ORIGINAL RESEARCH PAPER

Pathology

PATTERN OF PTEN IMMUNOREACTIVITY IN NORMAL, HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM

KEY WORDS: Endometrial hyperplasia, PTENimmunostain, Histopathology.

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ABSTRACT

Background: Atypical endometrial hyperplasia is generally considered as a precursor lesion of endometrial carcinoma. PTEN gene is the most commonly mutated gene in endometrial carcinoma as well as its precursor lesions. **Objective:** To study the prevalence of endometrial hyperplasia and carcinoma and also to determine the level of PTEN gene expression in normal, hyperplastic and neoplastic endometrium. **Material and Methods:** This cross-sectional study included 150 endometrial biopsy and hysterectomy specimen over two years duration. Patient's age,marital status,parity,bodyweight,duration of symptoms and history of exogenous estrogen therapy were taken.Specimens received in 10% buffered formalin were processed, slides prepared and routine haematoxylin and eosin(H & E) and PTEN immunostaining were done. **Results:** Endometrial hyperplasia and endometrial carcinoma were seen in 41-50 years age group. Maximum number of cases were seen in nulliparous women who presented with abnormal uterine bleeding.Loss of PTEN expression was observed in 22.2% of atypical endometrial hyperplasia and all cases of carcinoma. **Conclusion:** PTEN immunostaining should be done in all cases of endometrial hyperplasia to detect pre-malignant lesion which are likely to progress to carcinoma.

INTRODUCTION:

The Uterine Cavity is a triangular shaped cavity lined by endometrial mucosa having glandular and stromal components which undergoes physiologic and morphological changes with oestrogen and progesterone produced in the ovary.

Endometrial hyperplasia is classified by WHO(2014), into two types (i) Endometrial hyperplasia without atypia and (2) Atypical endometrial hyperplasia. Endometrioid intraepithelial neoplasia (EIN) is an alternative name for atypical hyperplasia proposed by Mutter[1,2].

Endometrial carcinoma is the 6th most common cancer of women and 14th leading cause of death in women worldwide[3].Common genetic alterations in endometrial carcinoma includes mutation of PTEN, K-RAS,microsatellite instability & β- catenin genes[4].

PTEN is a tumour suppressor gene located in chromosome 10q23 and is the most commonly mutated gene in endometrial carcinoma,advanced prostate cancer and glioblastoma.

PTEN has important role in inducing cell cycle arrest, programming apoptosis,regulation of cell adhesion, migration and differentiation. It has both lipid and protein phosphatase activity having different activities/ actions.

Many studies reported that loss of expression of PTEN is found in 50% of all endometrial carcinoma and 83% of tumours with adjacent premalignant lesions[5,6]. PTEN immunostaining can be used as a new and effective tool for screening of malignant and premalignant endometrial lesion[7].

MATERIAL & METHOD:

The study was done at a tertiary care centre in the North-Eastern India within a period of two years i.e.,1st August 2019 to 31st July 2021. The specimen collected were endometrial tissue from the specimen of hystrectomised uterus and endometrial biopsy cases.

The preparation of the preserved tissue samples in 10% buffer formalin(not more than 48 hours) for light microscopy involving the different steps was done. Thereafter staining with haematoxylin and eosin was done followed by PTEN immunostaining.

RESULTS & OBSERVATIONS:

A total of 150 cases of endometrial biopsy and endometrium from hysterectomy specimen were studied and the diagnosis is shown in Table-1.

Out of 150 cases, maximum are proliferative endometrium consisting of 118 (78.7%) cases followed by endometrial hyperplasia without atypia,11 cases (7.3%), atypical endometrial hyperplasia (Figure 1), 9 cases (6%), secretary endometrium 7 cases (4.7%) and endometrial carcinoma 5 cases (3.3%). All endometrial carcinoma cases in the study are well differentiated endometrial carcinoma of endometrioid type.

Table-1:Distribution of the cases and their frequency:

Histopathological Diagnosis	Frequency	Percentage
Proliferative Endometrium	118	78.7
Secretory Endometrium	7	4.7
Endometrial Hyperplasia without atypia	11	7.3
Atypical endometrial hyperplasia	9	6.0
Endometrial Carcinoma	5	3.3
Total	150	100.0

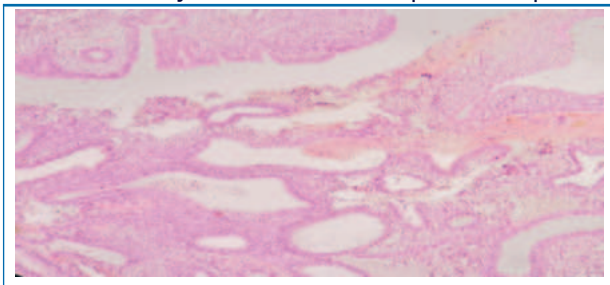

Figure 6. Photomicrogragh showing atypical endometrial hyperplasia(H&E,10X)

Age distribution of the patients in the study population ranges from 20-80 years with means age +SD, 45.5+ 9.2. The majority of the cases were in the age group of 41-50 years with 68 cases (45.3%), followed by 34 cases (22.7%), 30 cases (20.0%), 9 cases (6%) ,7 cases (4.7%) and 2 cases (1.3%) in the age group of 31-40 years, 51-60 years, 20-30 years, 61-70 years and >70 years respectively(Table -2).

