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Diagnostic Implication of Phosphatase and Tensin Homolog Expression in Endometrial Lesions

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ABSTRACT

Background: Expression of Phosphatase and Tensin homolog (PTEN) is lower in endometrial cancer in comparison with normal or hyperplastic endometrium. Loss of PTEN function by mutational or other mechanisms is an early event in endometrial tumorigenesis.

Objective: In this study, we evaluated PTEN gene expression in hyperplastic and malignant endometrium to assess its diagnostic implications.

Methods: This study was conducted on women presenting with abnormal uterine bleeding (AUB), attending the Outpatient and Inpatient Clinics of Obstetrics and Gynecology. Endometrial biopsy sections were routinely stained with HandE stain and immunohistochemical staining for PTEN expression was performed in all the endometrial lesions.

Results: The study comprised 278 cases of endometrial hyperplasias and carcinomas. In simple hyperplasia without atypia (n=143), 80.0% showed intense PTEN positivity and 20.0% showed moderate PTEN positivity. Simple hyperplasia with atypia (n=15), 93.3% cases showed moderate PTEN positivity and 6.7% case showed intense PTEN positivity. Complex hyperplasia without atypia (n=76), 14.4% cases showed mild PTEN positivity and moderate PTEN positivity was seen in 85.6% cases. Complex hyperplasia with atypia (n=34), 85.3% cases showed mild PTEN positivity and absent staining in 14.7% cases. Adenocarcinoma cases showed mild PTEN positivity in 10.0% and absent staining in 90.0% cases.

Conclusions: PTEN expression decreases as the lesion progress from benign to frank malignancy, and loss of PTEN function is an early event in the pathogenesis of endometrial carcinogenesis.

Key Words: PTEN, Endometrial hyperplasia, Carcinoma, Immunohistochemistry

INTRODUCTION

Endometrial diseases are the most common gynaecological disorders affecting women worldwide.¹ They constitute around 70.0% of all gynecologic consultancies in the peri-menopausal and postmenopausal age group.² They usually present with abnormal uterine bleeding (AUB), which is the most common and challenging problem presenting to the gynaecologist regardless of the age of the women.³ The International Federation of Gynaecology and Obstetrics (FIGO) working group on menstrual disorders developed a FIGO classification system for the causes of AUB in non-gravid women of reproductive age group.⁴ Its main categories are: polyp, adenomyosis, leiomyoma, hyperplasia and malignancy, coagulopathy, ovulation, endometrial, iatrogenic and not yet classified.

Endometrial hyperplasia and malignancy are important neoplastic lesions causing AUB. Endometrial hyperplasia is characterized by an increased proliferation of endometrial glands relative to stroma resulting in increased gland to stromal ratio than normal endometrium. The hyperplastic glands vary in shape and size and may shows cytologic atypia, which may progress or co-exist with endometrial carcinoma. Hyperestrogenic state is most commonly associated with endometrial hyperplasia. Prolonged estrogenic stimulation of the endometrium may be due to anovulation, obesity, polycystic ovarian disease, functional granulosa cell tumors of the ovary and estrogen replacement therapy.⁵

The WHO 2014 classification divides endometrial hyperplasia into 2 categories: hyperplasia without atypia and atypical

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hyperplasia/endometrioid intraepithelial neoplasia. This reduction in to 2 categories reflects a new understanding of molecular genetic changes. Hyperplasia without atypia showed no relevant genetic changes. They are benign entities and will regress with time after the endocrine milieu (physiological gestagen levels) has normalized. Atypical endometrial hyperplasias exhibit many mutations typical for invasive endometrioid endometrial carcinoma. In approximately 60.0% of such cases, patients have coexisting invasive cancer or are at increased risk of developing invasive cancer.^{6,7}

Phosphate and Tensin Homologue (PTEN) is a tumor suppressor gene. Expression of PTEN is lower in endometrial cancer than in normal endometrium. It is involved in the regulation of focal adhesion, cellular migration, and tumor cell proliferation and somatic mutations of this gene have been identified in a large number of other human malignancies.⁸ Although most pre-cancers and cancers had a mutation in only one PTEN allele, endometrioid endometrial adenocarcinomas showed complete loss of PTEN protein expression in 61% of cases, and 97.0% cases showed at least some diminution in expression. Cancers and most pre-cancers exhibited contiguous groups of PTEN negative glands, while endometria altered by unopposed estrogens showed isolated PTEN-negative glands. Loss of PTEN function by mutational or other mechanisms is an early event in endometrial tumorigenesis, and it may occur in response to known endocrine risk factors and offers an informative immune-histochemical biomarker for premalignant disease. Individual PTEN-negative glands in estrogen-exposed endometria are the earliest recognizable stage of endometrial carcinogenesis. Proliferation into dense clusters that form discrete premalignant lesion follows.⁹ PTEN mutations have been seen in about 55% of patients with endometrial hyperplasia.¹⁰ Loss of PTEN function has, therefore, been proposed as an early event in the pathogenesis of endometrioid carcinogenesis.

