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Tumor Suppression in Asymptomatic Postmenopausal Endometrial Polyps

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Abstract. *Background/Aim: To investigate tumor suppression as an indicator of malignization potential within endometrial polyps in asymptomatic postmenopausal women. Materials and Methods: Immunohistochemical studies of the phosphatase and tensin homolog (PTEN) were performed. Cases included 52 benign postmenopausal polyps, 19 endometrioid carcinomas with coexisting benign polyps, and 12 polyps with foci of carcinoma. Controls included 31 atrophic endometria and 32 benign premenopausal polyps. PTEN was scored by quantitative methods according to staining intensity. Results: The mean epithelial and stromal PTEN H-score in postmenopausal benign endometrial polyps (193.8 and 123.2, respectively) was significantly higher than that in the atrophic endometrium (135.5 and 90.2, p=0.008), and premenopausal benign endometrial polyps (100.7 and 198.7, p<0.001). Significant difference between postmenopausal endometrial polyps and endometrial carcinoma was noticed in the epithelial compartment (193.8 vs. 65.7, respectively, p<0.001). Conclusion: Asymptomatic benign postmenopausal polyps have a distinctively high tumor suppression compared with endometrial cancer, suggesting low malignization potential.*

Endometrial polyps (EPs) are localized projections of endometrial tissue found in approximately 30% of women in the western world and are commonly considered benign endometrial lesions (1-4).

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Hysteroscopic polypectomy is the gold standard to treat symptomatic endometrial polyps, to obtain specimens for histological evaluation, and to provide a relatively safe and cost-effective secondary prevention of endometrial cancer (EC) (5, 6). Widespread use of gynaecological ultrasonography has led to improved detection of asymptomatic endometrial polyps (6, 7). The management of asymptomatic postmenopausal women with incidentally identified EPs is still a matter of debate, as the association of these EPs with malignancy is low and accounts for 0.1-1.51%, compared with 4.47% in symptomatic EPs (4, 6, 8-11).

Phosphatase and tensin homolog (PTEN) mutations and deletions are the most frequent genetic alterations seen in endometrioid endometrial cancer with genetic inactivation found in approximately 80% of cases (12, 13). Immunohistochemical (IHC) analysis is a widely used technique capable to detect specific antigens in tissue samples, predict the course of the disease, and help in treatment decisions. Therefore, among the proteins that might be used as biological markers of endometrial cancer, one responsible for tumor suppression (PTEN), is expected to give a better understanding at the molecular level.

Accordingly, considering the high incidence of PTEN alterations in EC and given the lack of data on the potential for malignant transformation in asymptomatic postmenopausal polyps, the present study was conducted to investigate tumor suppression in postmenopausal endometrial polyps as one of the indicators of their malignization potential in asymptomatic women.

Materials and Methods

Design. A retrospective study done in the Lithuanian University of Health Sciences, Kaunas, Lithuania.

Patients and tissue collection. The selected cases were obtained from the Pathology Department at the Hospital of Lithuanian University of Health Sciences and included 52 benign postmenopausal endometrial polyps with no history of bleeding, 19 endometrial carcinomas which had coexisting benign endometrial polyps, and 12 endometrial polyps with foci of endometrial carcinoma. The controls included 31 cases of atrophic endometrium and 32 premenopausal benign endometrial polyps. Women were considered postmenopausal if they reported a period of amenorrhea of at least 12 months duration after the age of 45 years. Endometrial samples were submitted either as endometrial curetting, polypectomy or hysterectomy specimens of the patients diagnosed with endometrial polyps, endometrial carcinoma or endometrial atrophy. Two independent expert pathologists reviewed haematoxylin-eosin sections in order to confirm the histologic diagnosis. In the endometrial carcinoma cases, stage was determined by The International Federation of Gynaecology and Obstetrics (FIGO) surgical staging, 2009 (14).

Ethical approval. Kaunas Regional Biomedical Research Ethics Committee, Lithuanian University of Health Sciences, Lithuania, provided a consent waiver and approved the study (BE-2-14, 17 February 2016). All samples were coded using unique identifiers.

