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IMMUNOHISTOCHEMICAL PHOSPHOTENSIN TUMOUR SUPPRESSOR GENE(PTEN) STAINING IN ENDOMETRIAL HYPERPLASIAS IN COMPARISON TO ENDOMETRIAL CARCINOMAS-A 4 YEAR STUDY

Pathology

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ABSTRACT

Endometrial Hyperplasia is a precancerous lesion that can cause Endometrial carcinoma.Endometrial carcinoma is a common neoplasm of the female genital tract that is associated with a considerable degree of morbidity.The pathologist plays a vital role in identifying the subset or factors in endometrial hyperplasia that can lead to carcinoma.PTEN is one such factor that is important. **AIM:**Submission of Endometrial hyperplasia cases to determine the different patterns of PTEN expression by studying the PTEN intensity,% positivity,differences in the expression patterns of glandular and stromal staining and the variability in the number and distribution of PTEN null glands in various types of hyperplasias.To study the pattern of PTEN staining in endometrial carcinomas and comparing it with that of endometrial hyperplasia.**SETTINGS AND DESIGNS:**The study was done in the biopsy samples received in the Department of Pathology, Stanley Medical College and Vellore Medical College from January 2012-December 2015. **MATERIALS AND METHODS:**A retrospective and prospective study was done over 4 years in our study including a total of 132 cases which included 100 cases of endometrial hyperplasias and 14 cases of endometrial carcinomas.PTEN staining pattern was evaluated.Statistical analysis of analysing the P value was carried out by using Fischer's Exact Test. **RESULTS:**Endometrial hyperplasia with atypia showed a reduction in the number of strongly positive PTEN glands and increase in null gland clusters.**CONCLUSION:**PTEN expression in Endometrial hyperplasia is an early warning sign for increased cancer risk and potential target for preventive treatment.

KEYWORDS:

Endometrial hyperplasia, Endometrial carcinoma, PTEN tumour suppressor gene

INTRODUCTION:

The meaningful resolution of endometrial hyperplasias,a mixed group of diseases,has challenged pathologists for decades.Long envisioned as a continuous spectrum of morphologic changes of increasing severity,in reality they encompass only two discrete disease states,which can and should be diagnosed independently of carcinoma[1].This consensus has now been endorsed by the Clinical Practice Committee of the Society of Gynaecologic Oncologists[2] and the World Health Organization(WHO)[3].

Endometrial hyperplasia and endometrial carcinoma are common lesions of the female genital tract associated with considerable degree of morbidity[4],endometrial hyperplasia with atypia acting as a precancerous lesion.PTEN is the most commonly mutated gene in endometrial hyperplasias and carcinomas.This suggests that inactivation of the PTEN gene is very vital in the early diagnosis of endometrial hyperplasias and carcinomas.[5].

AIMS AND OBJECTIVES:

(a) To study the different types of endometrial hyperplasias leading to abnormal uterine bleeding.
(b) Submission of endometrial hyperplasia cases to determine the different patterns of PTEN expression by studying the PTEN intensity,% positivity,differences in the expression patterns of glandular and stromal staining and the variability in the number and distribution of PTEN null glands in various types of Endometrial hyperplasias.
© To study the pattern of PTEN staining in endometrial carcinoma and comparing it with that of endometrial hyperplasias.

MATERIALS AND METHODS:

A retrospective and prospective study was done on 132 cases which comprised 60% small biopsies and 40% hysterectomy specimens from January 2012-December 2015,age ranging from 20-60 years,under investigation for dysfunctional uterine bleeding.PTEN staining was carried out on 100 cases of endometrial hyperplasias which included 88 cases of endometrial hyperplasia without atypia,12 cases of endometrial hyperplasia with atypia;10 cases of proliferative phase endometrium and 8 cases of secretory phase endometrium served as the positive control and 14 cases of endometrial carcinomas served as the negative control.

Case selection:

Inclusion criteria:1)Surgical specimens of endometrium(both curettage and hysterectomy) in patients with abnormal uterine bleeding showing morphology of endometrial hyperplasias and carcinomas on H&E.

2)Family history of endometrial carcinoma.
3)Patients on hormonal therapy.

