activity of a protein complex called phosphoinositol 3 kinase (PI3K) that leads to the conversion of PIP2 (phosphoditylinositol-4, 5- diphosphate) to PIP3. Consequently, the inactivating mutations in PTEN result in increased levels of PIP3 which activates downstream molecules including phosphorylation of Protein Kinase B (Akt). Akt is a central regulator of numerous pathways and inactivates the proapoptotic factor Bcl-2 Associated Death (BAD) which suppresses apoptosis and promotes cell survival [12]. On the other hand, overexpression of PTEN inhibits cell growth and induces a G1 arrest with an increase in the cell cycle through down regulation of p27 [13]. These previous studies suggest that loss of PTEN expression contributes to carcinogenesis.

Mutter *et al.*, found that the altered PTEN expression in endometrial hyperplasias and carcinomas [14]. In his study, PTEN expression was completely absent in 61% of cases and 97% of cases revealed diminution in expression and has concluded that combination of loss of PTEN expression and histological features have got the greatest diagnostic utility in endometrial hyperplasias. Our study showed that absence of PTEN expression was minimal in simple hyperplasia without atypia (5%). This is consistent with Mutter *et al.*, [14] However, this is slightly higher when compared to Soheila sarmadi *et al.*, study which showed no negative PTEN expression in simple hyperplasia without atypia [15]. There were no negative PTEN expression in complex hyperplasia without atypia cases in our study. This may be due to the less number of cases analyzed.

In the studied done by Mutter et al and Soheila sarmardi, the incidence of negative PTEN expression in complex atypical hyperplasia was more when compared to simple hyperplasia without atypia [14, 15]. In our study, 21.2% of complex hyperplasia with atypia cases showed no PTEN expression whereas only 5% of simple hyperplasia without atypia cases showed no PTEN expression. This result in our study is similar to the results of studies done by Mutter et al and Soheila sarmardi *et al.*, [14, 15]. This shows that negative PTEN protein expression is seen much more in complex hyperplasia with atypia than compared to simple hyperplasia without atypia.

There was no loss of PTEN expression in complex hyperplasia without atypia which supports the findings of the study by Kurman *et al.*, which shows that hyperplasia with cytological atypia is more prone for development of carcinoma [16]. Our results showed lower PTEN activity (21.2%) in complex hyperplasia with atypia than in other studies (55-75%), which may be due to the use of polyclonal antibody in our study. However, Pallares *et al.*, showed that using monoclonal antibody was associated with more acceptable results than polyclonal antibody [17].

## CONCLUSION

We found significant differences in PTEN expression between simple hyperplasia and complex hyperplasia with atypia indicating that immunohistochemical identification of individual PTEN null glands in hyperplastic glands may be useful in detection of earliest progression to malignancy. The following are the key conclusions for our study:

- PTEN expression values were significantly higher in simple hyperplasia.
- However, there was statistically significant loss of PTEN expression in complex hyperplasia with atypia which is also a feature seen in endometrial carcinomas.
- Our study recommends evaluation of PTEN expression by immunohistochemistry, for all endometrial hyperplasias biopsy specimens. This immunohistochemistry is a simple and cost effective technique when compared to other molecular studies and therefore suitable to be carried out in developing countries like India.

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