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A CROSS-SECTIONAL STUDY OF PATHOLOGICAL SCORING OF PHOSPHATASE AND TENSIN HOMOLOG (PTEN) IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL CARCINOMA, ODISHA: DIAGNOSTIC, PROGNOSTIC AND THERAPEUTIC EFFICACY

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ABSTRACT

Background

Endometrial carcinoma (EC), the most common gynecologic cancer, is rising, especially in postmenopausal women. Identification of reliable prognostic indicators is essential for early diagnosis and patient management. EC is often linked to the tumor suppressor gene PTEN (Phosphatase and Tensin Homolog), suggesting it could be used as a diagnostic and prognostic marker. This study evaluated the expression of PTEN in endometrial tissues diagnosed with hyperplasia and carcinoma, and to correlate PTEN expression with the type and grade of EC.

Methods

Endometrial tissue samples from 66 patients were analyzed, including 30 cases of endometrioid endometrial carcinoma (EEC), 12 cases of proliferative endometrium (PE), 12 cases of endometrial hyperplasia (EH), and 12 cases of non-endometrioid uterine malignancies (NEUM). PTEN expression was assessed using immunohistochemistry on paraffin-embedded tissue sections, and statistical analysis was accomplished using SPSS version 20.

Results

The participants' ages ranged from 42 to 68 years, with a mean age of 55.4 years. PTEN expression was substantially lower in EEC in contrast to PE and EH ($p = 0.0001$). The mean PTEN scores were 235.83 ± 26.79 for PE, 94.17 ± 61.27 for EH, 31.67 ± 58.37 for EEC, and 61.67 ± 79.64 for NEUM. A significant correlation was found between reduced PTEN expression and higher tumor grade, increased myometrial invasion, and advanced tumor stage ($p < 0.05$).

Conclusion

PTEN expression is substantially reduced in EC, particularly in higher-grade tumors and those with extensive myometrial invasion. This study underscores the potential of PTEN as a prognostic marker in EC, which could be instrumental in guiding treatment strategies.

Recommendations

Further studies with higher sample numbers are recommended to validate PTEN as a routine diagnostic and prognostic marker in clinical practice. Additionally, exploring the role of PTEN in targeted therapies could provide new avenues for the treatment of EC.

Keywords: *Endometrial Carcinoma, Phosphatase and Tensin Homolog (PTEN), Immunohistochemistry, Prognostic Marker, Myometrial Invasion.*
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INTRODUCTION

Endometrial carcinoma (EC) is the most prevalent gynecological cancer, and its incidence rates are rising worldwide. Though it can also affect younger women, postmenopausal women are the ones who are most impacted. The deletion or mutation of the PTEN (phosphatase and tensin homolog) gene is a crucial factor in the pathophysiology of endometrial cancer, which is characterized by complicated genetic and molecular changes. PTEN is a tumor suppressor gene that is found on chromosome 10q23.3. It encodes a protein that inhibits the PI3K/AKT signaling pathway, which is essential for the growth and survival of cells [1].

Recent studies highlight the critical role of PTEN in endometrial carcinogenesis. PTEN mutations are prevalent in approximately 83% of endometrioid endometrial carcinomas (EEC), the most common subtype of endometrial cancer. These mutations include point mutations, deletions, and epigenetic silencing, leading to the functional loss of PTEN protein. The loss of PTEN function contributes to uncontrolled cellular proliferation, reduced apoptosis, and enhanced cell survival, thereby promoting tumor growth and progression [2].

The prognostic significance of PTEN expression in endometrial carcinoma has been extensively studied. Low

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PTEN expression is related to higher tumor grade, advanced stage, and poor clinical outcomes. For instance, a review reported a significant correlation between PTEN loss and the progression of endometrial hyperplasia to carcinoma, underscoring its role as a marker for malignancy risk in premalignant endometrial lesions [3]. Furthermore, recent studies suggest that PTEN status could be integrated with other molecular markers such as HER2 and hormone receptors to refine risk stratification and personalize treatment strategies [4].