Exclusion criteria:1)Patients with other causes of abnormal uterine bleeding.

Hematoxylin and eosin stained slides were studied.The impression based on H&E slides were categorized as endometrial hyperplasia without atypia and endometrial hyperplasia with atypia. Endom etrial carcinomas were graded.All the slides were submitted to monoclonal PTEN 28H6(Biogenex) antibodies designed for specific localization of PTEN in the nucleus of formalin fixed sections.PTEN stained sections were suitably graded for % of positive glands(6)(<10%,10-50%,>50%);intensity of staining of glandular epithelial cells(weak,moderate,strong);stromal staining(present or absent) and intensity(strong or weak);null gland number and arrangement(isolated or clustered).Endometrial glands in which less than 10% of epithelial cells were PTEN positive were taken as null glands.

Statistical correlation was done and P values were calculated using the Fischer's exact Test.Significant P values were obtained on comparing the intensity of glandular PTEN staining and null gland arrangement of endometrial hyperplasia without atypia and with atypia.

RESULTS:

Within the group of positive controls which included 10 proliferative

and 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(%)			**Intens ity of staini ng %**			
	<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 relationship between endometrial hyperplasia and abnormal ovarian function was first established by Schroedar in 1914[7,8].The sensitivity of endometrial biopsies to detect endometrial abnorm alities has been reported to be as high as 96%[9].According to Chonam hoon study[10],disordered proliferative pattern lies at one end of the spectrum of proliferative lesions of the endometrium and carcinoma falls at the other end of the spectrum,with the intervening stages comprising endometrial hyperplasia[11].Diagnosing the patients at the earliest stage of this spectrum will be of definitive help to the gynaecologists to prevent disease progression.

Endometrial hyperplasia is divided into 2 categories-Endometrial hyperplasia without atypia and Endometrial hyperplasia with atypia.Endometrial intraepithelial neoplasia(EIN) is synonymus with endometrial hyperplasia with atypia as per the new WHO classification.EIN is the monoclonal endometrial precancerous lesion associated with a heightened risk of development of carcinoma[12].

Endometrial carcinomas are currently classified into two types-Type I associated with PTEN mutation,microsatellite instability and K-ras mutation and Type II associated with p53 mutation.

PTEN with a genetic domain on chromosome 10q23 was discovered in 1997[13,14,15].PTEN is a tumour suppressor gene and the gatekeeper of endometrium.Inactivation of PTEN acts as an initiator of carcin ogenesis.In the endometrium,high expression of glandular PTEN is seen in the proliferative phase which decreases during the secretory phase.However,there is a high stromal expression throughout the cycle with the nucleus and cytoplasm showing variable intensity of expression.

Null glands are physiological in the premenopausal period[6,16]and can be found as isolated tubular glands in the normal endom etrium[6].

PTEN mutations in endometrial hyperplasias acts as an initiator to endometrioid endometrial adenocarcinoma.Hematoxylin and eosin stain can at times miss out on foci of endometrial hyperplasia that can lead to carcinoma.PTEN staining thus provides a pathologic tool of importance to seek out precancerous clones in endometriu m,s ignalling the development of malignancy[17].

Maximum number of cases showing endometrial hyperplasia in our study falls in 40-50 years age group showing 60% of the cases.The age group of patients with endometrial hyperplasias in our study correlated with studies done by Toshibo Jobo and team,Schroedar et al[18] and Shalini Rao et al[19].All these studies showed maximum number of cases in women belonging to 40-50 years age group.Number of endometrial adenocarcinomas in our study were 14 and the age group commonly involved was 50-60 years.This result correlated to the data mentioned by Yusuf et al[20] which states that the incidence of carcinoma increases with the age of the patient.

The most common symptoms included in our study were meno rrhagia(80%) and irregular cycles(70%) with 20% patients being

asymptomatic.Menorrhagia and irregular cycles could be due to anovulatory cycles or unopposed estrogen exposure.

Types of specimens in our study included endometrial curettage or biopsies and hysterectomy specimens.Out of the 132 cases that we studied,60% were small biopsies and 40% were hysterectomies.The clinical indication for curettage in our study was similar to that in the study of Mutter et al[16] and Anuradha et al[21],i,e.,DUB and menorrhagia predominate.