Table-2: Age distribution of the patients in the study population

Age Group (Years)	Frequency	Percentage
20-30	9	6.0
31-40	34	22.7
41-50	68	45.3
51-60	30	20.0
61-70	7	4.7
>70	2	1.3
Total	150	100.0
Mean + SD	45.5 + 9.2	

All the cases of endometrial hyperplasia without atypia, atypical endometrial hyperplasia and endometrial carcinoma were observed in the age group of 41-50 years.Mean age for endometrial hyperplasia without atypia, atypical endometrial hyperplasia and endometriod endometrial carcinoma was 49.8+11.4 years, 45.6 + 6.2 years and 47.0 + 15.4 years respectively as shown in Table-3.

Table-3: Age distribution of the cases in relation to histopathological diagnosis.

Histopathological Diagnosis	Frequency	Mean Age + SD	ANOVA
Endometrial hyperplasia without atypia	11	44.8 + 11.4	F=0.390
Atypical endometrial hyperplasia	9	45.6 + 6.2	df-2 P-0.682
Endometriod endometrial carcinoma	5	47.0 + 15.4	

Age distribution of the cases in relation to histopathological diagnosis is shown in Table-4

Table-4: Age distribution of the cases in relation to histopathological Diagnosis

Histopathological Diagnosis			
Age Group (Years)	Endometrial hyperplasia without atypia	Atypical endometrial hyperplasia	Endometrial Carcinoma
20-30	1	0	1
31-40	0	2	1
41-50	6	5	2
51-60	3	2	0
61-70	0	0	1
70 and above	1	0	0
Total	11	9	5

Frequency of the parity, symptoms and marital status of endometrial hyperplasia and carcinoma are described in Table 5 indicating maximum number of endometrial carcinoma occur in nulliparous married women and with abnormal uterine bleeding,the most common clinical presentation in endometrial hyperplasia and carcinoma.

Table-5: Frequency of the parity-symptoms and marital status of Endometrial hyperplasia and carcinoma.

	Endometrial hyperplasia without Atypia	Atypical Endometrial hyperplasic	Endometrial Carcinoma
1. Parity			
a). Multiparity	2	1	0
b). 1-2 issues	3	1	1
c) Nulliparity	6	7	4
a) Abnormal uterine bleeding	10	7	5
b) Other symptoms	1	0	1
3. Marital status			
a) Unmarried	2	2	1
b) Married	9	9	4

Immunohistochemical findings:

PTEN immunoexpression were evaluated following examination of H & E stained slides and their staining intensity and extents in benign and malignant endometrial lessons were studied in all the 150 cases.

The intensity of PTEN staining was scored from 0=absent, + 1=light brown, + 2 brown to dark brown in the nucleus or cytoplasm of glandular cells for each specimen(Table-6).

All the cases of proliferative and secretory endometrium showed intense cytoplasmic and nuclear staining (+2 intensity) in the glandular epithelial cells. In the 11 cases of endometrial hyperplasia without atypia 10 cases (90.9%) showed + 2 intensity in the glandular epithelial cells(Figure-2) and only one case (9.1%) showed scored of +1 intensity .

There was no significant statistical difference in PTEN immunoreactivity between normal endometrium, endometrial hyperplasia and endometrial carcinoma group.

Out of the 9 cases of atypical endometrial hyperplasia,2 cases(22.2%) were negative for PTEN immunostain.All cases of endometrial carcinoma 5(100%) showed loss of PTEN immunoexpression(Figure-3)

Table-6: Distribution of PTEN expression in relation to Histopathological Diagnosis.

Histopathological diagnosis	PTEN Expression			Chi-square test P-Value
	Negative	Positive		
	0 n (%)	1+ n (%)	2+ n (%)	
Proliferative Endometrium	0	0	118 (100)	NA
Secretory Endometrium	0	0	7(100)	
Endometrial hyperplasia without atypia	0(0.0)	1(9.1)	10(90-9)	
Atypical endometrial hyperplasia	2(22.2)	6(66.7)	1(11.1)	
Endometriod Endometrial Carcinoma	5(100.0)	0(0.0)	0(0.0)	

