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Research Protocol

Pathology Section

PTEN Immunoexpression in Atypical Endometrial Hyperplasia and Endometrioid Endometrial Carcinoma: A Research Protocol

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ABSTRACT

Introduction: One of the major cancers affecting women is of Endometrial Carcinoma (EC). Therefore, its molecular characterisation remains important. Phosphatase and Tensin Homolog (PTEN) gene defects are one such molecular pathology commonly seen in lesion of atypical endometrial hyperplasia and EC. However, studies on PTEN immunoexpression in India are scarce.

Aim: To study immunoexpression of PTEN and its correlation with various clinicopathologic parameters in lesion of atypical endometrial hyperplasia and EC.

Materials and Methods: The study will be conducted in Department of Pathology for duration of two years from April 2022 to March 2024. It will be carried out as a cross-sectional study with prospective and retrospective design with sample size of 20 cases each in two groups- cases diagnosed on biopsy or surgical specimen of atypical endometrial hyperplasia and endometrioid endometrial carcinoma type -1, dividing them into sample size of 20 for each group. PTEN immunoexpression will be observed by immunohistochemical method on paraffin section of the tissue. The study would make the observation on the PTEN positivity by IHC in atypical endometrial hyperplasia and type-1 EC. The positive research of IHC will be compared for various other clinicopathologic parameters will be included in the study for both the endometrial lesion. The statistics of comparison will be drawn by using standard statistical test.

Keywords: Endometrial hyperplasia, Immunohistochemistry, Phosphatase tensin homologue

INTRODUCTION

The EC is one of the most common invasive cancer of female genital tract which counts for 7% of all the invasive cancer in women [1-3]. The relationship between atypical endometrial hyperplasia and EC has been attempted through the evidences of molecular pathology. It's been known by now that few of molecular alterations in the tumour suppressor gene, as well as, few of the cell signalling pathway such as Pi3K/AKT has been shared by the pathologies of atypical endometrial hyperplasia and EC [4,5]. The endometrial pathology of the hyperplasia has recently been simplified by World Health Organisation (WHO) to be categorised as endometrial hyperplasia without atypia and Atypical Endometrial Hyperplasia (AEH). The latter is a precursor lesion for EC Type-I [1,2]. These two broad classes have a definite clinical implication for treatment and possible malignant transformation.

The molecular pathogenesis of EC types has different yet synergistic alteration to cell signalling pathway. The Type-I EC which arises in the situation of unopposed action of the oestrogen is due to acquisition of molecular alterations in tumour suppression gene and oncogenes [4]. One of the mutation acts to increase signalling is through the Pi3K/AKT pathway. Further more mutation in PTEN tumour suppressor gene or its inactivation has been cited as the initial molecular event is pathogenesis of type-1 EC [5-7]. The studies quoted above implied the prognostic, diagnostic and the predictive value of PTEN.

The other molecular event in pathogenesis of Type-I EC includes mutation to Kirsten Rat Sarcoma viral oncogene homolog (KRAS) ARID1A coupling with macrosatellite instability and defect in Deoxyribonucleic Acid (DNA) mismatch repair gene. The other molecular alteration happens especially in Type-II EC includes mutation to p53 gene and aneuploidy [1]. Most of the studies, PTEN detection was performed on immunohistochemistry on paraffin section. Among which, few studies bought out relationship between immunoexpression of PTEN with histological type of EC, its grade and stage of the disease, so also the relationship between PTEN immunoexpression was explored in AEH as nested marker in evolution of AEH to Type-I EC [8-15]. Hence, the present research protocol is planned with the aim to know and compare the frequency of PTEN immunoexpression in atypical endometrial hyperplasia and Type-I EC (endometrioid) as well as to study association of immunoexpression of PTEN with grade and stage of EC Type-1. The present research is done for culmination of research gap to understand PTEN demonstration on immunohistochemistry for knowing the association with various clinicopathological features in AEH and EC.

