Student's Journal of Health Research Africa
Vol. 4 No. 6 (2023): June 2023 Issue
https://doi.org/10.51168/sjhrafrica.v4i6.485
Original article

EVALUATION OF ALTERED PTEN EXPRESSION BY IHC AS A DIAGNOSTIC MARKER IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM. OBSERVATIONAL DESCRIPTIVE STUDY.

Dr. Bodhisatwa Behera, Dr. Jayanti Nayak, Dr. Saroj Ranjan Mohanty, Dr. Manoj Kumar Patro*, Dr. Sunita Rani Senapati, Dr. Neha Dash

Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Abstract

Introduction:

Endometrial carcinoma is a common cancer affecting women. Different molecular alterations have been described in endometrioid endometrial carcinoma (EC). Among them the most frequently altered is the loss of the PTEN protein, a tumor suppressor gene, leading to microsatellite instability. The purpose of this study was to evaluate the expression pattern of PTEN gene in normal proliferative, hyperplastic, and neoplastic endometrium. Objective: To observe the altered expression of PTEN in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma.

Methods:

This study was an observational descriptive study conducted in the Department of Pathology, M.K.C.G. Medical College, Brahmpur,Odhisha. over a two-year period in which immuno-histochemical evaluation of PTEN expression was done in 146 patients.

Results:

PTEN immunoreactivity was present in all normal proliferative endometrium, all simple hyperplasia, 50% of atypical hyperplasia, and in none of EC ($P < 0.001$). The intensity of PTEN reaction was significantly higher in the group with proliferative endometrium than atypical hyperplastic endometrium and EC ($P < 0.001$).

Conclusion:

PTEN expression was significantly higher in cyclical endometrium than in atypical hyperplasia and endometrioid carcinoma and PTEN immunostaining may be a new and effective tool for screening of malignant and premalignant endometrial lesions.

Keywords: Endometrial carcinoma, PTEN, IHC, Submitted: 2023-06-21 Accepted: 2023-06-24

1. Introduction:

Among the common malignancies of women Endometrial carcinoma is one. There are more than 1,89,000 new cases and 45,000 deaths worldwide each year.[1] The term "endometrial neoplasia" is a spectrum of morphologic alterations that range from endometrial hyperplasia to the different varieties of endometrial carcinoma. Endometrial hyperplasia and carcinoma represent a continuum in the spectrum of endometrial proliferations. Endometrial hyperplasia is defined by the

*Corresponding author.
Email address: manojkpatro@gmail.com (Dr. Manoj Kumar Patro)

World Health Organization (WHO) classification as a spectrum of morphologic alterations ranging from benign changes to premalignant disease, caused by an abnormal hormonal environment.[2]

According to clinical and pathologic characteristics, endometrial carcinomas are categorized into two subtypes. Type I tumors (about 70–80%) are endometrioid carcinomas (ECs), characterized by estrogen dependence, often accompanied by endometrial hyperplasia. On the contrary, type II (10–20%) tumors mainly include serous adenocarcinoma and clear cell carcinomas which are non-endometrioid carcinomas (NECs) with worse biological behavior and poor prognosis. [3,4]

The molecular mechanisms of ECs are mainly PTEN inactivation (50–80%), microsatellite instability (MSI, 20–40%), PIK3CA (30%), KRAS gene mutations, and beta-catenin overexpression.**5, 6** In NECs, the molecular changes include the p53 gene inactivation, p16 gene mutations, and human epidermal growth factor receptor 2 (c-erbB2/Her-2) overexpression.[7,8]

PTEN (the phosphatase and tensin homologue) is a tumor suppressor gene identified in the year 1997. Somatic mutations of this gene were later found to be frequently associated with multiple sporadic tumors and germline mutations in patients with cancer predisposition syndromes such as Cowden disease.[9] PTEN mutation has been associated with response to conventional standard-of-care chemotherapy. In contrast PTEN negative shows shorter survival rates in the post-docetaxel abiraterone treatment settings.[10] Due to frequent PTEN loss in endometrial tumours, the PTEN-PI3K –AKT pathway is a rational target for the treatment of endometrial cancer. Inhibitors to MTOR, PI3K, and AKT, all of which target this pathway, are currently in development.[11]

2. Objective and Aim:

The present study was an observational prospective study conducted to observe the pattern of expression of PTEN in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma.

3. Material and Method:

This study was an observational prospective study conducted in the Department of Pathology, M.K.C.G. Medical College and Hospital, Berhampur, Odhisha, India. over a two-year period (from October 2017 to September 2019) in which immuno-histochemical evaluation of PTEN expression was performed in 146 patients (113 proliferative endometrium, 24 endometrial hyperplasia, and 09 patients having endometrial carcinoma). The study was conducted after obtaining approval from the Institutional Ethical Committee.