The potential therapeutic implications of PTEN inactivation have also been explored. PTEN-deficient tumors exhibit resistance to certain therapies, including PI3K inhibitors and immunotherapies. The loss of PTEN has been linked to an immunosuppressive tumor microenvironment, reducing T-cell infiltration and enhancing immune evasion, which poses challenges for effective immunotherapy. This resistance emphasizes the need for comprehensive molecular profiling in endometrial carcinoma to identify patients who may benefit from alternative therapeutic approaches.

Given the significant role of PTEN in endometrial carcinogenesis and its implications for prognosis and therapy, this study aims to evaluate PTEN expression in endometrial tissue obtained from diagnostic curettage and hysterectomy specimens. By analyzing PTEN expression in cases diagnosed with hyperplasia and endometrial carcinoma, this research seeks to elucidate the correlation between PTEN loss and histological severity, thereby contributing to improved diagnostic and prognostic accuracy in endometrial carcinoma.

This study was conducted on endometrial tissue samples obtained from diagnostic curettage and hysterectomy specimens. The objective of this study is to evaluate the expression of PTEN in endometrial tissues diagnosed with hyperplasia and carcinoma and to correlate PTEN expression with the type, grade, and stage of endometrial carcinoma to assess its potential as a diagnostic and prognostic marker.

METHODOLOGY

Study Design

A hospital-based prospective cross-sectional study.

Study Setting

The study was conducted from July 2015 to August 2017 at the Department of Pathology and Obstetrics and Gynaecology, Shriram Chandra Bhanja Medical College, and Acharya Harihar Regional Cancer Centre, Cuttack.

Sample Size

The study included a total of 66 patients:
- 30 cases of endometrioid endometrial adenocarcinoma (EEC)
- 12 cases of proliferative endometrium (PE)
- 12 cases of endometrial hyperplasia (EH)
- 12 cases of non-endometrioid uterine malignancies (NEUM).

Inclusion Criteria

- All cases of endometrial hyperplasia and endometrial carcinoma (both endometrioid, non-endometrioid types, and carcinosarcoma).

Exclusion Criteria

- Pregnancy
- Post-abortal cases

Sample size

To calculate the sample size for this study, the following formula was used for estimating a proportion of a population:

$$n = \frac{Z2 \times p \times (1-p)}{E2}$$

Where:
- n = sample size
- Z = Z-score corresponding to the desired level of confidence
- p = estimated proportion in the population
- E = margin of error

Bias: There was a chance that bias would arise when the study first started, but it was avoided by giving all participants identical information and hiding the group allocation from the nurses who collected the data.

Collection of Specimens

Clinical data including patient demographics, clinical diagnosis, and investigation results were recorded. Endometrial biopsies were obtained using a Pipelle, a sterile and disposable plastic cannula for sampling the endometrium. Following specimen collection, histopathological examination was performed after proper processing.

Tissue Fixation and Processing

- The gross morphology of the supplied tissue was recorded, and it was fixed in 10% neutral buffered formal saline within 30 minutes and kept for 12-24 hours to preserve antigenicity.
- Endometrial curettage samples were completely embedded, and representative tissues from hysterectomy specimens were embedded and processed in an automatic tissue processor.

Immunohistochemical Analysis

Immunohistochemistry (IHC) was performed to evaluate PTEN expression in the endometrial tissue sections using the following steps:

1. Deparaffinization and Rehydration:
    - Tissue sections embedded in paraffin were deparaffinized in xylene and rehydrated using a graduated ethanol series.
2. Antigen Retrieval:
    - To reveal the antigenic sites, antigen retrieval was carried out in a

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microwave oven with citrate buffer (pH 6.0).

