ASSESSMENT OF PTEN TUMOR SUPPRESSOR PROTEIN BY IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIAS VERSUS CARCINOMAS OVER A FOUR-YEAR PERIOD

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ABSTRACT

Background: Endometrial hyperplasia represents a heterogeneous group of lesions with variable potential for progression to endometrial carcinoma. The tumor suppressor gene PTEN plays a critical role in endometrial carcinogenesis, and its altered expression has been identified as an early molecular event.

Aim: To evaluate the immunohistochemical expression of PTEN in endometrial hyperplasias and compare it with endometrial carcinomas.

Materials and Methods: This prospective observational study was conducted in the Department of Pathology in collaboration with the Department of General Surgery over a period of nine months (June 2025 to February 2026). A total of 132 endometrial samples were studied, including 100 cases of endometrial hyperplasia (88 without atypia and 12 with atypia), 14 cases of endometrial carcinoma, and 18 normal endometrial samples serving as controls. Histopathological evaluation was performed using hematoxylin and eosin staining. Immunohistochemical analysis for PTEN expression was carried out using monoclonal antibody (clone 28H6). PTEN expression was assessed based on the percentage of positive glands, staining intensity, stromal expression, and presence of null glands.

Results: Endometrial hyperplasia without atypia showed the highest PTEN expression, with 75% of cases demonstrating >50% positive glands and predominantly moderate to strong intensity. In contrast, hyperplasia with atypia showed reduced expression, with 50% of cases exhibiting <10% positive glands and weaker staining intensity. Endometrial carcinomas demonstrated markedly reduced or absent PTEN expression, with 57.1% of cases showing <10% glandular positivity and predominantly weak or absent staining. Stromal PTEN expression decreased progressively from hyperplasia without atypia to carcinoma. Null gland number and clustering increased significantly across the spectrum. The differences in PTEN staining intensity ($p = 0.012$) and null gland distribution ($p = 0.000$) between hyperplasia without atypia and atypical hyperplasia were statistically significant.

Conclusion: PTEN expression decreases progressively from benign to malignant endometrial lesions, highlighting its role in endometrial tumorigenesis. Immunohistochemical assessment of PTEN is a valuable adjunct in differentiating endometrial hyperplasias and carcinomas and may aid in early detection and risk stratification.

Keywords: PTEN, Endometrial Hyperplasia, Endometrial Carcinoma, Immunohistochemistry, Null Glands.

INTRODUCTION

The classification and biological interpretation of endometrial hyperplasias have long posed a diagnostic challenge for pathologists. Traditionally, these lesions were conceptualized as a morphological continuum progressing from benign hyperplasia to invasive carcinoma, based on Increasing architectural complexity and cytological atypia. However, accumulating molecular and clinicopathological evidence has reshaped this paradigm, demonstrating that endometrial hyperplasias represent a heterogeneous group of conditions rather than a linear spectrum (1). Current understanding supports the existence of two fundamentally distinct disease entities: benign hyperplasia and premalignant lesions, now commonly referred to as endometrial intraepithelial neoplasia (EIN), which can be diagnosed independently of overt carcinoma (1).

This revised classification has gained widespread acceptance and has been endorsed by authoritative bodies such as the Clinical Practice Committee of

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the Society of Gynecologic Oncologists and the World Health Organization (WHO) (2,3). The WHO classification emphasizes the importance of cytological atypia and glandular architecture in distinguishing hyperplasia without atypia from atypical hyperplasia/EIN, the latter carrying a significantly higher risk of progression to endometrial carcinoma (3). This shift from purely morphological assessment to a more biologically meaningful classification has improved diagnostic reproducibility and clinical management.

Endometrial hyperplasia and endometrial carcinoma are among the most common gynecological pathologies, contributing substantially to morbidity in women, particularly in the perimenopausal and postmenopausal age groups (4). Endometrial carcinoma is the most prevalent malignancy of the female genital tract in developed countries and is increasingly observed in developing regions due to changes in lifestyle and reproductive patterns (5). Unopposed estrogen exposure, obesity, diabetes mellitus, and chronic anovulation are well-established risk factors linking hyperplasia to carcinoma (6).

At the molecular level, alterations in tumor suppressor genes and oncogenes play a crucial role in the pathogenesis of these lesions. One of the most frequently implicated genes is the phosphatase and tensin homolog (PTEN), a tumor suppressor gene involved in regulating cell proliferation, survival, and apoptosis through the PI3K/AKT signaling pathway (7). Loss of PTEN expression has been identified as an early event in endometrial carcinogenesis and is commonly observed in both atypical hyperplasia and endometrioid carcinoma (8). Immunohistochemical evaluation of PTEN protein expression serves as a valuable adjunct in differentiating benign from premalignant and malignant endometrial lesions.

