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Study of P53 and Pten Immunohistochemical Markers in Hyperplastic and Neoplastic Endometrial Lesions

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Abstract
Endometrial carcinoma is a common malignancy in women, with a rising incidence due to improved awareness and screening programs. The molecular events in its carcinogenesis, particularly involving the tumor suppressor genes PTEN and p53, are not fully understood. Te aim of the study was to analyze the expression of PTEN and p53 in cases of endometrial carcinoma and endometrial hyperplasia and to understand their roles in the disease's pathogenesis. An observational study was conducted with 50 cases (35 endometrial carcinoma and 15 endometrial hyperplasia). Routine processing and H&E staining were followed by immunohistochemical analysis of PTEN and p53. In endometrial hyperplasia, PTEN was positive in 6 of 8 cases without atypia and in 2 of 7 cases with atypia. Among the 35 endometrial carcinoma cases, 28 were Type 1 and 7 were Type 2. PTEN expression was less frequent in carcinoma cases, with most either negative or showing heterogeneous expression. p53 positivity was notably higher in Type 2 carcinomas (71%) and increased with tumor stage and grade, with 8 of 10 Stage II carcinoma cases being p53 positive (Grade 3). PTEN inactivation and p53 over expression are significant in the progression of endometrial carcinoma. PTEN serves as an early marker for carcinogenesis, while p53 mutations are linked to advanced and aggressive tumor stages. These findings highlight the importance of these tumor suppressor genes in the pathogenesis of endometrial adenocarcinoma and underscore their potential as targets for therapy.

INTRODUCTION

Endometrial carcinoma is one of the most common gynecological malignancies in the developed countries. In India, although the incidence of endometrial carcinoma is low when compared to developed countries, there has been a steady increase, making it the fourth leading cancer and seventh leading cause for cancer deaths in women[1].

Endometrial carcinomas predominantly occur in postmenopausal women. The occurrence in premenopausal age group constitutes to <25%. There has been a proven strong association between estrogen exposure and development of endometrial carcinoma. The risk factors include obesity, diabetes, hypertension, infertility and nulliparity[2].

Endometrial hyperplasias are classified as Endometrial hyperplasia without atypia and Endometrial Atypical Hyperplasia/EIN. Endometrial carcinomas are classified into Type 1 which are estrogen dependent and usually exhibit endometrioid morphology and Type 2 which are estrogen independent and have serous/ clear cell morphology[3]. Endometrial carcinoma develop from a continuum of premalignant lesions ranging from endometrial hyperplasia without atypia to atypical hyperplasia to finally well differentiated adenocarcinoma[4].

Endometrioid type (Type1) endometrial adenocarcinoma occurs in a setup of endometrial hyperplasia in mostly postmenopausal women and carries good prognosis. Whereas non endometroid type (Type2) endometrial adenocarcinoma arises in the background of atrophic endometrium and has poor prognosis[5].

In the recent past there has been a development in analysing the additional prognostic and predictive factors at the molecular level such as PTEN and P53. The gene profile of gynecological malignancy provides a new field of investigation towards achievement of early diagnosis and better prognosis thereby improving years of survival rates.

P53 and PTEN signalling pathways play an important role in the pathogenesis of endometrial carcinoma. The tumour suppressor genes like PTEN, P53, Kras support the hypothesis that both the types of endometrial carcinomas (Type 1 and Type 2) involve different molecular carcinogenic pathways[6]. The previous studies have shown that P53 alteration independently predicts poor outcome in patients with endometrial carcinoma suggesting that there is a synergistic effect of PTEN and Kras signalling pathway during carcinogenesis of endometrial carcinoma[7]. p53 mutations correlated with non endometrioid (Type2) histolological type, undifferentiated type and also absence of progesterone receptors. The aim of this study is to analyse the immunohistochemical expression of PTEN and P53 in endometrial carcinomas and to study the correlation between PTEN and P53 expression with tumour grade and type.