All patients having a histological diagnosis of proliferative endometrium, endometrial hyperplasia & endometrial carcinoma from endometrial curettage samples or hysterectomy specimens were included. All cases with evidence of pregnancy-related complications as well as cases with systemic diseases like diabetes mellitus, hypertension, chronic liver & kidney diseases & organic genital tract lesion were excluded.

Immunohistochemistry protocol: Paraffin block sections of 4 μm in thickness were taken, deparaffinized in xylene, and rehydrated through a series of graded alcohols. Antigen retrieval was achieved by heat treatment at 980C in citrate buffer (MERCK, PH = 6.4) for 20 minutes. This was followed by washing with TBS buffer (MERCK, pH = 7.6). Next, the slides were incubated in serum blocking solution to block all the endogenous peroxidase activity. This was followed by incubation with monoclonal mouse Anti-Human PTEN Clone 6H2.1 (Dako North America, Inc. 6392 Via Real Carpinteria, CA 93013 USA), wash with TBS buffer, treatment with super-enhancer, wash with TBS buffer, the addition of HRP, washing with TBS buffer and then freshly prepared DAB Chromogen (DAB 20μl + Substrate buffer 1ml) was added to develop a brown color. Lastly, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. In each case normal endometrium was used both as the positive control and the negative control, in the latter the addition of the primary antibody was omitted.

Brown staining localized to the nuclei or cyto-

plasm of normal endometrial glandular cells [internal control] or tumor cells was considered to be immunoreactive. The scoring system followed is as follows: negative if < 10%, + 1 if 10 – 50%, and +2 if >50% of the slide's area was stained positive. The intensity of PTEN staining was graded from 0 to +2 as follows, 0 = absent, +1 = light brown, and +2 = brown to dark brown staining of the nucleus or cytoplasm of glandular cells.12, 13 Statistical analyses were carried out by Statistical Package for Social Sciences v 18.0 (SPSS Inc., Chicago, IL, USA) software for Windows and a p-value of < 0.05 was considered to be significant.

4. Results:

The age of the patient in the present study ranged from 35 years to 70 years; with 40 – 49 years being the most prevalent age group (73.3%). More prevalence was noted in the postmenopausal age group, with 82.2% of cases. Of the 146 cases studied, 113 cases (77.3%) were diagnosed as proliferative endometrium; 24 cases (16.4%) as endometrial hyperplasia which included 16 cases of hyperplasia without atypia and 8 cases of atypical hyperplasia; and 9 cases (6.2%) as endometrial carcinoma which included 7 cases of endometrioid type carcinoma (EC) while the rest two were non-endometrioid type (NEC) (1 case each of serous carcinoma and clear cell carcinoma).

PTEN immunoreactivity pattern observed is depicted in Table 1 which shows 100% reactivity in cases of proliferative endometrium and endometrial hyperplasia without atypia, 113 / 113 cases and 16 / 16 cases respectively; the same was 50% in cases of endometrial hyperplasia with atypia (8 / 8 cases) and NEC (2 / 2 cases). In cases of EC, the PTEN immunoreactivity was 0% (0 / 7 cases). The difference observed in the PTEN immunoreactivity pattern between proliferative endometrium, endometrial hyperplasia with atypia, and EC was found to be statistically significant p <0.001. The PTEN color intensity of immunostaining and the percentage of area covered was found to be a heterogeneous presentation as shown in Tables 2 & 3. The normal proliferative endometrium showed intense cytoplasm and/ or nucleus staining in the glandular epithelial cells. The lowest PTEN intensity of immunostaining was detected in cases of EC and the difference was statistically significant, p < 0.001). There was no statistically significant difference between the PTEN expression in proliferative endometrium & hyperplasia without atypia and also between atypical hyperplasia & endometrial carcinoma.

