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RESEARCH ARTICLE

STUDY OF EXPRESSION OF PTEN IN ENDOMETRIUM AT A TERTIARY CARE CENTRE.

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Abstract

Background: To study the expression of PTEN (Phosphatase and Tensin homologue) in normal, hyperplastic and neoplastic endometrium by immunohistochemistry in normal to neoplastic endometrial lesions including endometrial carcinoma.

Materials and Methods: Formalin fixed paraffin embedded (FFPE) sections of spectrum of endometrium in hundred different cases were taken from secretory phase to endometrial carcinoma and subjected to Immunohistochemistry using PTEN.

Results: Immunoreactivity was taken as positive when a brown colour was noted in the nuclei or cytoplasm with intensity of staining being graded from 0 to 3+. Also if <10% of cells were positive a score of 0 was given, if 11 % to 30% cell were positive a score of 1+ , 31% to 60 % positive, a score as 2+ and > 60% positive cells a score of 3+ was given. Statistical analysis was performed with Chi-Square test and significant differences were noted between these 3 groups (p value < 0.05).

Conclusion: A decreased expression of PTEN is a marker of the earliest endometrial premalignant lesions, and therefore we in our study suggest that PTEN immunostaining can be helpful in identifying premalignant conditions that are likely to progress to carcinoma.

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Introduction:-

One of the most common malignancies of female genital tract is the endometrial carcinoma which accounts for ~7 % of all invasive cancers.^{1,2} The pathogenesis of endometrial carcinoma is complex involving several genetic alterations. Estrogen has been found to have an effect in the development of endometrial carcinoma with studies showing that an unopposed intake of estrogen for more than two years has a two to three fold higher risk of developing endometrial cancer.³ PTEN gene is a tumor suppressor gene that is located on chromosome 10 (10q23) which causes suppression of cell division and enable apoptosis.^{4,5} This gene negatively regulates the PI3K-AKT pathway and its downregulation is implicated in the pathogenesis of endometrial carcinoma.⁶ The expression of

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PTEN varies with phase of the menstrual cycle. It is always expressed in the estrogenic proliferative phase whereas in secretory phase its expression is variable with a post ovulatory secretory phase showing an increased PTEN expression in relation to the estrogenic proliferative phase whereas in the mid secretory phase there is loss of PTEN expression in the epithelium and an increased expression of PTEN in stroma. In the late secretory phase there is loss of PTEN expression. Stimulation of endometrium for long intervals with estrogen results in a clonal outgrowth of PTEN-depleted epithelium resulting in a pre-malignant state.⁷ In around 20% of precancerous hyperplastic lesions PTEN mutations are noted.

Our study was conducted to see the pattern of PTEN expression in normal, hyperplastic, and neoplastic endometrium evaluate its role in the pathogenesis of preneoplastic and neoplastic lesions of the endometrium.

Materials And Methods:

The study was carried out in the Department of Pathology, Adesh Institute of Medical Sciences and Research, Bathinda over a period of two years from February 2015 to August 2017. A total of 100 cases were taken with 18 cases of proliferative phase of endometrium, 22 cases of secretory phase, 23 cases of simple hyperplasia without atypia, 15 cases of complex hyperplasia without atypia, 9 cases of complex hyperplasia with atypia and 13 cases of endometrial carcinoma.

Immunohistochemical (IHC) procedure was employed on formalin fixed paraffin embedded tissue sections to study the expression of PTEN. A mouse anti-PTEN monoclonal antibody (Biogenex, USA) was used.

Scoring system:

A brown colour in the nuclei or cytoplasm was taken as positive with intensity of staining being graded from 0 to 3+ from no staining to weak, to moderate to mark staining. Also if <10% of cells were positive a score of 0 was given, if 11 % to 30% cell were positive a score of 1+ , 31% to 60 % positive, a score as 2+ and > 60% positive cells a score of 3+ was given.

Statistical analysis:

Calculation of association using chi square test and a level of $p < 0.05$ was considered as statistically significant.