MATERIALS AND METHODS

Study Design and Sample: This observational study was conducted in the Department of Pathology. A minimum of 50 cases were included, focusing on endometrial hyperplasia and neoplasia. Specimens with myometrial lesions without obvious endometrial pathology were excluded.

Specimen Preparation: Specimens were fixed in 10% buffered formalin, grossed meticulously and sampled from representative sites. Tissues were processed in an automated tissue processor and embedded in paraffin wax. Sections of 3-4 mm thickness were cut and stained with Hematoxylin and Eosin (H and E).

Routine Hematoxylin and Eosin (H and E) Staining: Sections of 4-5 micron thickness were prepared from the corresponding paraffin blocks on albumin-coated slides for H and E staining. The procedure involved deparaffinization, hydration through graded alcohols, staining with Harris's hematoxylin, differentiation in acid alcohol, bluing, counterstaining with eosin, dehydration, clearing in xylene and mounting with DPX. Nuclei stained blue, while the cytoplasm showed varying shades of pink.

Immunohistochemical Staining:

Specimen Preparation and Staining: Sections of 4-5 micron thickness were prepared on poly-L-lysine coated slides. Deparaffinization was followed by quenching of endogenous peroxidase activity, antigen retrieval using Tris buffer and incubation with primary and secondary antibodies. Detection was performed using DAB chromogen, followed by counterstaining with hematoxylin. The primary antibodies used were Mouse monoclonal Anti-human PTEN and p53, with DAKO hrp as the secondary antibody.

Data Analysis: Data analysis included calculating frequencies, means, and Chi-square test values. PTEN staining was interpreted based on the Memorial Sloan Kettering Cancer Centre criteria. Positive staining indicated strong positivity in the entire tumor or majority of the tumor, while negative staining indicated no staining in the tumor with strong positivity in adjacent normal endometrium or stromal cells. Heterogeneous staining showed both positive and negative staining within the tumor.

p53 scoring was performed by counting the number of positively stained nuclei of tumor cells and expressing it as a percentage of the total number of

tumor cells. Scores were classified as follows: Grade 1 (<5%, negative), Grade 2 (=5% and <50%, weak positive) and Grade 3 (=50%, strong positive).

Correlation: The expression of PTEN and p53 was correlated with histopathological features such as histological type, tumor grade and lymphovascular invasion, as well as clinical parameters including stage and extrauterine spread.

RESULTS AND DISCUSSIONS

A total number of 50 patients of endometrial hyperplasia (15) and endometrial carcinoma (35) are included in the study .These cases were categorized and subjected to IHC staining by PTEN and p53. The mean age of endometrialhyperplasiawas54yrsand the mean age of endometrial carcinoma was 56yrs. There was peak incidence of cases in the age group 40-60yrs.

Out of 50 patients, 39 were postmenopausal, with the primary complaint being postmenopausal bleeding. This symptom often leads to further clinical investigation and diagnosis of endometrial hyperplasia or neoplasia.

Patients with endometrial hyperplasia were subdivided into two categories: endometrial hyperplasia without atypia and endometrial hyperplasia with atypia. Out of the 15 patients with endometrial hyperplasia, 8 cases were classified as endometrial hyperplasia without atypia and 7 cases were classified as endometrial atypical hyperplasia.

Out of 35 patients diagnosed with endometrial carcinoma, 28 patients had Type 1 endometrial carcinoma, and 7 patients had Type 2 endometrial carcinoma.

Out of 28 patients with Type 1 endometrial carcinoma, 13 had Grade 1 tumors, 9 had Grade 2 tumors, and 6 had Grade 3 tumors. Additionally, there were 7 cases of Type 2 endometrial carcinoma.

In accordance with FIGO staging, 16 patients were classified as Stage IA, 9 patients as Stage IB and 10 patients as Stage II.

PTEN expression was positive in 6 cases of endometrial hyperplasia (EH) without atypia. As the atypia increased, PTEN expression decreased. Out of 7 cases of atypical hyperplasia/endometrial intraepithelial neoplasia (EIN), 5 were negative for PTEN.