Immunohistochemistry. Paraffin-embedded blocks were obtained and 4 μm sections cut and placed on slides. Immunohistochemistry was performed on sections using the avidin-biotin-peroxidase complex method for the identification of the antigen binding sites. Monoclonal mouse anti-human PTEN antibody (Anti-PTEN clone 6H2.1; Dako Corp., Glostrup, Denmark) was used for the identification of the PTEN protein. Sections were heated at 62˚C for 1 h, de-waxed in xylene and rehydrated in graded alcohol, and boiled in buffer (pH 9.0) for 3 min at 120˚C. After blocking of endogenous peroxidase, they were incubated with the pre-diluted primary antibody Anti-PTEN clone 6H2.1 (Dako Corp., Glostrup, Denmark) using 1:100 dilution. After 30 min incubation, the slides were treated with anti-mouse IgG as the secondary antibody. Reactions were visualized using an avidin-biotin-peroxidase complex with 3,3' diaminobenzidine (DAB, Sigma Chemical Co., St. Louis, MO, USA) as a chromogen. Counterstaining was performed with Mayer's haematoxylin. Finally, the sections were dehydrated and mounted. For each batch of slides, when staining with the primary antibody, negative and positive controls were used according to the manufacturer's recommendation.

Scoring of immunohistochemical staining. Image capture and analysis was performed using an Olympus BX 53F (Tokyo, Japan) light microscope and the digital camera QImaging EXi AQUA (Surrey, CO, Canada). Digital image analysis was performed using the image analysis software Image-Pro Plus (version 7.0). The overall stain intensity and the proportion of positive and negative glandular and stromal cells in the captured fields were counted under 40× magnification, and the semi-quantitative histo-score (H-score) was obtained per field. H-score was obtained by applying the following formula: H-score=1×(% light staining)+2×(% moderate staining)+3×(% strong staining).

Statistical analysis. Calculations were carried out using Statistical Package of Social Science, Windows version 23 (Statistical Package for the Social Sciences (SPSS), IBM, Brøndby, Denmark). As data were distributed normally, pairwise comparisons were performed by the Student's *t*-test. The numerical data are presented with mean and standard deviation and the results were considered significant when *p*-value was less than 0.05.

Results

PTEN immunoreactivity was heterogeneous (Figure 1). Some cells within a gland or some glands were negative for PTEN staining (PTEN null glands). The least proportion of PTEN null glands cases was noted among benign postmenopausal EP patients (4%), and this proportion was significantly lower than that in endometrial atrophy (19%, $p<0.05$), premenopausal polyps (28%, $p<0.05$) and EC (39%, $p<0.05$) (Figure 2).

The lowest epithelial PTEN immunoreactivity (mean H-score 65.7) was detected in EC and the differences were significant compared with postmenopausal ($p<0.001$) and premenopausal ($p=0.006$) EPs and atrophic endometria ($p<0.001$) (Figure 2, Table I). The mean epithelial and stromal PTEN H-score in postmenopausal benign EPs (193.8 and 123.2, respectively) was significantly higher than in atrophic endometrium (135.5 and 90.2, $p=0.008$), and premenopausal benign EPs (100.7 and 198.7, $p<0.001$), however, a significant difference between postmenopausal EPs and EC was noticed only in the epithelial compartment (193.8 *versus* 65.7, respectively, $p<0.001$) with no difference between stromal components (Table I).

When benign EPs were found in association with EC, epithelial PTEN expression was significantly lower in the cancer tissue, than in the polyp, at 76.8 *versus* 138.9; $p=0.005$ (Figure 3).

The group of EPs with foci of EC revealed a statistically significantly higher PTEN level in the polyp tissue itself compared with the EC focus, with overall H-scores of PTEN in epithelial and stromal components at 107.4 and 118.4 *versus* 48.1 and 68.8, respectively, $p<0.05$ (Table I, Figure 3).

Interestingly, the tissue of malignant polyps (including polyp tissue of cases where polyp was found with coexisting EC or had EC focus inside) in the epithelial compartment showed high tumor suppression (126.7), which had insignificant difference from the atrophic endometrium (135.5, $p=0.543$) or premenopausal polyps (100.7, $p=0.089$), and was significantly lower than that in benign postmenopausal polyps (193.8, $p<0.0001$) and higher compared with EC (65.7, $p<0.0001$).