MATERIALS AND METHODS

This study was performed on 278 women attending the Outpatient and Inpatient Departments of Obstetrics and Gynecology and Pathology with complaints of abnormal uterine bleeding (AUB), after approval from the hospital ethics committee. A thorough clinical history of patients was collected and complete physical examination findings and other relevant investigations were recorded. All the endometrial biopsies/curettage and hysterectomy specimen sent for histopathological evaluation with a history of abnormal uterine bleeding were included in the study, while all other malignancies of the female genital tract and patients not giving informed consent were excluded. Specimens were processed in 10% neutral buffered formalin (10 ml of 40% formaldehyde diluted in 90 ml of water). Tissues were fixed for 24 hours with two changes of formalin and embedded in paraf-

fin which was further cut into 4-5 μ m thick sections. The sections were routinely stained with haematoxylin and eosin (H&E) stain. Immunohistochemical staining using the PTEN monoclonal antibodies was performed on diagnosed cases of endometrial lesions. The various histomorphological and immunohistochemical patterns were identified and classified according to the percentage of cell stained and intensity of staining on immunostained slides. PTEN staining pattern was evaluated as: absent staining- 0, mild-1+, moderate-2+, Intense- 3+.¹¹

Statistical analysis was performed using Fisher's exact test. Data was statistically analyzed using SPSS software version (20.0). Chi-square tests were used in the analysis of dichotomous or categorical variables. When expected cell frequencies were <5, the Fisher exact test was used. p-value of 0.05 or less was considered to be statistically significant.

RESULTS

The present study included 278 patients between ages of 11-70 who presented with abnormal uterine bleeding (AUB). The maximum number of patients were in the 5th decade, 112 (40.3%) followed by 83 (29.9%) patients in the 4th decade and 38 (13.7 %) patients in the 3rd decade of life.

Majority of the patients in our study were parous 261 cases (93.8%) and nulliparous constituted only 17 cases (6.1%). The incidence of abnormal uterine bleeding was high in grand multipara 109 (39.2%) and multipara 104 (37.4%) as compared with nulliparous 17(6.1%) and primiparous 48 (17.3%) females.

Out of the 278 cases, there were 268 (96.4%) cases of endometrial hyperplasia and 10 (0.36%) cases of endometrial cancer. Table 1 Endometrial hyperplasia was the commonest pathology seen in the perimenopausal age group (41-50 years). This included simple hyperplasia without atypia in 74 (51.7%) cases followed by complex hyperplasia without atypia in 46(60.5%) cases. The other common lesions encountered were endometrial carcinoma in 8 (80.0%) cases and atypical hyperplasia in 3 (6.2%) cases. Table 2

Of the 268 cases of endometrial hyperplasia, the most common subtype was simple hyperplasia without atypia in 143 (51.4%) cases (Figure 1) followed by complex hyperplasia without atypia in 7 (27.3%) cases. (Figure 2) Complex hyperplasia with atypia was seen in 34 (12.2%) cases (Figure 3) and the least common variant was simple hyperplasia with atypia seen in 15 (5.4 %) cases. Out of 10 cases of endometrial carcinoma 7(70.0%) cases presented with grade II, 2 (20.0%) cases presented with grade III, 1 (10.0%) case presented with grade I. 4 (40.0%) cases presented with <1/2 myometrial invasion, 3 (30.0%) cases presented with >1/2 myometrial invasion and 3 (30.0%) cases did not show myometrial invasion. 3 (30.0%)

cases presented with lymphovascular invasion and 7 (70.0%) cases showed no lymphovascular involvement. Hence, accordingly 6 (60.0%) cases presented with stage II, 2 (20.0%) cases presented with stage III, 1 (10.0%) case each presented with stage I and stage IV.