In our study, out of 28 cases of endometrial carcinoma (EC), the majority of cases were either heterogeneous or negative for PTEN expression.

In our study, Type 1 endometrial carcinoma (EC) cases were mostly Grade 1 or Grade 2. Most of the Type 2 EC cases were Grade 3 and positive for p53.

In our study, p53 expression varied with the FIGO stage of endometrial carcinoma. Among Stage IA tumors, 12 were p53 Grade 1, 2 were p53 Grade 2 and 2 were p53 Grade 3. For Stage IB tumors, 3 were p53 Grade 1, 4 were p53 Grade 2 and 2 were p53 Grade 3. In Stage II tumors, 1 was p53 Grade 1, 1 was p53 Grade 2 and 8 were p53 Grade 3. These results show that higher p53 grade expression is more common in advanced tumor stages, particularly in Stage II.

Endometrial carcinoma is the fifth most common malignancy in women worldwide, with an annual incidence of 10-20% per 100,000 women according to Globocan 2016. This incidence is increasing due to better awareness and screening programs, though the molecular events in its carcinogenesis are still not well understood.

Our study included patients aged 35-80 years, with a mean age of 54 years for endometrial hyperplasia and 56 years for endometrial carcinoma. These

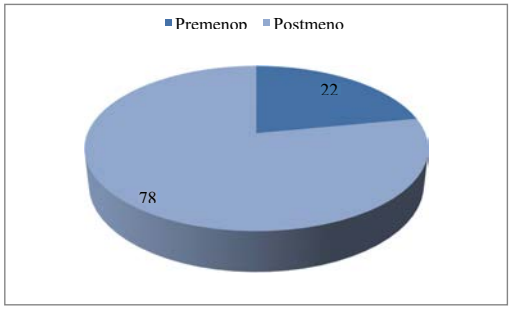

Fig. 1: Distribution of cases according to menopausal status

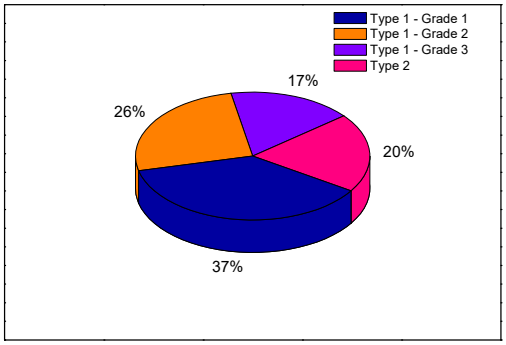

Fig. 2: Distribution of Cases According to Histopathological Grade

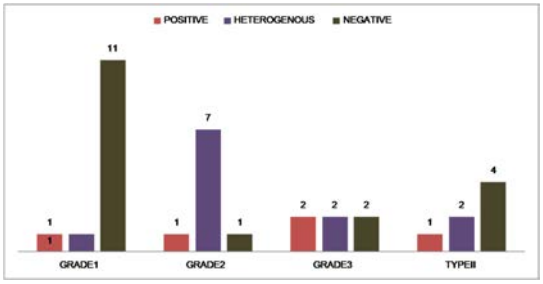

Fig. 3: Expression of PTEN in Endometrial Carcinoma According to Grade of Tumor

Table 1: Distribution of cases according to age group

Agegroup	Endometrial Hyperplasia	Endometrial Carcinoma
21-30	-	-
31-40	1	1
41-50	6	17
51-60	7	12
61-70	1	3
71-80	-	2
Total Cases-50	15	35

Table 2: Distribution of Cases of Endometrial Hyperplasia

Category	Number of Cases
Total Cases of Endometrial Hyperplasia	15
Endometrial Hyperplasia Without Atypia	8
Endometrial Atypical Hyperplasia	7

Table 3: Distribution of Cases of Endometrial Carcinoma

Endometrial Carcinoma	Number of Cases
Type 1	28
Type 2	7

Table 4: Distribution of Cases According to Stage of Tumor

FIGO Staging	Number of Cases
Stage IA	16
Stage IB	9
Stage II	10
Stage III	-
Stage IV	-