Results:

A total of 100 cases were considered with 18 cases of Proliferative Endometrium and 22 cases in secretory phase (Figure 1A & B). 23 cases were found in the group Simple Hyperplasia without atypia (Figure 1C). 15 cases were reported in the group Complex hyperplasia without atypia (Figure 1D) and 9 cases were there in the Complex Hyperplasia with atypia group (Figure 1E). 13 cases belonged to Endometrial Carcinoma group (Figure 1F). The age of the patients ranged from 26-79 years with a median age being 46.73 ± 11.55 years.

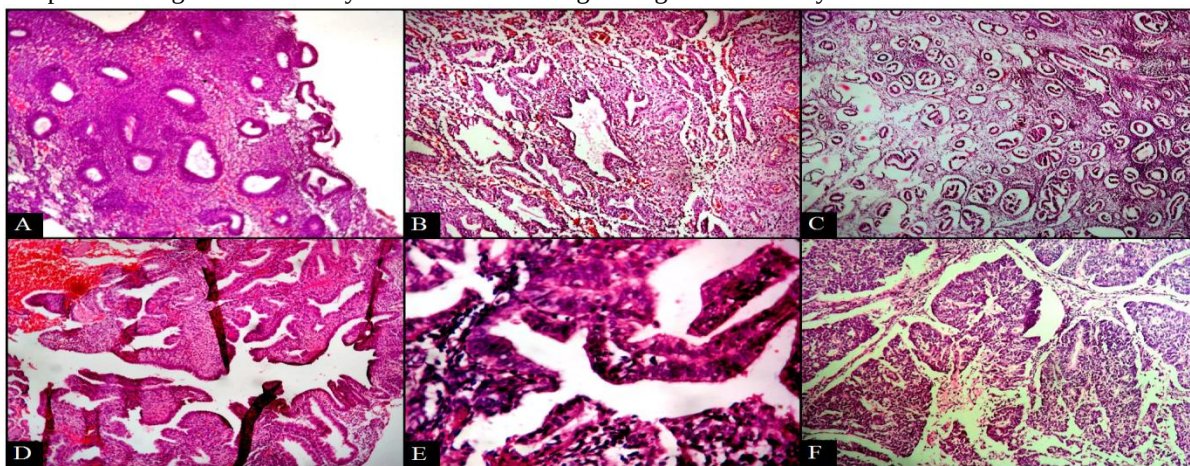


Figure1:-Hematoxylin & Eosin (H & E) pictures of Endometrium. (A) Normal Proliferative Endometrium (4x). (B) Endometrial glands and stroma in Secretory Phase (4x). (C) Simple Hyperplasia without atypia (4x). (D) Complex Hyperplasia without atypia (4x). (E) Complex Hyperplasia with atypia (10x). (F) Endometrial Carcinoma (4x).

In the 18 cases of Proliferative Phase, five cases (27.78%) showed 3+ intensity, thirteen cases (72.22%) cases showed 2+ intensity (Figure 2a). In the 22 cases of Secretory Phase, two cases (9.09%) showed 3+ intensity, eighteen cases (81.81%) cases showed 2+ intensity and two case (9.09%) showed 1+ intensity (Figure 2b). Overall 100% of cases of proliferative and secretory endometrium were positive for PTEN. In the 23 cases of Simple Hyperplasia without Atypia, two cases (8.69%) showed 3+ intensity, twelve cases (52.17%) cases showed 2+ intensity, six cases (26.08%) showed 1+ intensity and 3 cases (13.04%) were negative for PTEN (Figure 2c). In a total fifteen cases of Complex Hyperplasia without Atypia, three case (20%) showed 2+ intensity, eleven cases (73.33%) cases showed 1+ intensity and one case (6.66%) was negative for PTEN (Figure 2d). In a total nine cases of Complex Hyperplasia with atypia all cases were negative for PTEN. In a total of thirteen cases of Carcinoma Endometrium, five cases (38.46%) showed 1+ intensity and the remaining eight cases (61.53%) were negative for PTEN. The findings are summarized in Table 1.

