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Immunohistochemical Assessment of PTEN Expression: A Possible Tool to Screen Premalignant Endometrial Lesions

Umer Farooq[1], Wajiha Khalid[2], Saba Shamim[1*]

[1]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Defence Road Campus, Lahore, Pakistan*

[2]*Department of Pathology, Postgraduate Medical Institute, Lahore, Pakistan*

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Abstract

This study was carried out to observe the PTEN Expression in normal, hyperplastic and neoplastic endometrial tissues using Immunohistochemistry (IHC). In the present study, PTEN expression was assessed in 75 endometrial biopsies by using immunohistochemistry (IHC). Histopathologically, there were 15 cases of normal endometrium, 24 cases of simple hyperplasia, 22 cases of complex hyperplasia and 14 cases of endometrial carcinoma. PTEN proportionate score (PS) was +2 in all cases of the normal endometrium, +2 in 36, +1 in 6 and 0 in 4 cases of endometrial hyperplasia, +2 in 4, +1 in 4 and 0 in 4 cases of complex hyperplasia without atypia and +2 in 2, +1 in 3 and 0 in 9 cases of endometrial carcinoma. SPSS 21 was used for analyzing the data. A highly significant difference of PTEN PS ($p < .001$) was among these groups of endometrial lesions. It is concluded that there is a close association between loss of PTEN expression and malignant transformation. Therefore, PTEN expression assessed by immunohistochemistry (IHC) can be used as a possible tool to screen premalignant endometrial lesions.

* **Corresponding Author:** Saba Shamim ✉ sabashamimgenetics@gmail.com

Introduction

Endometrial carcinoma (EC) is the most common malignancy of female reproductive system in Europe and North America. It is fourth among all the malignancies in females, after breast, lung and large intestinal cancer (Samulak *et al.*, 2013). It has two main types. Type I endometrial carcinoma is estrogen dependent and type II endometrial carcinoma is estrogen independent (Cristofano and Ellenson, 2007). Type I endometrial carcinoma is the most frequent type (75-85%) among all endometrial carcinomas (Garg and Soslow, 2014). Endometrial hyperplasia is regarded as a precursor lesion of endometrial adenocarcinoma (Kurman and Mazur, 1994). It is a proliferation of endometrial glands with irregular shape and size, having highest proportion of glands to stroma as a result of excessive exposure to estrogen (Ayala *et al.*, 2010). Grading of hyperplastic lesions of endometrium is done on the basis of histological classification into simple hyperplasia, complex hyperplasia without atypia and complex hyperplasia with atypia (Kurman and Mazur, 1994). The risk for development of endometrial cancer rises from 1% in simple hyperplasia to up to 45% in complex hyperplasia with atypia (Keichle *et al.*, 2000). Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene located on chromosome 10 and is regarded as the most mutated one among tumor suppressor genes in human malignancies (Romano and Schepis, 2012). Mutations in PTEN tumour suppressor gene are common in diverse carcinomas (Yevgeniya *et al.*, 2012). It was found that 24 out of 70 endometrial cancers had PTEN mutations (Risinger *et al.*, 1998). Somatic PTEN mutations are reported in 34–55% of endometrial cancers mainly in the endometrioid histotype (Djordjevic *et al.*, 2012). It is also mutated in 50% of premalignant lesions, atypical endometrial hyperplasia (Mutter *et al.*, 2000). Unfortunately, cytological atypia which is a main criterion to diagnose a premalignant lesion (endometrial hyperplasia with atypia) has poor reproducibility (Mutter, 2002). Consequently, these problems may be solved by new insights into the morphology of endometrial hyperplasia with atypia (Mutter *et al.*, 2000). An attempt has been made to observe the PTEN expression in normal, hyperplastic and neoplastic endometrial tissue in this study by using Immunohistochemistry (IHC) technique.