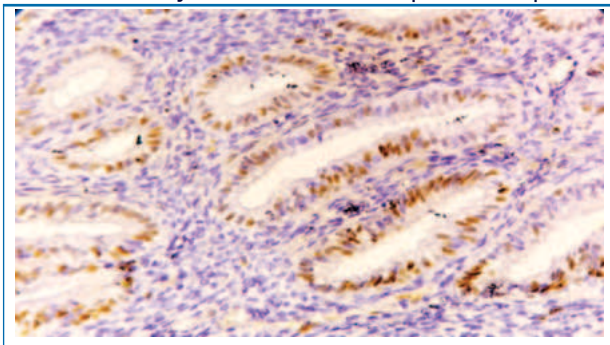

Figure 2. Photomicrograph showing strong PTEN positivity in endometrial hyperplasia without atypia (40X)

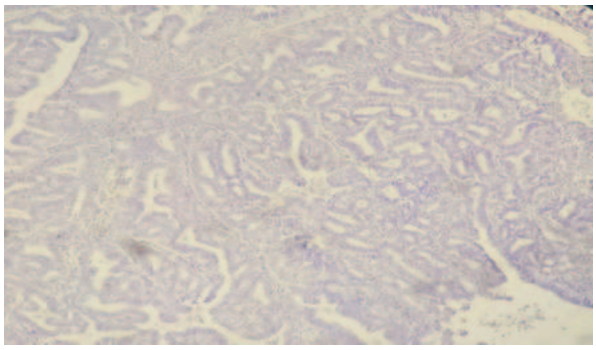

Figure 3. Photomicrograph showing loss of PTEN expression in endometriod endometrial carcinoma(10X)

DISCUSSION:

Histopathological evaluation is the gold standard for the diagnosis of endometrial hyperplasia and endometrial carcinoma. It is important to identify benign from premalignant and malignant conditions as the treatment modalities will be different for each category[8].

In the present study, frequency of endometrial hyperplasia without atypia was 7.3% and was more frequently noted in the age group 41-50 years with a mean age of 45.5± 9.2,which are comparable with that of Shawana et al[9] and Suri V et al[10] even though higher number of cases(37%) are observed in their studies.

The frequency of atypical endometrial hyperplasia in the present study was 6% and is more frequently observed in the age group of 41-50 years with mean age of 45.6 + 6.2 years. Shawana et al[9] and Suri V. et al[10]also found most of the atypical hyperplasia in women in the age group of 41-55 years and 41-50 years respectively which co-relates well with our study.

In the present study,endometrial carcinoma was observed in 3.3% with maximum number of cases in 41-50 years age group. However, Shawana et al[9] and Suri V et al[10] observed higher number of cases in age group of 61-65 years and 51-60 years respectively. The differences may be attributed to the variation in the sample size.

In the present study, endometrial hyperplasia without atypia showed +2 intensity PTEN immunoreactivity in 90.9% cases and 9.1% cases showed +1 intensity but none showed negative immune expression which is comparable to the studies of EI Sheikh et al[11],Sarmadi et al[7] and Sithara et al[12] but in the studies conducted by Tantbirojn P et al[13], Shanmugapriya et al[14]., Thukhral et al[15] and Shawana et al[9] PTEN positive was seen in 76%, 89% 85.7% and 86.6% respectively.

In atypical hyperplasia, 2 cases (22.2%) showed loss of PTEN and 7 cases were positive, of which 6 cases (66.7%) showed +1 intensity and 1 case (11.1%) showed +2 intensity. This is comparable to the finding of EI Sheikh et al[11],Sarmadi et al[7] and Sithara et al[12] where there were 25%, 33% and 37% loss of PTEN immunoreactivity respectively. In the current study,all cases of proliferative endometrium, secretory endometrium and endometrial hyperplasia without atypia showed 100% immunoreactivity to PTEN which is comparable to other studies[7,11,14,12,9,15].

PTEN negative immunoreactivity was detected in all cases (100%) of endometrioid endometrial carcinoma which is much higher to studies conducted by Tanbirojn P et al[13],Sarmadi et al [7],Sithara et al[12],Thukhral et al [15]and Shawana et al[9].The difference in PTEN expression may be due to different histological type and smaller sample size in our study.

Risinger JI et al[16] observed association of PTEN mutation with early stage,non-metastatic disease and more favourable survival in endometrial carcinoma.Another study conducted by Bussaglia E et al[17] observed more frequent PTEN mutation in endometrioid type than the non-endometrioid type of endometrial carcinoma.

PTEN is down regulated in endometrioid type of endometrial carcinoma and combination of PTEN expression and histological features are of greatest diagnostic utility in endometrial hyperplasia and carcinoma.

CONCLUSION:

Accurate diagnosis of pre-malignant lesions of the endometrium is of great clinical value in clinical management PTEN is the most frequently altered gene in atypical endometrial hyperplasia and endometrial carcinoma. Use of PTEN immunostaining as an adjunct to histopathological diagnosis in the evaluation of the endometrial lesions are of great value in diagnosis as well as for prediction of endometrial preneoplastic lesions evolving into endometrial carcinoma for the benefit of the patients.

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