5. Discussion:

It has been difficult to determine the malignant potential of the various types of endometrial hyperplasia, due to problems in terminology and lack of follow-up data. Microsatellite instability, mutations of PTEN and K-ras, and nuclear accumulation of β-catenin are the most characteristic molecular alterations associated with these tumors.[2]

PTEN gene inactivation is the most frequently altered gene in endometrial tumors of endometrioid histology which suggest a clonal growth pattern in cases of endometrial hyperplasia with atypia and carcinoma.[13–15] PTEN loss occurred differently in different population groups as well as in different histological types, but no significant difference based on hormone receptor (ER, PR) status, myometrium invasion, lymph node metastases, vessel invasion, grade of ECs, or FIGO staging subgroups. This suggests that patients that have a loss of PTEN protein expression might have a better prognosis.[62]

In the present study, the percentage of PTEN immunoreactivity, intensity of staining, and homogeneity of staining was 100% in cases of proliferative endometrium and endometrial hyperplasia without atypia with relatively more in the former. This observation is in concordance with that of several other studies.[2,13,17,18] Loss of PTEN expression was observed in all cases of endometrioid endometrial carcinoma in this study is similar to the findings in other studies[2,17,19] and also 50% cases of atypical endometrial hyperplasia, which is also in concordance to several studies.[2,17,20] Both the cases of non- endometrioid

carcinoma showed

TABLE 1: PTEN EXPRESSION IN ALL ENDOMETRIAL SAMPLES

Type of endometrium	Positive Number	Negative	Negative Number	%	Total
Proliferative endometrium	113	100%	0	0%	113
Endometrial hyperplasia					
Without atypia	16	100%	0	0%	16
Atypical hyperplasia	04	50%	04	50%	08
Endometrial carcinomas					
Endometrioid type	0	0%	07	100%	07
Non endometrioid type	02	100%	0	0%	02
Total	**135**	**93%**	**11**	**07%**	**146**

TABLE 2: COLOR INTENSITY OF PTEN EXPRESSION

Type of endometrium	Color intensity 0	+1	+2	Total
Proliferative endometrium	0	11	102	**113**
Hyperplasia without atypia	0	04	12	**16**
Hyperplasia with atypia	04	03	01	**08**
Endometrial carcinoma	07	01	01	**09**

TABLE 3: SLIDE AREA STAINING BY PTEN

Type of endometrium	Color intensity 0	+1	+2	Total
Proliferative endometrium	0	11	102	**113**
Hyperplasia without atypia	0	04	12	**16**
Hyperplasia with atypia	04	03	01	**08**
Endometrial carcinoma	07	01	01	**09**

PTEN positivity. Similar findings were observed by Risingerji et al[21] who also didn't find PTEN mutation in all 5 cases of non-endometrioid carcinoma.

PTEN immunoreactivity was heterogeneous in PTEN positive cases of atypical endometrial hyperplasia, in which PTEN- negative hyperplastic glands were scattered among PTEN- positive glands. This intermittent pattern was detected at a variety of gland densities, ranging from the null gland with closely packed architecture to PTEN-positive glands in the low densities of a disordered proliferative endometrium. This suggests that loss of PTEN expression begins in the earliest stages of endometrial tumorigenesis, under conditions of excess estrogen exposure. PTEN inactivation initiates in precancers from a normal background state, and additional PTEN damage accumulates in the transition from premalignant to malignant disease.[13,2]**2**

Regarding hyperplasia and atypia, the results of this study showed lower PTEN activity (25%) than in other studies (55-75%); which may be due to use of polyclonal antibodies in our study. As Pallares et al showed, using monoclonal antibodies was associated with more acceptable results than a polyclonal antibody.**23**

6. Conclusion:

Decreased PTEN expression tended to associate with malignant features of the endometrium with significant statistical differences in PTEN immunoreactivity between groups of normal endometrium, hyperplastic changes & carcinoma. This suggests that loss of PTEN expression is partly associated with endometrial cancers through a premalignant phase. Therefore, altered PTEN expression can be used as a diagnostic marker in hyperplastic and neoplastic endometrium, and that PTEN immunostaining may be a new and effective tool for screening of malignant and premalignant endometrial lesions.

7. Conflict of interest:

None.

8. List of Abbreviations:

EC- Endometrioid endometrial carcinoma
NEC- Non-endometrioid carcinomas
SPSS- Statistical Package for Social Sciences
PTEN- The phosphatase and tensin homologue