Discussion

Asymptomatic postmenopausal endometrial polyps are a frequently reported incidental finding. Data concerning the malignization potential of asymptomatic postmenopausal EPs, however, remain unclear and sparse. This study was conducted to pursue evidence whether asymptomatic postmenopausal EPs show any early and the most common

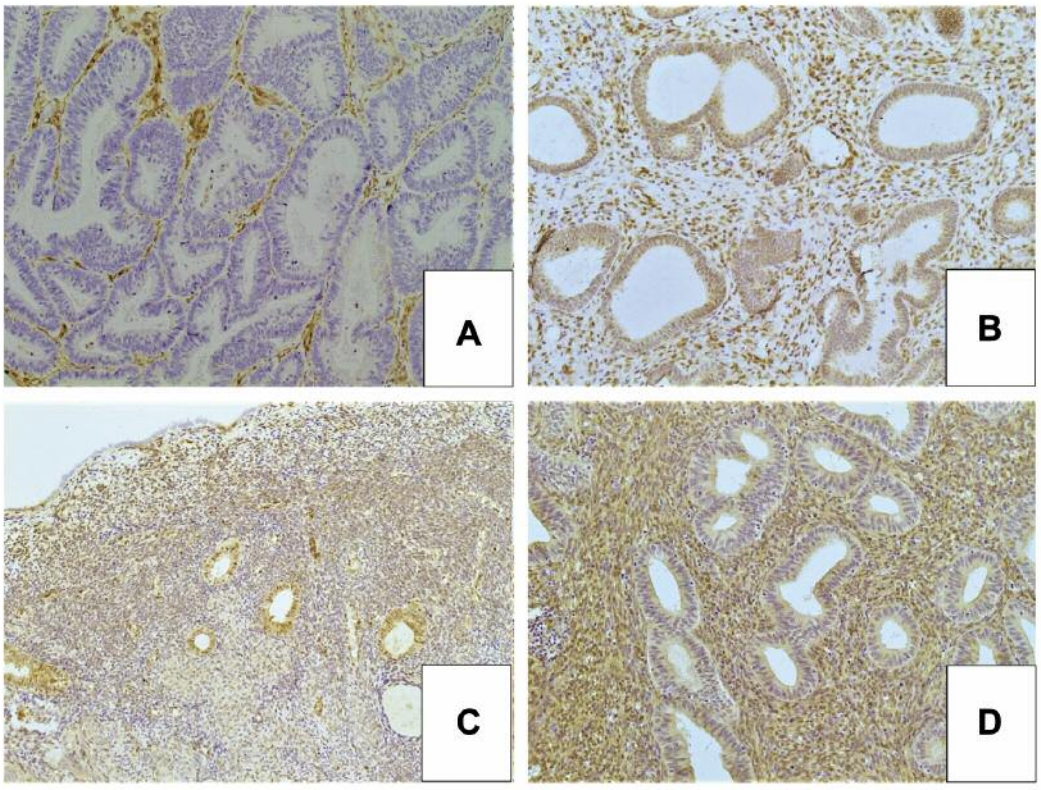

Figure 1. *PTEN over the spectrum of endometrium lesions. A: A case of endometrial cancer showing PTEN-null glands and PTEN positive stroma. B: A case of postmenopausal benign polyp showing PTEN positive glands and stroma. C: A case of atrophic endometrium showing PTEN positive glands and heterogeneous stroma. D: A case of premenopausal benign polyp showing PTEN positive glands and stroma. Original magnification ×10 (A, B, C, D).*