3. Blocking Endogenous Peroxidase Activity:
    - The slices were incubated in 3% hydrogen peroxide to inhibit the activity of endogenous peroxidase.
4. Primary Antibody Incubation:
    - Sections were incubated with a primary rabbit monoclonal antibody against PTEN (dilution 1:100).
5. Secondary Antibody and Detection:
    - Following washing, slices were treated with a streptavidin-horseradish peroxidase complex and then a biotinylated secondary antibody.
6. Chromogen Development:
    - The chromogenic reaction was developed using a 3,3'-diaminobenzidine (DAB) substrate.
7. Counterstaining:
    - Hematoxylin was used as a counterstain, sections were dried, and then mounted.

Scoring of PTEN Expression

The following grading method was used to assess PTEN expression based on the intensity and percentage of positively stained cells:
- Intensity: 0 (no staining), 1 (weak), 2 (moderate), 3 (strong).
- Percentage of Positive Cells: 0 (0-5%), 1 (6-25%), 2 (26-50%), 3 (51-75%), 4 (76-100%).
The total immunohistochemical score (IHS) was obtained by multiplying the intensity and percentage scores, ranging from 0 to 12.

Statistical Analysis

The analysis of the data was done with SPSS version 20. To compile the data, descriptive statistics were employed. The chi-square test and nonparametric Kruskal-Wallis test were used to examine the relationship between PTEN expression and the kind and grade of endometrial cancer. < 0.05 was the threshold for statistical significance.

Ethical considerations

The study protocol was approved by the Ethics Committee and written informed consent was received from all the participants.

RESULT

The study included a total of 66 cases, which were distributed as follows: 30 cases of EEC, 12 cases of PE, 12 cases of EH, and 12 cases of NEUM.

Table 1: Distribution of Cases

Group	Number of Cases	Age Range (years)	Mean Age (years)
EEC	30	45 - 68	56.2
PE	12	42 - 58	50.3
EH	12	44 - 63	54.1
NEUM	12	47 - 65	55.8
Total	**66**	**42 - 68**	**55.4**

Among the 30 cases of EEC, 10 cases (33.33%) were Grade I (well-differentiated), 14 cases (46.66%) were Grade II (moderately differentiated), and 6 cases (20%) were Grade III (poorly differentiated). Myometrial invasion was evaluated in 20 cases, with 15 cases (75%) having more than 50% myometrial invasion and 5 cases (25%) having < 50% myometrial invasion.

The distribution of cases according to the FIGO staging system was as follows: 5 cases (25%) were Stage IA, 6 cases (30%) were Stage IB, 4 cases (20%) were Stage II, 2 cases (10%) were Stage IIIA, 2 cases (10%) were Stage IIIB, 1 case (5%) was Stage IIIC, and there were no cases in Stage IV.

Table 2: Grading of Endometrial Carcinoma (EEC)

Grade	Number of Cases	Percentage (%)
I	10	33.33
II	14	46.66
III	6	20

Table 3: Myometrial Invasion in EEC Cases

Myometrial Invasion	Number of Cases	Percentage (%)
>50%	15	75
<50%	5	25

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Table 4: Staging of Endometrial Carcinoma (EEC)

FIGO Stage	Number of Cases	Percentage (%)
IA	5	25
IB	6	30
II	4	20
IIIA	2	10
IIIB	2	10
IIIC	1	5
IV	0	0

PTEN expression was analyzed across different groups, with the mean PTEN score calculated for each group. The differences in PTEN scores among these groups were found to be statistically significant (p = 0.0001).

Table 5: Mean PTEN Scores by Group

Group	Mean PTEN Score ± SD
PE	235.83 ± 26.79
EH	94.17 ± 61.27
EEC	31.67 ± 58.37
NEUM	61.67 ± 79.64

Significant differences in PTEN scores were observed between various groups. The mean difference in PTEN scores between Group A (EEC) and Group B (PE) was 204.167 (p = 0.000), and between Group B (PE) and Group C (EH) was 141.667 (p = 0.0001). However, the differences between Group A (EEC) and Group C (EH), as well as between Group A (EEC) and Group D (NEUM), were not statistically significant.