Out of 7 cases of stage I/II endometrial carcinoma 1/7 (14.3%) showed mild PTEN positivity and 6/7 (85.7%) showed absent PTEN staining. All of the 3 cases of stage III/IV endometrial carcinomas showed absent PTEN staining. 3 cases with absent lymphovascular invasion showed absent PTEN staining and 7 cases of lymphovascular invasion showed mild PTEN positivity. Histologically, 9 (90%) cases of endometrioid endometrial carcinomas showed absent PTEN staining (Figure 4) and 1 (10%) case of serous papillary carcinoma showed mild PTEN positivity.

Out of 143 cases of simple hyperplasia without atypia, 115 (80%) cases showed intense (3+) PTEN positivity and 28 (20%) cases showed moderate (2+) PTEN positivity. Out of 15 cases of simple hyperplasia with atypia, 14 (93.3%) cases showed moderate 2+ PTEN positivity, and 1 (6.7%) case showed intense (3+) PTEN positivity. Out of 76 cases of complex hyperplasia without atypia 11 (14.4) cases showed mild (1+) PTEN positivity and moderate (2+) PTEN positivity was seen in 65 (85.6%) cases. Complex hyperplasia with atypia was seen in 34 cases, 29 (85.3%) cases showed mild (1+) PTEN positivity and absent staining in 5 (14.7%) cases. Adenocarcinoma cases showed mild (1+) PTEN positivity in 1/10 (10%) case and absent staining in 9/10 (90%) cases. Table 3

DISCUSSION

Abnormal uterine bleeding is one of the most commonly encountered complaints in gynaecological practice. It comprises more than two-thirds of all gynecological consultations in the peri and postmenopausal years.¹² The causes may vary with respect to the age of patients. Histopathological diagnosis of endometrial biopsy and curettage play an important role in identifying the various disease spectrum causing uterine bleeding, especially in developing countries with limited resources. 40.3% of our patients were in the 5th decade of life, a finding in concordance with the study of Abdullah and Bondagji and Saraswathi et al., who have reported incidence of 32.1% and 33.5% respectively in the 5th decade of life.^{13,14} A slightly higher incidence of 48.1% was reported by Zhang et al and 59.1% by Rena et al.^{15,16}

93.8% of our patients were parous, and the incidence of abnormal uterine bleeding was highest in grand multipara, who constituted 39.2% cases. These findings were consistent with reports of Sharma et al.¹⁷

Out of 278 patients, 39.6% of the patients presented with menorrhagia followed by 12.6% cases of infertility, find-

ings concordant with Kaur et al.¹⁸ However Zhang et al have reported a slightly higher percentage of patients with menorrhagia at 51.9%.¹⁵ We reported 7.9% incidence of postmenopausal bleeding in our study, which is comparable with Agrawal et al.¹⁹ However, Rifat and Mahmoud had reported a slightly higher percentage (16.3%) of postmenopausal bleeding.²⁰ This might be probably due to lesser number of patients in our study in this age group. Our study revealed that the occurrence of menstrual disorders increases with advancing age. Excessive bleeding was seen in 41–50 years age group in our study. A similar incidence was reported by Zhang et al and Mahapatra and Mishra et al. in their study.^{15,21}

The maximum incidence of endometrial hyperplasia was found in the age group 40-49 years, which was similar to the study by Doraiswami et al.²² Hyperplasias occur usually in perimenopausal age group due to failure of ovulation. It is due to persistent unripened follicles which exposes the endometrium to an abnormally and excessive prolonged estrogenic secretion. Our study comprised of 51.4% cases of simple hyperplasia without atypia, with majority seen in the perimenopausal age group, a finding similar to Jairajpuri et al and Munro, who have reported 64.4% to 66.6% cases respectively.^{23,24} Complex hyperplasia without atypia (CH) was seen in 76/278 (27.3%) cases similar to Rifat and Mahmoud's reports.^[20] There were 5.4% cases of simple atypical hyperplasia and 12.2% cases of complex atypical hyperplasia in our study, findings concordant with reports of Sajitha et al.²⁵ Endometrial carcinoma was the least common pathology in our study, found in 10 (0.5%) cases. Similar results have been reported by Riaz et al and Abdullah et al.^{26,27}

Diagnosis of endometrial hyperplasia is important because they are thought to be a precursor of endometrial carcinoma. The overall increased risk of progression of hyperplasia to cancer is 5-10.0%.²⁸ Simple, complex, simple atypical (SAH) and complex atypical hyperplasia (CAH) have progression risks of 1.0%, 3.0%, 8.0% and 29.0% respectively to carcinoma.²⁸

The mean scoring of PTEN staining decreased from simple hyperplasia without atypia to complex hyperplasia with atypia and was the least in adenocarcinoma. It can be seen that the intensity of PTEN decreased from benign to malignant lesions. Our finding was in concordance with the study by Scully et al, Westin et al and Ramalingam et al.²⁸⁻³⁰

CONCLUSION

Loss of PTEN function by mutational or other mechanisms is an early event in endometrial tumorigenesis and offers an informative immune-histochemical biomarker for premalignant and malignant endometrial lesions. Hence, PTEN antibodies can be used to detect cancer at an early stage and can be used to target adjuvant therapy in endometrial cancers.