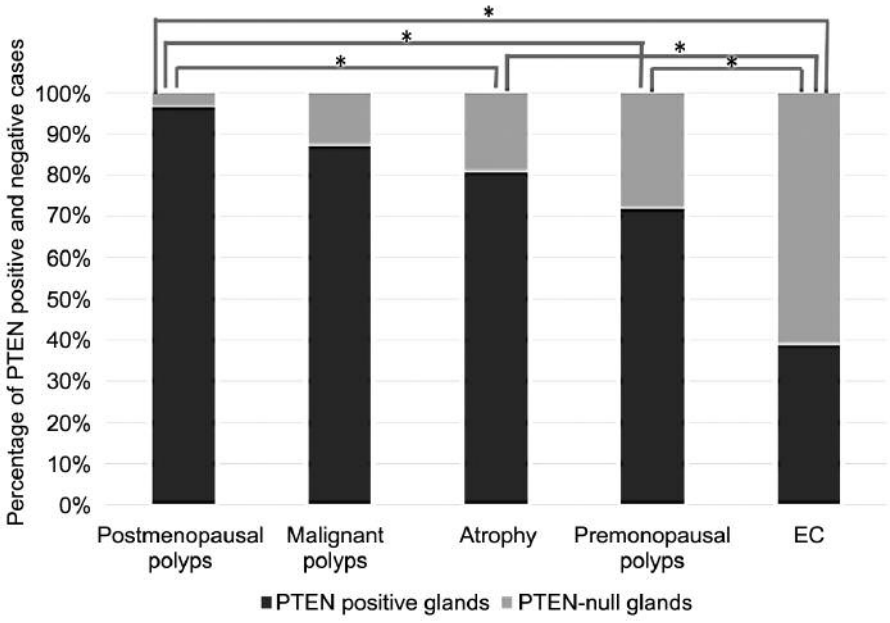

Figure 2. *Differential expression of PTEN over the spectrum of endometrium lesions. *p<0.05.*

Table I. *Mean values of H score for PTEN in different endometrial lesions.*

Histology type	n	PTEN			
		Mean (±SD) H-score		*p*-Value* in epithelium	*p*-Value* in stroma
		Epithelial	Stromal		
Postmenopausal benign polyps	52	193.8 (±62.2)	123.2 (±55.7)	-	-
Premenopausal benign polyps	32	100.7 (±54.5)	198.7 (±42.3)	**<0.001**	**<0.001**
Endometrial cancer:	35	60.2 (±62.3)	95.2 (±71.3)	**<0.001**	**0.017**
EC with coexisting benign polyps:	19				
Polyps		138.9 (±63.8)	129.1 (±57.9)	**0.001**	0.697
EC		76.8 (±63.3)	115.9 (±80)	**<0.001**	0.487
Polyps with foci of EC	12				
Polyp tissue		107.4 (±63.2)	118.4 (±59.5)	**<0.001**	0.791
EC focus		48.1 (±63.2)	68.8 (±56.1)	**<0.001**	0.003
Atrophic endometrium	31	135.5 (±48.4)	90.2 (±49.4)	**<0.001**	**0.008**

*Compared with asymptomatic postmenopausal endometrial polyps (Student's *t*-test). EC: Endometrial carcinoma; SD: standard deviation; H-score: histopathological score. Bold values denote statistical significance at the $p<0.05$ level.

carcinogenetic alteration characteristic to endometrial cancer precursors. To the best of the authors' knowledge, in this article, we report the largest series of IHC of asymptomatic benign postmenopausal EPs and the first IHC investigation of malignant EPs.

PTEN is the second most frequently mutated tumor suppressor gene in human cancers (15, 16). Loss of PTEN expression is considered an early event in endometrial pathogenesis and the most common genetic defect in endometrioid carcinoma, seen in up to 80-83% of tumors that are preceded by a histologically discrete premalignant phase (17, 18). In 2016, a multidisciplinary panel of 40 leading experts of European Society for Medical Oncology (ESMO), European SocieTy for Radiotherapy & Oncology (ESTRO) and European Society of Gynaecological Oncology (ESGO) developed a consensus in the management of endometrial cancer and recommended immunohistochemical assessment of PTEN to distinguish premalignant endometrial changes from benign mimics (19). As the new era of personalized medicine is on the horizon, postmenopausal patients with asymptomatic EPs have a great perspective to benefit from a less aggressive treatment and, thus, reduction in morbidity and complications related with invasive procedures could be achieved.