Materials and methods

Present study was carried out at Post Graduate Medical institute (PGMI), Lahore. Seventy five (75) cases of endometrial biopsies from Lahore General Hospital and Lady Willingdon Hospital Lahore, diagnosed histologically as Normal Endometrium (NE), Endometrial Hyperplasia (EH) and Endometrial Carcinoma (EC), were included. SPSS 21 was used for analysing the data.

Methodology

The biopsies were fixed in neutral buffered formalin. Gross examination was done as per protocol (Rosai and Ackerman, 2011). Tissue processing was done in automated tissue processor and 3-4 µm thick sections slides were made (Spenceer and Bancroft, 2008). The sections were stained with Hematoxylin and Eosin (H&E) as per standard protocol (Gamble, 2008).

Immunohistochemical staining for PTEN

The 4 micrometer thick sections were taken on albumenized slides, deparaffinized with xylene, rehydrated with graded alcohol, treated with hydrogen peroxide (3%) for 10 minutes and then washed in distilled water. The antigen was retrieved with heat treatment in citrate buffer (pH 6) for 15 minutes, cooled for 20 minutes, washed in distilled water, and then treated with phosphate-buffered saline (PBS) for 5 minutes. Incubation with anti-PTEN monoclonal antibody (Biocare Medical, Concored, CA, USA) (1:200 dilution) was done at 37 °C for 35 minutes. The detection of immunoreactivity was done with the use of Dako Envision kit. The reaction was visualized by incubation with liquid DAB. After counterstaining with hematoxylin, sections were dehydrated and cleared with xylene. Finally, mounting of sections was done with Eukitt (Pallares *et al.*, 2005; Sarmadi *et al.*, 2009). The stromal cells serve as a positive internal control (Djordjevic *et al.*, 2012). The negative control was achieved by omitting primary antibody.

Microscopic examination

Microscopic examination of slides stained with Hematoxylin and eosin (H&E) was done under the light microscope for histological diagnosis of normal, hyperplastic and neoplastic endometrial lesions. The cytoplasm was stained pink by eosin stain and nucleus was stained purplish blue by hematoxylin stain. Immunohistochemically stained slides for PTEN were examined for immunoreactivity. It was considered as positive immunoreactivity when brown staining was found in endometrial cells' nuclei or cytoplasm (Sarmadi *et al.*, 2009).

Proportionate Scoring (PS)

It was done as percentage of slide area stained +ve for PTEN (Sarmadi *et al.*, 2009) as follows:

0 (negative) < 10%

+1 = 10%-50%

+2 > 50%

Results

On the basis of histology, endometrial lesions were diagnosed as 15 cases of normal endometrium, 24 cases of simple hyperplasia, 10 cases of complex hyperplasia without atypia, 12 cases of complex hyperplasia with atypia, 5 cases of Type I (endometrioid) EC and 9 cases of Type II (non-endometrioid) EC (Figure 1). The histological diagnosis of normal, hyperplastic and neoplastic endometrial lesions is shown in Figure 2 (a-d) and Figure 3 (a-d). PTEN score was calculated in every histological group and was statistically analysed as PTEN Proportionate Score (PS). PTEN PS was found +2 in all 15 (100%) cases of the normal endometrium. PTEN PS was +2 in 36 (78.3%) cases, +1 in 6 (13%) cases and 0 in 4 (8.7%) cases of endometrial hyperplasia and +2 in 2 (14.3%) cases, +1 in 3 (21.4%) cases and 0 in 9 (64.3%) cases of endometrial carcinoma. PTEN PS was +2 in 22 (91.7%) cases while it was +1 in 2 (8.3%) cases of simple hyperplasia. PTEN PS was +2 in all 10 (100%) cases of Complex hyperplasia without atypia. PTEN PS was +2 in 4 (33.3%) cases and +1 in 4 (33.3%) cases while 0 in remaining 4(33.3%) cases of complex hyperplasia with atypia. PTEN PS was +2 in 1 (20%) case and 0 in 4 (80%) cases of Type I endometrial carcinoma. PTEN PS was +2 in 1 (11.1%) case and +1 in 3 (33.3%) cases and 0 in 5 (55.5%) cases of Type II endometrial carcinoma. A highly significant difference of PTEN PS ($p < .001$) was among group of lesions (Table 1).