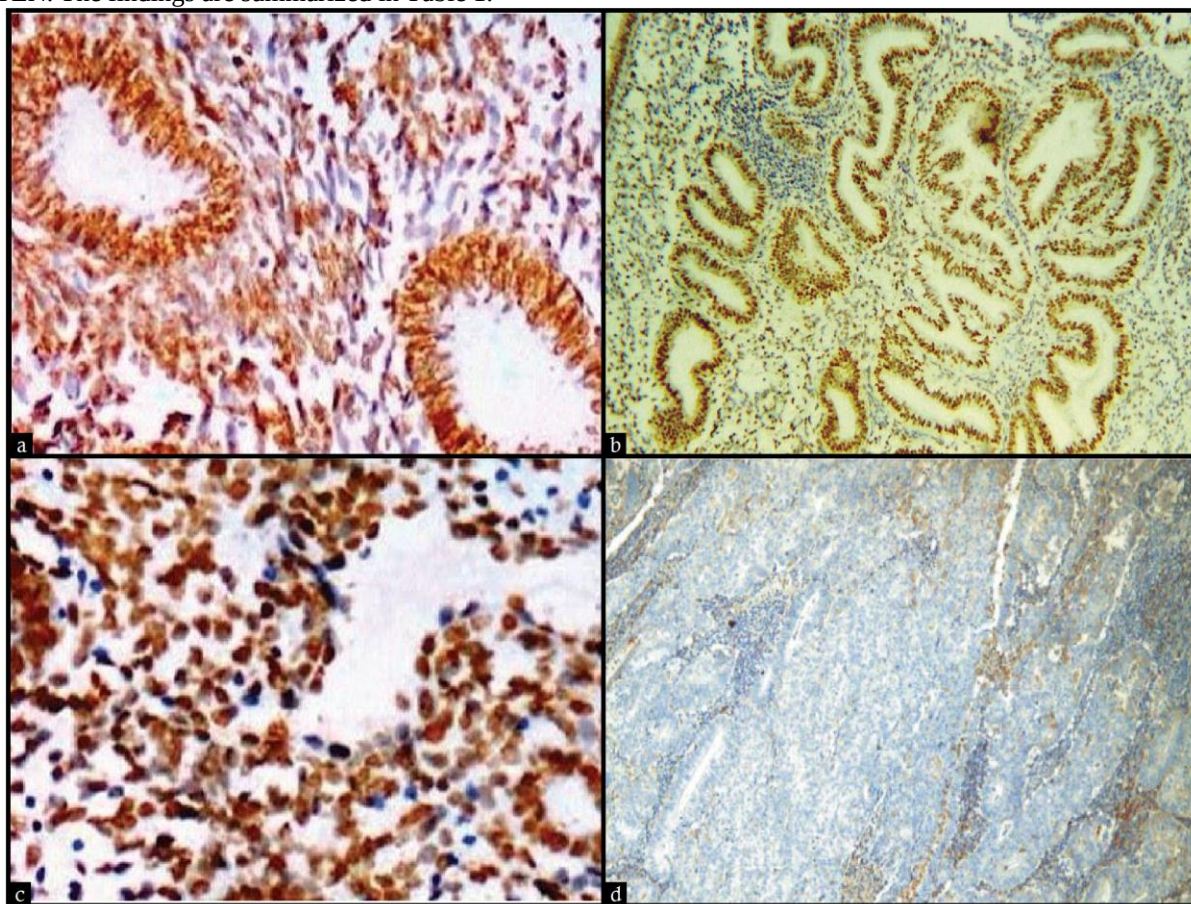


Figure 2:-PTEN immunostaining in Endometrium. (a) High power view showing normal Proliferative Endometrium. (b) Low power view showing normal Secretory phase. (c) Low power view showing Simple Hyperplasia. (d) Scanner view showing Endometrial Carcinoma.