REVIEW OF LITERATURE

The EC is the most common gynaecological malignancy in developed counties. More than 80% cases of EC are preceded by endometrial hyperplasia. The study conducted by Allithy A et al., evaluated the immunohistochemical expression of PTEN in normal, hyperplastic and neoplastic endometrial tissues. They observed that intensity of PTEN immunostaining was down regulated with increasing cytological atypical features, that is all included normal endometrium and EH without atypia showed strong cytoplasmic expression of PTEN, while only 6% of atypical EH showed it and none of endometrioid EC showed strong PTEN expression [2]. When compared with study conducted by Shanmugapriya M et al., evaluated that PTEN immunoreactivity was strongly expressed in all cases of proliferative, secretory phase of endometrium and downregulated in atypical EH and endometriod EC [4]. Similar findings of Allithy A et al., were observed with Sarmadi S et al., were all normal, proliferative and hyperplastic without atypia showed strong positivity for PTEN while atypical EH and EC showed (75% and 48%) positivity, respectively [2,8]. In a study by Rani E et al., PTEN immunoexpression was noted in all cases of proliferative and secretory phase of endometrium [3], which was similiar to findings way by Shanmugapriya M et al., but slightly lower to study by Sarmadi S et al., [4,8]. Correlation of PTEN immunostaining with tumour grading and myometrial invasion of examined EC was also studied. According to observations noted by Allithy A et al., found

that PTEN immunostaining was accentuated in higher tumour grades. A 28.6% of Grade-I were negative for PTEN, while 100% of Grade-II and III showed mild to moderate staining intensity [2].

These findings were against to what was observed by Ray S et al., where PTEN expression in ECs was 93.7% in Grade-I and II, compared to only 8.6% in Grade-III [11]. Association of PTEN expression with tumour stage was also evaluated and in the study of Allithy A et al., negative and mild expression was observed in stage-I and II while stage-III showed increased intensity of immunostaining, concluding that PTEN expression was positively correlated with advanced tumour stage ($p<0.0001$) [2]. This finding i.e., positive correlation of PTEN expression with tumour stage was supported when PTEN expression was compared with myometrial invasion [12,13]. They detected that 40% of ECs without myometrial invasion were negative for PTEN expression, while 100% of myometrial invasive ECs showed positive PTEN expression [14]. Ray S et al., found 29 cases to be positive for myometrial invasion out of which four cases showed ($<\frac{1}{2}$ myometrial invasion [11]. Thus, these studies discussed above showed that there is downregulation of PTEN expression from hyperplasia with atypical changes to carcinoma. Therefore, PTEN can be used as screening tool in EH to detect potentially cancerous tissues and since, there was a strong correlation between expression of PTEN in EH and EC along with correlation of mutation status with histological differentiation noted that PTEN gene may contribute to characterisation of tumour behaviour in EC.

MATERIALS AND METHODS

This will be a cross-sectional study carried out in the prospective and retrospective design with sample size of 20 for each of the two groups especially from April 2019 to March 2024, involving women aged 35 to 80-year-old whose endometrial biopsy and resected samples will be obtained for histological diagnosis. (IEC Number-IEC/2022/1060). The study will be conducted in Department of Pathology for duration of two years from April 2022 to March 2024.

Inclusion criteria: The cases diagnosed on biopsy or surgical specimen of EC with correlating clinicopathological features.

Exclusion criteria: Those cases with endometrial Biopsy with inflammatory histomorphology, endometrium without representative histomorphology of pre-cancerous and cancerous lesion, those cases with deficient clinical data and details were excluded from the study.

Sample size: The sample size as tentatively calculated is as follows:

Cochran formula for sample size:

$$n=(Z\frac{\alpha}{2})^2\times p\times(1-p)/E^2$$

where,

$Z\frac{\alpha}{2}$=is the level of significance at 5% i.e., 95% confidence interval=1.96

P=Incidence of Endometrial Cancer=4.5%=0.045 [16]

d=Desired error of Margin=10%=0.1 n=1.962×0.045×(1-0.045)

0.102=16.50 n=20 patients in each group for precancerous and cancerous.

Hispathological analysis: The grading and TNM staging and involvement of adenocarcinoma of endometrium will be performed by its degree of differentiation.

Histopathologic Grades (G) are classified as: Gx- Grade cannot be assessed; G1-welldifferentiated; G2- moderately differentiated; G3- poorly or undifferentiated [16].