Table 6: Comparison of PTEN Scores Between Groups

Comparison	Mean Difference	p-value
EEC vs. PE	204.167	0.000
EEC vs. EH	30.000	0.038
PE vs. EH	141.667	0.0001
PE vs. NEUM	174.167	0.0001
EEC vs. NEUM	30.000	0.796

The mean PTEN score decreased significantly from Grade I (60) to Grade III (0) in EEC cases (p = 0.001). Furthermore, the mean PTEN score was significantly higher in cases with less than 50% myometrial invasion (146.00 ± 50.29) compared to those with more than 50% invasion (6.00 ± 11.83) (p = 0.0001). The PTEN score also showed a substantial decrease from Stage IA (146 ± 50.29) to Stage IV (0) (p = 0.007).

DISCUSSION

The study comprised 66 cases, categorized into 30 cases of EEC, 12 cases of PE, 12 cases of EH, and 12 cases of NEUM. Among the EEC cases, a significant portion (46.66%) were moderately differentiated (Grade II), with a smaller percentage being well-differentiated (Grade I, 33.33%) and poorly differentiated (Grade III, 20%).

Myometrial invasion was assessed in 20 EEC cases, revealing that 75% had more than 50% invasion, while the remaining 25% had less than 50% invasion. The FIGO staging system indicated that most EEC cases were in the early stages (IA and IB, 55%), with fewer cases in advanced stages (III and above, 25%).

PTEN expression varied significantly across different groups. The mean PTEN scores were highest in proliferative endometrium (235.83 ± 26.79) and progressively lower in endometrial hyperplasia (94.17 ± 61.27), endometrioid endometrial carcinoma (31.67 ± 58.37), and non-endometrioid uterine malignancies (61.67 ± 79.64). These differences were statistically considerable (p = 0.0001), indicating a clear trend of decreasing PTEN expression from benign to malignant conditions.

Statistical analysis showed significant differences in PTEN scores between various groups. Notably, there was a substantial difference between EEC and PE (mean difference = 204.167, p = 0.000) and between PE and EH (mean difference = 141.667, p = 0.0001). However, the differences between EEC and EH, and between EEC and NEUM, were not statistically considerable, suggesting similar PTEN expression patterns in these groups.

A notable finding was the significant reduction in PTEN scores with increasing tumor grade. The mean PTEN score decreased from 60 in Grade I to 0 in Grade III EEC cases (p = 0.001). Similarly, cases with less than 50% myometrial invasion had significantly higher PTEN

scores (146.00 ± 50.29) compared to those with more than 50% invasion (6.00 ± 11.83) (p = 0.0001). The PTEN score also decreased significantly from Stage IA (146 ± 50.29) to Stage IV (0), highlighting its potential role as a prognostic marker (p = 0.007).

The results demonstrate a clear association between reduced PTEN expression and the progression of endometrial pathology from proliferative endometrium to endometrial hyperplasia and carcinoma. The significant decrease in PTEN expression with higher tumor grades, greater myometrial invasion, and advanced stages of endometrial carcinoma underscores its potential as a valuable diagnostic and prognostic marker. These findings suggest that PTEN immunostaining could be an effective tool in the early detection and management of endometrial carcinoma, guiding therapeutic decisions and improving patient outcomes.

The diagnostic accuracy of D&C and office hysteroscopy (OH) for atypical endometrial hyperplasia (AEH) was examined in a study. OH had an 87% diagnostic coincidence with the final histology diagnosis, but D&C's was only 14%, according to the study. This suggests that OH has a much higher diagnostic accuracy for AEH [5].