Diagnosis	No. of Cases (n)	PTEN Positive Cases	Percentage (%)
Proliferative Phase	18	18	100%
Secretory Phase	22	22	100%
Simple Hyperplasia without Atypia	23	19	82.60%
Complex Hyperplasia without Atypia	15	13	86.66%
Complex Hyperplasia with Atypia	09	00	00%
Endometrium Carcinoma	13	05	38.46%

Table 1. PTEN Expression in Endometrium

Discussion:-

Endometrial cancer is the most common malignancy of the female genital tract. The development of endometrial carcinoma is a stepwise acquisition of several genetic alterations which involves the tumour suppressor genes and the oncogenes. Estrogen and its metabolites result in DNA damage and are therefore associated with higher risk of developing endometrial cancers.⁸ Loss of PTEN function results in excessive proliferation of cells which is a feature of many types of cancers, including endometrial cancers.^{9,10} Hyperplasia on the other hand is a heterogeneous group of lesions, which is considered by some to be reversible and truly neoplastic by some, so attempts have been made to sub classify to justify their role as a precancerous event.¹¹ The aim of our study was to study the expression of PTEN in normal, hyperplastic and neoplastic endometrium. The mean age of our study is in closest resemblance to the study done by Aziz et al.¹² PTEN immunoreactivity was noted in all cases of proliferative and secretory phase which was similar to study conducted by Erkanli S et al and Rao et al.^{13,14} PTEN expression in simple hyperplasia without atypia was 82.60 % which is slightly lower to study by Soheila Sarmadi et al but in a study by Anuradha Rao et al PTEN expression in simple hyperplasia without atypia showed 87% positivity which correlated well with our current study.¹⁵ In a study by Anuradha et al on the study group of hyperplasias found that simple hyperplasia without atypia had the maximum number of PTEN positive glands with the number of glands reducing as the number of complex hyperplastic glands increased, the least number of PTEN positive glands was seen in complex hyperplasia with atypia. Study conducted by Lee H et al showed the percentage of PTEN loss was significantly higher in endometrial carcinoma compared with simple hyperplasia and it was also higher in Complex atypical hyperplasia.¹⁶ In a study by Samar A El Sheikh et al PTEN immunoreactivity was noted in all normal proliferative endometrium and all Simple hyperplasia cases whereas Complex atypical hyperplasia 66.7% showed positive immunoreactivity. The difference was highly statistically significant.¹⁷ In a study by Tantbirojn P et al, 70 % cases of simple hyperplasia and 47 % cases of complex hyperplasia were positive.¹⁸ In Endometrial Carcinoma, loss of PTEN expression is seen in 61.53% of cases. Orbo et al reported loss of PTEN protein expression in 55% of specimens in patients with subsequent endometrioid endometrial carcinoma.¹⁹ In a study by Kanamori et al out of 98 advanced cases, 64 (65.3%) showed negative or mixed PTEN staining; their survival rate was significantly lower than that of PTEN positive cases.⁴ In a study by Terakawa et al out of 98 advanced cases, 64 (65%) showed negative or heterogeneous PTEN staining; their survival rate was significantly lower than that of PTEN-positive cases.²⁰ (Table 4).

Study	Loss of PTEN expression in Carcinoma
Kanamori et al ⁴	65.3%
Orbo et al ¹⁹	55%
Bueno et al ²¹	50%
N Terakawa et al ²⁰	65%
Erknali S et al ¹³	80%
Soheila Sarmadi et al ¹⁵	52%
Our study	61.54 %

Table 4. Comparison of loss of PTEN expression in Endometrial Carcinoma with other studies.

Conclusion:-

The current study showed that the loss of PTEN expression is an early event in endometrial carcinogenesis. An altered PTEN function is responsible for majority of endometrial cancers. The loss of PTEN expression is seen in the premalignant phase of the disease as well and is seen in the progression to carcinoma. Therefore a decreased PTEN expression is a marker of progression of premalignant lesions to endometrial carcinoma. It is therefore proposed that the use of PTEN immunostaining in a clinical setting will help in identifying premalignant lesions that are likely to progress to carcinoma.

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