Conflict of interest: Nil

Source of funding: Nil

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Table 1: Histopathological distribution of cases in patients of AUB

Endometrial Histopathology	No. of patients	Percentage
Endometrial hyperplasia	268	96.4
Endometrial cancer	10	0.36
Total	278	100

Table 2: Histopathological distribution of endometrial lesions according to age in years

Histopathological diagnosis	Reproductive (15-40 years)	Perimenopausal (41-50 years)	Postmenopausal (>50 years)	Total
				No of cases
Simple hyperplasia without atypia	60 (42.0)	74 (51.7)	9 (6.3)	143 (100)
Complex hyperplasia without atypia	28 (36.8)	46 (60.5)	2 (2.6)	76 (100)
Atypical hyperplasia	10 (20.4)	35 (71.4)	4 (8.2)	49 (100)
Endometrial carcinoma	-	2 (0.2)	8 (0.8)	10 (100)
Total	98	157	23	278 (100)

Table 3: Immunostaining pattern of PTEN based on intensity of staining

Category	No of cases	PTEN staining			
		Absent (%)	1+ (Mild) (%)	2+ (Moderate) (%)	3+ Intense (%)
Simple Hyperplasia without atypia	143	-	-	28 (20.0)	115 (80.0)
Simple Hyperplasia with atypia	15	-	-	14 (93.3)	1 (6.7)
Complex Hyperplasia without atypia	76	-	11(14.4)	65 (85.6)	-
Complex Hyperplasia with atypia	34	5 (14.7)	29 (85.3)	-	-
Adenocarcinoma	10	9 (90.0)	1 (10.0)	-	-
Total	278	14	41	107	116

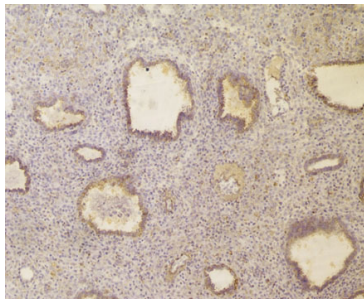


Figure 1: Simple Hyperplasia without Atypia. PTEN immuno-expression shows intense (3+) cytoplasmic staining in the lining epithelial cells. IHC PTEN x 10X.

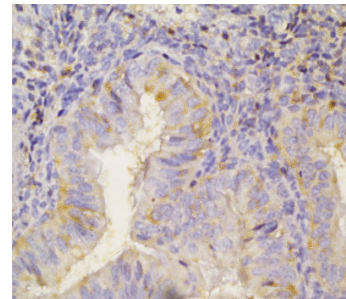


Figure 3: Complex Hyperplasia with Atypia. Photomicrograph shows weak focal (1+) PTEN cytoplasmic positivity. IHC PTEN x 40X.

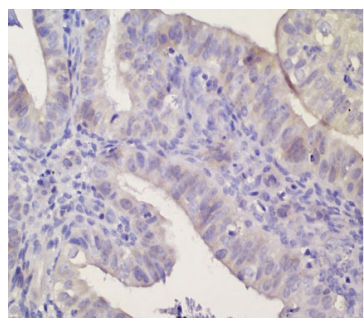


Figure 2: Complex Hyperplasia without Atypia. Photomicrograph shows moderate (2+) PTEN cytoplasmic positivity. IHC PTEN x 40X.

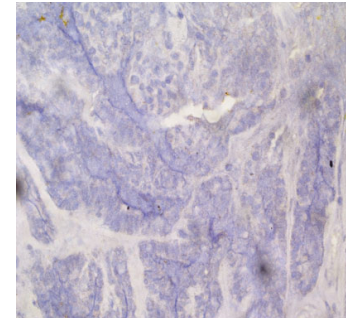


Figure 4: Adenocarcinoma Endometrium. Photomicrograph shows absent PTEN staining. IHC PTEN x 40X