In our study group of hyperplasias,endometrial hyperplasia without atypia had the maximum number of PTEN positive glands and the number reduced as atypia increased.Intensity of PTEN staining was strongest in endometrial hyperplasia without atypia .Likew ise,stromal staining was strong in majority of endometrial hyp erplasia without atypia.Null gland arrangement and number increased from hyperplasia without atypia to with atypia and endometrial carcinoma.

Our study correlated well with studies done by Mutter et al[16], Sarmadi et al,Nehad et al[22] and Taranger charpin et al[23] in exhibiting a differential PTEN expression in various phases of the normal menstrual cycle.Similar to the study by Mutter et al,PTEN null glands were conspicuously absent in proliferative endometrium in our study.Using the FIGO grading system in endometrial carcinomas, Piero et al[24] documented PTEN positivity in less than half the glands,irrespective of grade.However,a reduction in PTEN positive glands,and an increase in null glands was seen in our study with increasing grade of carcinoma which correlated well with studies done by Inaba's series[25].Numerous PTEN null gland clusters were a consistent feature of Mutter's[16] study,which correlated well with ours.A finding of significance in our study was the presence of PTEN null glands in all atypical hyperplasias,correlateing with Mutter's study[16].

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 hyperplasia without atypia and with atypia,the P value derived is 0.000,both of which are highly significant.

Statistical correlation was done and P values were calculated using the Fisher's Exact test and applied wherever needed.Significant P values were obtained on comparison of intensity of glandular PTEN staining and null gland arrangement of endometrial hyperplasia without atypia and with atypia.

PTEN null glands were absent in normal endometrium,appeared in endometrial hyperplasia,clustering together with increased complexity of glands and increasing clusters towards the malignant end of the spectrum,which is an important finding.Thus,PTEN null glands could become an important indicator to malignancy.

CONCLUSION:

Hence,we conclude that IHC identification of loss of PTEN express ion and null glands in endometrial hyperplasia will help in the early detection of progression to malignancy,and the clinician could be alerted.

Our study recommends that PTEN staining pattern should be done in all cases of endometrial hyperplasia in the biopsy specimens which is a simple and cost effective technique when compared to other molecular modalities,for early detection of malignant transfor mation,and for close follow-up of patients.

Figure 1:10 x view-Proliferative Endometrium-Strong PTEN positivity

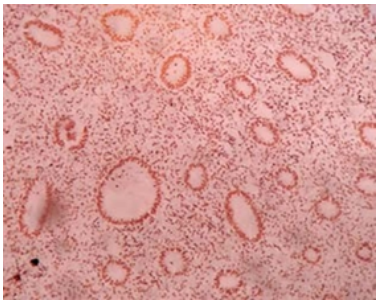


Figure 2:10 x view-Endometrial Hyperplasia without Atypia-Strong PTEN positivity

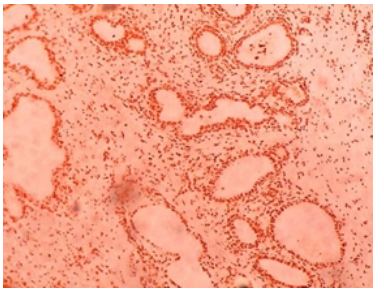


Figure 3:10 x view-Endometrial Hyperplasia with Atypia-Weak PTEN positivity

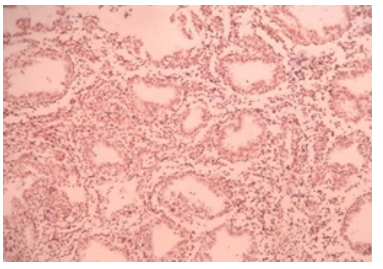
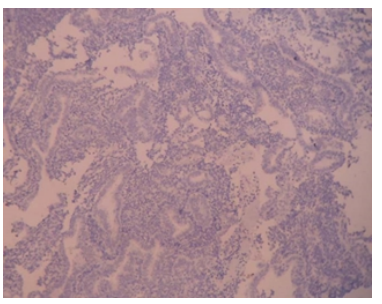


Figure 4:10 x view-Endometrioid Endometrial Adenocarcinoma Grade II-Absent PTEN staining



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