Federation Internationale de Gynecolgie et d'Obstetrique (FIGO) staging classification for cancer of corpus uteri:

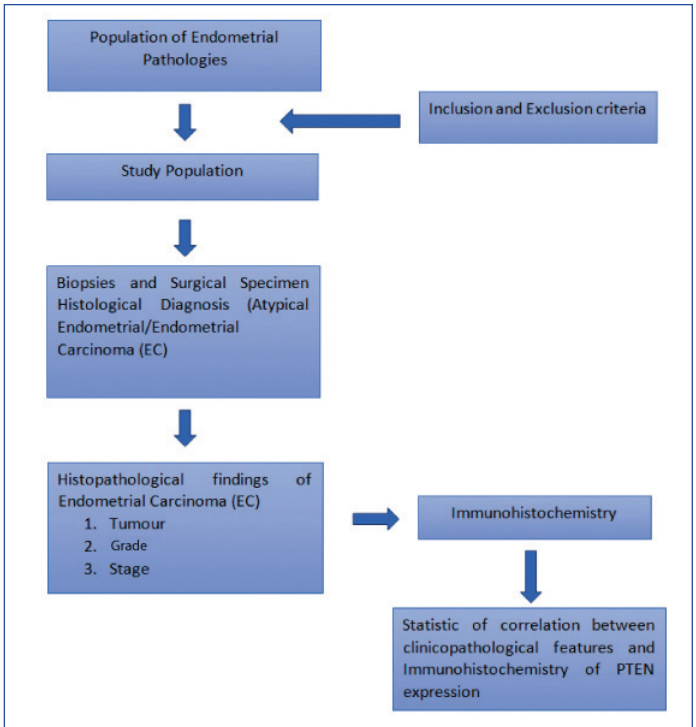
- Ia- Tumour confined to corpus uteri,
- IAa- No or less than half myometrial invasion,
- IBa- Invasion is equal to or more than half of myometrium,
- IIa- Tumour invades to cervical stroma but not beyond uterus,
- IIIa- Local or Regional spread of tumour,
- IIIAa- Tumour Invades serosa of endometrium or adnexa,
- IIIBa- Vaginal involvment and/parametrial involvement,
- IIICa - Metastases to pelvic and/para-aortic lymph nodes,
- - IIIC1a- Positive pelvic nodes,
  - IIIC2a- Positive para aortic nodes,
- IVa- Tumour invades to bladder and/bowel/distant metastasis,
- IVAa- Tumour invasion to bladder and/bowel mucosa,
- IVBa- Distant metastasis [17].

Immunohistochemical staining of PTEN: Deparaffinisation of tissue section will be done. Antigen retrieval with treatment of buffer and microwave processing and then incubated with PTEN primary antibody (i.e., monoclonal PTEN antibody– Dako, clone 6H2.1). The secondary antibody in principle to streptavidin biotin method will be used. Colour developed by peroxidase – DAB (3,3'-diaminobenzidine) chromogen.

Interpretation [5]:

1. Positive (pos) immunohistochemistry for PTEN- diffused cytoplasmic staining (>90%) of tumour cells.
2. Negative (neg) immunohistochemistry for PTEN- only scattered tumour cells and (<1%) have cytoplasmic staining.
3. Heterogenous immunohistochemistry for PTEN- These tumours have distinct positive and negative staining.

Complete study will be conducted as depicted in [Table/Fig-1] step by step.



[Table/Fig-1]: Flowchart of research work methodology.

STATISTICAL ANALYSIS

The statistics of association and correlation between the immunoexpression of PTEN with clinicopathological characters will be carried out by applying Chi-square test with value of significance (Pearson's correlation coefficient, p-value) using Statistical Package for Social Sciences (SPSS) version 27.0.

EXPECTED OUTCOMES/RESULTS

According to the existing literature, the potential outcome of the study would suffice to immunoexpression of PTEN and its relationship

and comparison in groups of AEH and EC type 1. The relationship between PTEN immunopositivity and grade and stage of EC may correlate for its expression. The research protocol is presently at data collection stage.

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