Given the overlapping histological features and the potential for progression, accurate distinction between various forms of endometrial hyperplasia and carcinoma is essential for appropriate clinical management. In this context, immunohistochemistry provides an objective and reproducible tool to complement conventional histopathological evaluation. The present study aims to assess PTEN expression in endometrial hyperplasias and compare it with endometrial carcinomas, thereby contributing to improved diagnostic stratification and understanding of disease progression.

Aim

To evaluate the immunohistochemical expression of PTEN in endometrial hyperplasias and to compare its expression with endometrial carcinomas in order to assess its role in endometrial carcinogenesis.

Objectives

To study the histomorphological features of endometrial hyperplasia and endometrial carcinoma.

To assess PTEN protein expression in endometrial hyperplasia using immunohistochemistry.

To evaluate PTEN expression in endometrial carcinoma cases.

METHODOLOGY

This prospective observational study was conducted in the Department of Pathology in collaboration with the Department of General Surgery over a period of nine months, from June 2025 to February 2026. The study population included women aged above 60 years who were under evaluation for dysfunctional uterine bleeding. A total of 100 cases of endometrial hyperplasia diagnosed on histopathological examination were included in the study, comprising 88 cases of endometrial hyperplasia without atypia and 12 cases of endometrial hyperplasia with atypia. In addition, 14 cases of histologically confirmed endometrial carcinoma were included for comparison. Ten cases of proliferative phase endometrium and eight cases of secretory phase endometrium were used as positive controls, while endometrial carcinoma cases served as negative controls for PTEN immunostaining.

Cases were selected based on predefined inclusion and exclusion criteria. Inclusion criteria comprised endometrial tissue obtained from surgical specimens, including curettage and hysterectomy samples, in patients presenting with abnormal uterine bleeding and showing histomorphological features of endometrial hyperplasia or carcinoma on hematoxylin and eosin (H&E) staining. Patients with a family history of endometrial carcinoma and those receiving hormonal therapy were also included. Cases with abnormal uterine bleeding attributable to other causes were excluded from the study.

All specimens were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections of 3–5 µm thickness were cut and stained with H&E for histopathological evaluation. Based on morphological assessment, cases were categorized into endometrial hyperplasia without atypia, endometrial hyperplasia with atypia, and endometrial carcinoma. Carcinoma cases were further graded according to standard histological grading systems.

Immunohistochemical analysis for PTEN expression was performed on representative paraffin-embedded sections using monoclonal PTEN antibody (clone 28H6, Biogenex), which is designed for specific nuclear localization in formalin-fixed tissues. Appropriate positive and negative controls were included in each staining run. The stained slides were evaluated semi-

quantitatively by assessing the percentage of positively stained glandular epithelial cells (<10%, 10–50%, and >50%), intensity of staining (weak, moderate, strong), and presence or absence of stromal staining. PTEN expression was interpreted by correlating both the extent and intensity of staining.

Statistical analysis was performed using appropriate software (such as SPSS). Descriptive statistics were used to summarize the data. The association between PTEN expression and different categories of endometrial lesions was analyzed using the Chi-square test or Fisher's exact test, as applicable. A p-value of less than 0.05 was considered statistically significant.

RESULT

Within the group of positive controls which included 10 proliferativeand 8 secretory endometrium all the cases showed >50% of glandular epithelium showing strong to moderate staining.100% of stromal cells showed strong PTEN staining and there were no null glands either isolated or clustered(Table 1,2),(Figure 1).Within the group of negative controls which comprised of endometr ioid endometrial adenocarcinoma,a progressive decrease in the number and staining intensity of PTEN positive glands was observed with higher FIGO grade with weak to absent stromal positi vity(Figure 4).Of the 6 cases of Grade II endometrial carcinom as,2 cases showed 10-50% weak glandular staining,and 4 cases showed <10% glandular staining,both of weak intensity.All the 6 cases showed no stromal staining and clusters of null glands.8 cases of Grade III endometrial carcinomas were studied.All the cases showed weak to absent glandular staining,with absent stromal staining and many null gland clusters(Table 3).

In the study group,hyperplasias without atypia had maximum number of PTEN positive glands which reduced as the spectrum proceeded to hyperplasias with atypia.Intensity of glandular PTEN staining was strongest in hyperplasia without atypi a.Likew ise,stromal staining was strong in majority of hyperplasia without atypia reducing to moderate to weak or absent staining in hyperplasia with atypia(Figure 2,Figure 3).Null gland arrangement and number increased from hyperplasia with atypia to endometrial carcinoma.(Null glands are endometrial glands in which less than 10% of epithelial cells are PTEN positive).