9. References:

1. Jemal A, Bray F, Center MM, et al. Global cancer statistics. Cancer J Clin. 2011;61:69–90. [
2. Shanmugapriya M, Sudha M,Geetha P et al. A study of PTEN expression in endometrial hyperplasia and endometrioid type of endometrial carcinoma. Trop J Path Micro 2017;3(1):39-45.
3. Murali R, Soslow RA, Weigelt B. Classification of endometrial carcinoma: More than two types. Lancet Oncol.2014;15:e268–78. [
4. Kurman RJ, Carcangiu ML, Simon HC, Young RH. WHO Classification of tumours of female reproductive organs. Lyon, France: IARC Press; 2014. pp. 122–35.
5. Ioffe YJ, Chiappinelli KB, Mutch DG, et al. Phosphatase and tensin homolog (PTEN) pseudogene expression in endometrial cancer: A conserved regulatory mechanism important in tumorigenesis? Gynecol Oncol.2012;124(2):340–46. [
6. Nout RA, Bosse T, Creutzberg CL, et al.
7. Yu CG, Jiang XY, Li B, et al.
8. Smolle MA, Bullock MD, Ling H, et al. Long non-coding RNAs in endometrial carcinoma. Int J Mol Sci.2015;16:26463–72. [
9. Tan MH, et al. Clin Cancer Res. 2012;18:400–407. [
10. Ferraldeschi R, et al. Eur Urol. 2015;67:795–802. [
11. Kong D, Yamori T. Phosphatidylinositol 3-kinase inhibitors: promising drug candidates for cancer therapy.
12. Tantbirojin P ,Triratanachat S, Trivijitsilp P ,et al. Detection of PTEN immunoreactivity in endometrial hyperplasia and adenocarcinoma. J Med Assoc Thai 2008;91(8):1161-5

13. Sarmadi S, Izadi-Mood N, Sotoudeh K, Tavangar SM. Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. Diagnostic pathology. 2009 Nov 25;4(1):41.
14. Hayes MP, Wang H, Espinal-Witter R, Douglas W, Solomon GJ, Baker SJ, et al.: PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. Clin Cancer Res 2006, 12:5932-5.
15. Athanassiadou P, Athanassiades P, Grapsa D, Gonidi M, Athanassiadou AM, Stamati PN, et al.: The prognostic value of PTEN, p53, and beta-catenin in endometrial carcinoma: a prospective immunocytochemical study. Int J Gynecol Cancer 2007, 17:697-704.
16. Wenting Li, Ying Wang, Xinzhi Fang, Mei Zhou, Yiqun Li, Ying Dong, andRuozheng Wang.Differential Expression and Clinical Significance of DNA Methyl transferase 3B (DNMT3B), Phosphatase and Tensin Homolog (PTEN) and Human MutL Homologs 1 (hMLH1) in Endometrial CarcinomasWenting Li, Ying Wang, Xinzhi Fang, Mei Zhou, Yiqun Li, Ying Dong, andRuozheng Wang.Differential Expression and Clinical Significance of DNA Methyl transferase 3B (DNMT3B), Phosphatase and Tensin Homolog (PTEN) and Human MutL Homologs 1 (hMLH1) in Endometrial Carcinomas
17. Dr. Bela Sharda , Malik R, Jain P. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancerous changes.2017, International Journal of medical research and review.
18. Sitara S, Sheela Varghese, Sankar S et al.PTEN expression patterns in endometrial hyperplasia hyperplasias and endometrioid carcinoma.J. Evolution Med. Dent. Sci. 2019.,8(07):403-406
19. Erkanli S, Kayaselcuk F, Kuscu E, Bagis T, Bolat F, Haberal A, Demirhan B. Expression of survivin, PTEN and p27 in normal, hyperplastic, and carcinomatous endometrium. International Journal of Gynecological Cancer. 2006 May 1;16(3):1412-8.
20. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, Weng LP, Eng C. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. Journal of the National Cancer Institute. 2000 Jun 7;92(11):924-30.
21. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer research. 1997 Nov 1;57(21):4736-8.
22. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, et al.: Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst 2000, 92:924-30.
23. Pallares J, Bussaglia E, Martínez-Guitarte JL, Dolcet X, Llobet D, Rue M, et al.: Immunohistochemical analysis of PTEN in endometrial carcinoma: a tissue microarray study with a comparison of four commercial antibodies in correlation with molecular abnormalities. Mod Pathol 2005, 18:719-27.

10. Publisher details:

Publisher: Student's Journal of Health Research (SJHR)
(ISSN 2709-9997) Online
Category: Non-Governmental & Non-profit Organization
Email: studentsjournal2020@gmail.com
WhatsApp: +256775434261
Location: Wisdom Centre, P.O.BOX. 148, Uganda, East Africa.

Author biography

Dr. Bodhisatwa Behera Associate Professor of Pathology, SLN MCH Koraput, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Dr. Jayanti Nayak Associate Professor, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Dr. Saroj Ranjan Mohanty Assistant Professor, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Dr. Manoj Kumar Patro Associate Professor, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Dr. Sunita Rani Senapati Assistant Professor, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.

Dr. Neha Dash PG Resident, Department of Pathology, MKCG Medical College and Hospital, Brahmapur, Odisha.