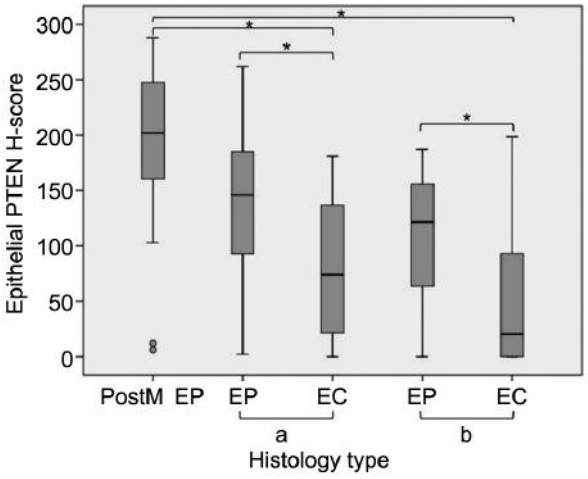

Figure 3. *Box and whisker plot showing epithelial expression of PTEN over the spectrum of endometrial lesions in selected cases. *p<0.05. PostM: Postmenopausal polyps; EP: endometrial polyp; EC: endometrial carcinoma. a: Cases of endometrial carcinoma with coexisting benign endometrial polyps; b: Cases of endometrial polyps with foci of endometrial carcinoma.*

Patterns of tumor suppression in postmenopausal endometrial polyps. In the present study, we found that PTEN expression was significantly reduced in endometrioid endometrial cancer compared to benign endometrial lesions. This is consistent with the findings from previous studies (20-27).

A surprising finding was that malignant polyps (including polyps from cases where a polyp was found with coexisting endometrioid cancer or had a focus of cancer inside) showed high tumor suppression in the epithelial compartment of the polyp tissue, which was not significantly different from that in atrophic endometrium or premenopausal polyps but was significantly lower than that in benign postmenopausal polyps and higher compared with endometrioid cancer. These results demonstrate a different expression of tumor suppressors in polyp tissue compared with endometrioid EC regardless of the proximity of the EP to the surrounding malignant endometrium.

Additionally, our previous study revealed a very low proliferative activity in postmenopausal EPs, nevertheless they were benign, asymptomatic or a polyp tissue surrounding an EC focus (28).

Accordingly, we have two types of speculations - either our findings do not provide evidence that EPs have any genetic changes associated with early carcinogenesis, or EP is not a precursor lesion to EC, but has the same causes and risk factors as EC (epidemiological association).

Conclusion

Asymptomatic benign postmenopausal polyps have a distinctive profile, indicating high levels of tumor suppression compared with endometrial cancer. Our study findings support the idea that resection of incidentally found endometrial polyps in asymptomatic postmenopausal women should not be recommended routinely. Further studies are needed to determine whether endometrial polyps are true premalignant precursors or whether they are benign endometrial disease with the potential for endometrial cancer as any other locus of benign endometrium. The establishment of a wider genetic panel and new biomarkers could shed more light upon this disease and clarify the detailed carcinogenetic mechanism and the potential for it.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Conceptualization: L.A.; Data curation: A.C., J.P.; Formal analysis: L.A.; Investigation: L.A., J.P., M.N.A.; Methodology: L.A., R.N., A.C., C.H, M.W.S., M.N.A, J.P.; Supervision: M.W.S., R.N.; Writing original draft: L.A.; Writing, review, editing: J.P., M.W.S., C.H.

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References

1 Dreisler E and Rahimi S: The oncogenic potential of endometrial polyps: a systematic review and meta-analysis. Obstet Gynecol *118(2 Pt 1)*: 361; author reply 361-362, 2011. PMID: 21775864. DOI: 10.1097/AOG.0b013e3182273b6e

2 Anastasiadis PG, Koutlaki NG, Skaphida PG, Galazios GC, Tsikouras PN and Liberis VA: Endometrial polyps: prevalence, detection, and malignant potential in women with abnormal uterine bleeding. Eur J Gynaecol Oncol *21(2)*: 180-183, 2000. PMID: 10843481.