Table 1. Comparison of PTEN expression based on Proportionate Score (PS) in endometrial lesions. Key for Proportionate Score 0 < 10% of slide area +1= 10-50% of slide are +2 = 51-100% of slide area.

Sr. No.	H&E Diagnosis	PTEN Proportionate Score			Total
		0	+1	+2	
1	Normal Endometrium	0(0%)	0(0%)	15(100%)	15
2	Endometrial Hyperplasia	4(8.7%)	6(13%)	36(78.3%)	46
	a- Simple Hyperplasia	0(0%)	2(8.3%)	22(91.7%)	24
	b- Complex Hyperplasia without Atypia	0(0%)	0(0%)	10(100%)	10
	c- Complex Hyperplasia with Atypia	4(33.3%)	4(33.3%)	4(33.3%)	12
3	Endometrial Carcinoma	9(64.3%)	3(21.4%)	2(14.3%)	14
	a-Type I Endometrial Carcinoma	4(80%)	0(0%)	1(20%)	5
	b- Type II Endometrial Carcinoma	5(55.6%)	3(33.3%)	1(11.1%)	9
	Total	13(17.3%)	9(12%)	53(70.7%)	75

$p < .001$.

Discussion

Endometrial cancer is one of the most frequent gynecolological cancers in the developed countries (Salvesena *et al.*, 2009). Endometrial hyperplasia is considered as a precursor lesion of adenocarcinoma of the endometrium (Kurman and Mazur, 1994). On the basis of PTEN mutations in endometrial hyperplasia, it is suggested that inactivation of PTEN

may be the early step in endometrial carcinogenesis and is indulged in developing atypical features in endometrial hyperplasia (Sun *et al.*, 2002).

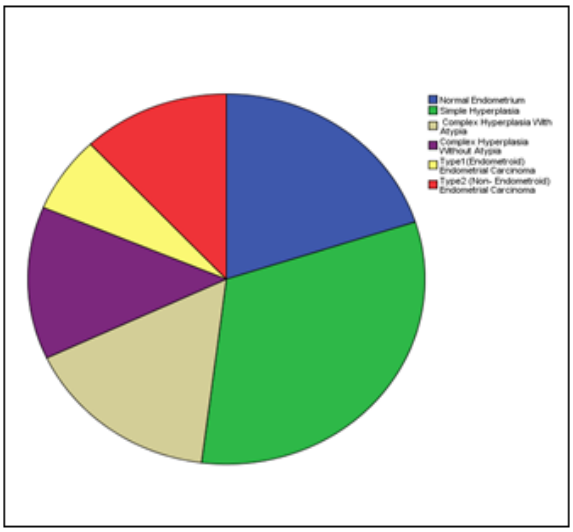

Fig. 1. Frequency of histological diagnosis of endometrial lesions.

It is observed in present study that PTEN expression is highest in normal endometrium, simple hypeplasia (with few cases with diminished expression) and complex hypeplasia without atypia. But its expression is lost in many cases of complex hyperplasia with atypia and majority of cases of endometrial carcinoma specially Type I endometrial carcinoma. The present study was compared with Shawana *et al.* (2014) who conducted a retrospective study that showed results similar to our results. The percentage of PTEN loss (heterogenous and negative) was higher in endometrial carcinoma as compared to simple hyperplasia and also found higher in complex hyperplasia with atypia as compared to simple hyperplasia. A study conducted by Lee *et al.* (2012) supported this study. Djordjevic *et al.* (2012) described that 57 cases (57%) of Type I endometrial carcinoma and 14 cases (26%) of Type II endometrial carcinoma had negative PTEN expression.