Before a hysterectomy, a study examined the diagnostic accuracy of D&C and aspiration biopsy in individuals with endometrial hyperplasia. Overall diagnostic concordance for D&C and aspiration biopsy was 51.0% and 41.3%, respectively, according to the study. Patients who had aspiration biopsy had a considerably higher chance of having their preoperative specimen upgraded than those whose material was collected via D&C (21.0% vs. 36.7%, P = 0.008). Additionally, D&C had a decreased final pathological upgrading rate to endometrial cancer (15.0% vs. 27.3%, P = 0.022) [6].

Studies looked at the potential predictive value of histologic subtyping of complex atypical hyperplasia (CAH) for endometrial adenocarcinoma (ADCA). They discovered that 38.3% of CAH cases at hysterectomy had ADCA. After categorizing CAH into Type A and Type B subtypes, it was found that Type A had a far stronger correlation with ADCA (75.9%) than Type B (26.2%) [7].

The effectiveness of immunohistochemistry (IHC) for mismatch repair proteins (MMRPs) in detecting Lynch syndrome (LS) in cases of endometrioid endometrial cancer was assessed in a study. The MMRP expression patterns of paired hysterectomy specimens and endometrial biopsy/curettage specimens were shown to be 100% concordant. This study supported routine LS screening on endometrial biopsy/curettage tissues by demonstrating that IHC for MMRPs can be conducted on either sample type with reliability [8].

A different study evaluated the efficaciousness of endometrial biopsy performed as an outpatient procedure for the diagnosis of endometrial cancer. Following a negative endometrial biopsy result, the posttest likelihood of having EC was 0.74%. The investigation also revealed a notable lack of sensitivity in endometrial sampling for the detection of EC, with further tissue levels not showing a discernible improvement in diagnostic yield [9].

Additionally, a study examined the effectiveness of complete and selective sampling in identifying EC in hysterectomy tissues. Complete sampling involves examining every other block of the endometrium. In 92% of patients, including all high-grade/high-stage carcinomas, selective sampling revealed EC. The diagnostic agreement between selective and total sampling increased to 96% when interobserver variability was taken into account, indicating that selective sampling is a workable and resource-efficient approach [10].

Generalizability

The external validity of this study is potentially limited by its hospital-based, single-center design, which may not fully represent the broader population of patients with endometrial carcinoma. The specific demographic characteristics of the study population, such as the predominance of postmenopausal women from urban areas, may not be generalizable to all geographic or socioeconomic groups. Additionally, the relatively small sample size further restricts the ability to generalize the findings to a larger, more diverse population. However, the study's findings are still valuable for similar clinical settings and can inform further research in more varied populations to enhance external validity.

CONCLUSION

The results indicate that PTEN expression is significantly lower in endometrioid endometrial carcinoma compared to proliferative endometrium and endometrial hyperplasia. Additionally, reduced PTEN expression is associated with higher tumor grade, increased myometrial invasion, and advanced tumor stage. This suggests that PTEN could serve as a valuable prognostic marker in endometrial carcinoma.

Limitations

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack
of a comparison group also poses a limitation for this study's findings.

Recommendation

Further studies with higher sample numbers are recommended to validate PTEN as a routine diagnostic and prognostic marker in clinical practice. Additionally, exploring the role of PTEN in targeted therapies could provide new avenues for the treatment of EC.

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List of abbreviations

EC: Endometrial Carcinoma
PTEN: Phosphatase and Tensin Homolog
EEC: Endometrioid Endometrial Carcinoma
PE: Proliferative Endometrium
EH: Endometrial Hyperplasia
NEUM: Non-Endometrioid Uterine Malignancies
IHC: Immunohistochemistry
IHS: Immunohistochemical Score
DAB: 3,3'-Diaminobenzidine
D&C: Dilatation and Curettage
OH: Office Hysteroscopy
AEH: Atypical Endometrial Hyperplasia
CAH: Complex Atypical Hyperplasia
ADCA: Adenocarcinoma
MMRPs: Mismatch Repair Proteins
LS: Lynch Syndrome

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Conflict of interest

The authors have no conflicting interests to declare.

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