3 Savelli L, De Iaco P, Santini D, Rosati F, Ghi T and Pignotti E: Histopathologic features and risk factors for benignity, hyperplasia, and cancer in endometrial polyps. Am J Obstet Gynecol *188*: 927-931, 2003. PMID: 12712087. DOI: 10.1067/mob.2003.247

4 Costa-Paiva L, Godoy CE Jr., Antunes A Jr., Caseiro JD, Arthuso M and Pinto-Neto AM: Risk of malignancy in endometrial polyps in premenopausal and postmenopausal women according to clinicopathologic characteristics. Menopause *18(12)*: 1278-1282, 2011. PMID: 21926931. DOI: 10.1097/gme.0b013e31821e23a1

5 Lev-Sagie A, Hamani Y, Imbar T, Hurwitz A and Lavy Y: The significance of intrauterine lesions detected by ultrasound in asymptomatic postmenopausal patients. BJOG *112(3)*: 379-381, 2005. PMID: 15713160. DOI: 10.1111/j.1471-0528.2004.00444.x

6 Ferrazzi E, Zupi E, Leone FP, Savelli L, Omodei U and Moscarini M: How often are endometrial polyps malignant in asymptomatic postmenopausal women? A multicenter study. Am J Obstet Gynecol *200*: 235.e1-6, 2009. PMID:19027096. DOI: 10.1016/j.ajog.2008.09.876

7 Bel S, Billard C, Godet J, Viviani V, Akladios C, Host A, Faller E, Boisrame T, Hummel M, Baldauf JJ, Lecointre L and Garbin O: Risk of malignancy on suspicion of polyps in menopausal women. Eur J Obstet Gynecol Reprod Biol *216*: 138-142, 2017. PMID: 28763739. DOI: 10.1016/j.ejogrb.2017.07.013

8 Lee SC, Kaunitz AM, Sanchez-Ramos L and Rhatigan RM: The oncogenic potential of endometrial polyps: a systematic review and meta-analysis. Obstet Gynecol *116(5)*: 1197-1205, 2010. PMID: 20966706. DOI: 10.1097/AOG.0b013e3181f74864

9 Golan A, Cohen-Sahar B, Keidar R, Condrea A, Ginath S and Sagiv R: Endometrial polyps: symptomatology, menopausal status and malignancy. Gynecol Obstet Invest *70(2)*: 107-112, 2010. PMID: 20332644. DOI: 10.1159/000298767

10 Wethington SL, Herzog TJ, Burke WM, Sun X, Lerner JP, Lewin SN and Wright JD: Risk and predictors of malignancy in women with endometrial polyps. Ann Surg Oncol *18(13)*: 3819-3823, 2011. PMID: 21701931. DOI: 10.1245/s10434-011-1815-z

11 Uglietti A, Mazzei C, Deminico N, Somigliana E, Vercellini P and Fedele L: Endometrial polyps detected at ultrasound and rate of malignancy. Arch Gynecol Obstet *289(4)*: 839-843, 2014. PMID: 24091484. DOI: 10.1007/s00404-013-3037-z

12 Kanamori Y, Kigawa J, Itamochi H, Shimada M, Takahashi M, Kamazawa S, Sato S, Akeshima R and Terakawa N: Correlation between loss of PTEN expression and Akt phosphorylation in endometrial carcinoma. Clin Cancer Res *7(4)*: 892-895, 2001. PMID: 11309338.

13 Latta E and Chapman WB: PTEN mutations and evolving concepts in endometrial neoplasia. Curr Opin Obstet Gynecol *14(1)*: 59-65, 2002. PMID: 11801878. DOI: 10.1097/00001703-200202000-00010

14 Pecorelli S: Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet *105(2)*: 103-104, 2009. PMID: 19367689. DOI: 10.1016/j.ijgo.2009.02.012

15 Gil A, Rodriguez-Escudero I, Stumpf M, Molina M, Cid VJ and Pulido R: A functional dissection of PTEN N-terminus: implications in PTEN subcellular targeting and tumor suppressor activity. PLoS One *10(4)*: e0119287, 2015. PMID: 25875300. DOI: 10.1371/journal.pone.0119287