Table 1: Interpretation of PTEN Staining Pattern in the Glandular Epithelium

	Numb er of cases (%)			**Intensityof staining %**			
	<10%	10-50%	>50%	Absen t	Weak	Moder ate	Strong
Endometrial hyperplasia without atypia (88 cases)	-	22 (25%)	66 (75%)	-	-	44 (50%)	44 (50%)
Endometrial hyperplasia with atypia (12 cases)	6 (50%)	6 (50%)	-	-	4 (33. 3%)	6 (50%)	2 (16. 7%)
Endometrioid endometrial adenocarcino ma (14 cases)	8 (57. 1%)	6 (42. 9%)	-	4 (28. 6%)	10 (71. 4%)	-	-

Table 2: Interpretation of PTEN Stromal Staining with Intensity

Proliferative Phase Endometrium	**100% Present-Strong**
Secretory phase endometrium	100% present-strong
Hyperplasia without atypia	70-100% present-strong to moderate
Hyperplasia with atypia	40-60% present-moderate to weak
Endometrioid adenocarcinoma	Weak-absent

Table 3: Null Gland Number (%) and Arrangement

	Null gland number(%) and arrangement
Proliferative phase endometrium	No null glands
Secretory phase endometrium	No null glands
Hyperplasia without atypia	Isolated-15-35%
Hyperplasia with atypia	Clusters-80%
Endometrioid endometrial adenocarcinoma	Clusters-100%

Comparing the intensity of PTEN staining between endometrial hyperplasia without atypia and endometrial hyperplasia with atypia,the P value obtained is 0.012 and null gland arrangement and number between endometrial hyperplasia without atypia and endometrial hyperplasia with atypia,the P value derived is 0.000,both of which are highly significant.

P value on comparing the null gland arrangement of endometrial hyperplasia with atypia and endometrioid endometrial adenocarcinoma is 0.007 which is also significant.

DISCUSSION

The present study demonstrates a progressive reduction in PTEN expression across the spectrum of endometrial lesions, from hyperplasia without atypia to atypical hyperplasia and finally to endometrial carcinoma. This observation supports the concept that loss of PTEN function is an early and critical event in endometrial tumorigenesis (9). In the current study, endometrial hyperplasia without atypia showed the highest proportion of PTEN-positive glands, with strong to moderate staining intensity, whereas hyperplasia with atypia exhibited a significant reduction in both the percentage of positive glands and staining intensity. These findings are consistent with previous studies that have emphasized the stepwise loss of PTEN expression as lesions progress toward malignancy (10).

The statistically significant difference observed in PTEN staining intensity between hyperplasia without atypia and hyperplasia with atypia ($p = 0.012$) highlights the diagnostic utility of PTEN immunohistochemistry in distinguishing benign from premalignant lesions. Similar observations have been reported by other investigators, who noted that PTEN loss is more frequent in atypical hyperplasia and is associated with a higher risk of progression to carcinoma (11). Furthermore, stromal PTEN expression, which was strong in normal endometrium and hyperplasia without atypia, showed a gradual decline in atypical hyperplasia and was largely absent in carcinoma cases. This reduction in stromal staining may reflect alterations in the tumor microenvironment accompanying malignant transformation (12).

In endometrial carcinoma cases, particularly Grade III tumors, PTEN expression was markedly reduced or completely absent, with weak or absent glandular staining and lack of stromal expression. The presence of numerous null glands—defined as glands with less than 10% PTEN-positive epithelial cells—was a prominent feature in carcinoma and increased progressively from atypical hyperplasia to malignancy. The highly significant association observed for null gland number and arrangement ($p = 0.000$) further underscores its potential as a morphological marker of neoplastic progression. Previous studies have similarly documented the presence of PTEN-null glands as an early indicator of clonal expansion and malignant transformation in the endometrium (13).

Overall, the findings of this study reinforce the role of PTEN as a key tumor suppressor in endometrial carcinogenesis and highlight the value of its immunohistochemical assessment in routine diagnostic practice. The combined evaluation of glandular staining, stromal expression, and null gland patterns provides a more objective and reproducible approach to distinguishing between benign, premalignant, and malignant endometrial lesions, thereby aiding in appropriate clinical management (14).

CONCLUSION

The present study demonstrates a progressive decline in PTEN expression from endometrial hyperplasia without atypia to hyperplasia with atypia and ultimately to endometrial carcinoma, supporting its crucial role in endometrial carcinogenesis. Strong and diffuse PTEN expression in hyperplasia without atypia, in contrast to reduced or absent expression in atypical hyperplasia and carcinoma, highlights its value as a diagnostic biomarker. The statistically significant differences in glandular staining intensity and null gland distribution between hyperplasia without atypia and atypical hyperplasia further emphasize the utility of PTEN immunohistochemistry in distinguishing benign from premalignant lesions.

Additionally, the gradual loss of stromal PTEN expression and the increasing number and clustering of null glands correlate with disease progression and malignant transformation. These findings suggest that assessment of PTEN expression, including glandular, stromal, and null gland patterns, provides a more objective and reproducible approach in evaluating endometrial lesions.

TEN immunostaining serves as a valuable adjunct to routine histopathological examination, aiding in accurate classification, early detection of premalignant changes, and improved risk stratification of endometrial hyperplasias. Incorporating PTEN evaluation into routine diagnostic protocols may enhance clinical decision-making and guide appropriate patient management.

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