Table 5: Expression of PTEN in Endometrial Hyperplasia

Hyperplasia Type	Total Cases (15)	Positive	Heterogeneous	Negative	
Without Atypia	8	6	1	1	P < 0.05
Atypical Hyperplasia/EIN	7	1	1	5	

Table 6: Expression of p53 According to Grade of Tumor

Tumor Grade	p53 Grade 1	p53 Grade 2	p53 Grade 3
Type I - Grade 1	11	1	1
Type I - Grade 2	3	3	3
Type I - Grade 3	1	2	3
Type II	1	1	5

Table 7: Expression of p53 According to Stage of Tumor

Stage of Tumor	p53 Grade 1	p53 Grade 2	p53 Grade 3
Stage IA	12	2	2
Stage IB	3	4	2
Stage II	1	1	8

findings are consistent with other studies, such as those by Shanmugapriya *et al.* (2017) and Rasty *et al.* (1998), who reported mean ages of 54 and 63.3 years, respectively[8,9].

PTEN expression was positive in 6 of 8 cases of endometrial hyperplasia without atypia but decreased as atypia increased, with 5 of 7 atypical hyperplasia/EIN cases negative for PTEN. This aligns with studies by Soheila Sarmadi *et al.* (2009) who also noted reduced PTEN expression with increasing atypia[10]. The pathogenesis of endometrial hyperplasia and carcinoma is closely linked to hormones like estrogen and progesterone. Previous studies suggested that 75% of women with endometrial carcinoma were postmenopausal, often presenting with postmenopausal bleeding. In our study, 39 out of 50 patients were postmenopausal, most presenting with postmenopausal bleeding and did not receive hormone replacement therapy (HRT).

Rasty *et al.* (1998) suggested that low parity or nulliparity is a risk factor for endometrial hyperplasia and carcinoma, whereas most patients in our study were parous[9]. Bokhman described two pathogenetic types of EC: Type 1 tumors, which are usually low-grade endometrioid adenocarcinomas, and Type 2 tumors, which are more aggressive and less common[11]. Lax proposed that these types are pathogenetically distinct, with molecular pathways involving stepwise genetic changes[12]. Our study classified 35 cases of endometrial carcinoma into 28 Type 1 and 7 Type 2, following FIGO recommendations. We had 13 Grade 1, 9 Grade 2 and 6 Grade 3 carcinomas. Pathological staging revealed 16 cases in Stage IA, 9 in Stage IB and 10 in Stage II.

Substantial lymphovascular invasion is a significant risk factor for recurrence in endometrial carcinomas, though our study did not compare this with survival rates. PTEN is a tumor suppressor gene, crucial in the development of Type 1 endometrioid carcinoma. Inactivation of PTEN is an early event in carcinogenesis, associated with advanced stage and aggressive behavior. Risinger *et al.* suggested that PTEN inhibits invasion and metastasis through cytoskeleton modulation[13].

The tumor suppressor gene p53, located on chromosome 17p13.1, mutates in 90% of Type 2 and 10-20% of Type 1 endometrial carcinomas, influencing the progression of these tumors[14]. Our study found PTEN expression in 53.5% of Type 1 and 14.2% of Type 2 endometrial carcinomas, with p53 positivity primarily in Stage II endometrial carcinomas. As tumor stage increased, so did p53 positivity. Our findings align with previous research indicating a strong correlation between p53 and PTEN expressions in endometrial adenocarcinoma, highlighting their roles in the disease's pathogenesis[15].

CONCLUSION

Conclusion, present study analyzed 50 cases of endometrial carcinoma and endometrial hyperplasia. PTEN positivity decreased with increasing atypia and tumor grade/stage, while p53 positivity increased with tumor grade/stage. These findings suggest that PTEN inactivation and p53 overexpression play crucial roles in the progression of endometrial carcinoma, indicating their potential as markers for diagnosis and targets for therapy.

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