16 Nero C, Ciccarone F, Pietragalla A and Scambia G: PTEN and gynecological cancers. Cancers (Basel) *11(10)*: 10.3390/cancers

11101458, 2019. PMID: 31569439. DOI: 10.3390/cancers 11101458

17 Troncon JK, Meola J, Candido-Dos-Reis FJ, Poli-Neto OB, Nogueira AA and Rosa-E-Silva JC: Analysis of differential genetic expression in endometrial polyps of postmenopausal women. Climacteric *20(5)*: 462-466, 2017. PMID: 28622040. DOI: 10.1080/13697137.2017.1335701

18 Hecht JL and Mutter GL: Molecular and pathologic aspects of endometrial carcinogenesis. J Clin Oncol *24(29)*: 4783-4791, 2006. PMID: 17028294. DOI: 10.1200/JCO.2006.06.7173

19 Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, Marth C, Nout R, Querleu D, Mirza MR, Sessa C and ESMO-ESGO-ESTRO Endometrial Consensus Conference Working Group: ESMO-ESGO-ESTRO Consensus Conference on endometrial cancer: Diagnosis, treatment and follow-up. Int J Gynecol Cancer *26(1)*: 2-30, 2016. PMID: 26634381. DOI: 10.1093/annonc/mdv484

20 Abd El-Maqsoud NM and El-Gelany S: Differential expression patterns of PTEN in cyclic, hyperplastic and malignant endometrium: Its relation with ER, PR and clinicopathological parameters. J Egypt Natl Canc Inst *21(4)*: 323-331, 2009. PMID: 21415869.

21 Lee H, Choi HJ, Kang CS, Lee HJ, Lee WS and Park CS: Expression of miRNAs and PTEN in endometrial specimens ranging from histologically normal to hyperplasia and endometrial adenocarcinoma. Mod Pathol *25(11)*: 1508-1515, 2012. PMID: 22766795. DOI: 10.1038/modpathol.2012.111

22 Yang HP, Meeker A, Guido R, Gunter MJ, Huang GS, Luhn P, d'Ambrosio L, Wentzensen N and Sherman ME: PTEN expression in benign human endometrial tissue and cancer in relation to endometrial cancer risk factors. Cancer Causes Control *26(12)*: 1729-1736, 2015. PMID: 26376893. DOI: 10.1007/s10552-015-0666-5

23 Shawana S, Kehar SI, Masood S and Aamir I: Immuno-expression of cyclin D1 and PTEN in various endometrial pathologies. J Coll Physicians Surg Pak *26(4)*: 277-282, 2016. PMID: 27097697.

24 Feng ZZ, Chen JW, Yang ZR, Lu GZ and Cai ZG: Expression of PTTG1 and PTEN in endometrial carcinoma: correlation with tumorigenesis and progression. Med Oncol *29(1)*: 304-310, 2012. PMID: 21181309. DOI: 10.1007/s12032-010-9775-x

25 Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N and Ciris M: Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathol Res Pract *203(3)*: 153-162, 2007. PMID: 17317031. DOI: 10.1016/j.prp.2007.01.003

26 Sarmadi S, Izadi-Mood N, Sotoudeh K and Tavangar SM: Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. Diagn Pathol *4*: 41, 2009. PMID: 19930726. DOI: 10.1186/1746-1596-4-41

27 Skrzypczak M, Lattrich C, Haring J, Schuler S, Ortmann O and Treeck O: Expression of SCUBE2 gene declines in high grade endometrial cancer and associates with expression of steroid hormone receptors and tumor suppressor PTEN. Gynecol Endocrinol *29(12)*: 1031-1035, 2013. PMID: 24053619. DOI: 10.3109/09513590.2013.829441

28 Adomaitiene L, Nadisauskiene R, Nickkho-Amiry M, Cizauskas A, Palubinskiene J, Holland C and Seif MW: Proliferation in postmenopausal endometrial polyps-a potential for malignant transformation. Medicina (Kaunas) 55(9), 2019. PMID: 31466367. DOI: 10.3390/medicina55090543

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