The discrepancy can be explained on the basis of geographical distribution because the study done by Djordjevic included population of white race. So there is strong possibility of difference in gene sequencing which needs to be studied in future.

Other reason could be the difference of sample size because the sample size taken by them was greater than the current study. In 2008, Tantbirojn *et al.* carried out a study for investigating the PTEN expression in endometrial lesions.

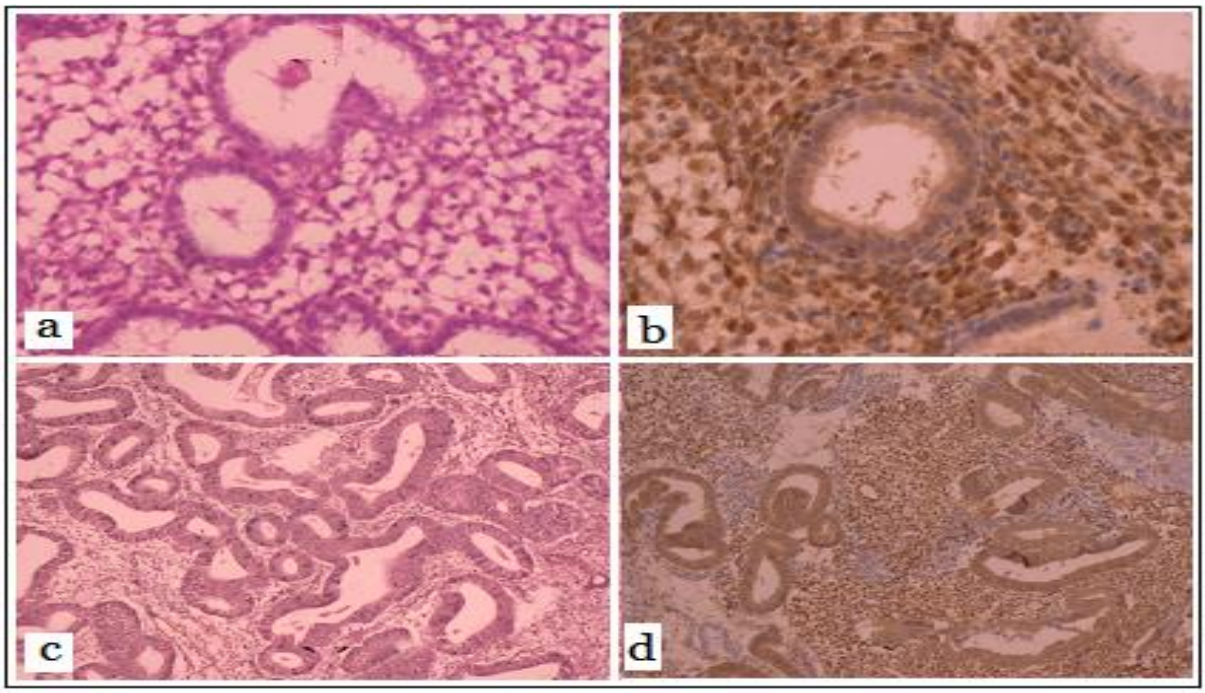

Fig. 2. (a) Normal endometrium (H&E; 40x); (b) PTEN IHC showing strong reactivity in normal endometrium; (c) Complex hyperplasia without atypia (H&E; 10x) and (d) PTEN immunohistochemistry showing strong reactivity in complex hyperplasia without atypia (PTEN stained;10x).

The PTEN expression was similar to the findings of the present study in endometrial carcinoma cases but in contrast in case of complex hyperplasia with atypia and complex hyperplasia without atypia. Greater loss of PTEN expression in atypical endometrial hyperplasia and endometrial hyperplasia without atypia in the above study maybe due to the highest estrogen exposure at the time when endometrial biopsies were taken. Because protracted estrogen stimulation causes loss of PTEN (by mutation) in endometrial glands that may initiate the development of precancerous lesion (Mutter *et al.*, 2000).

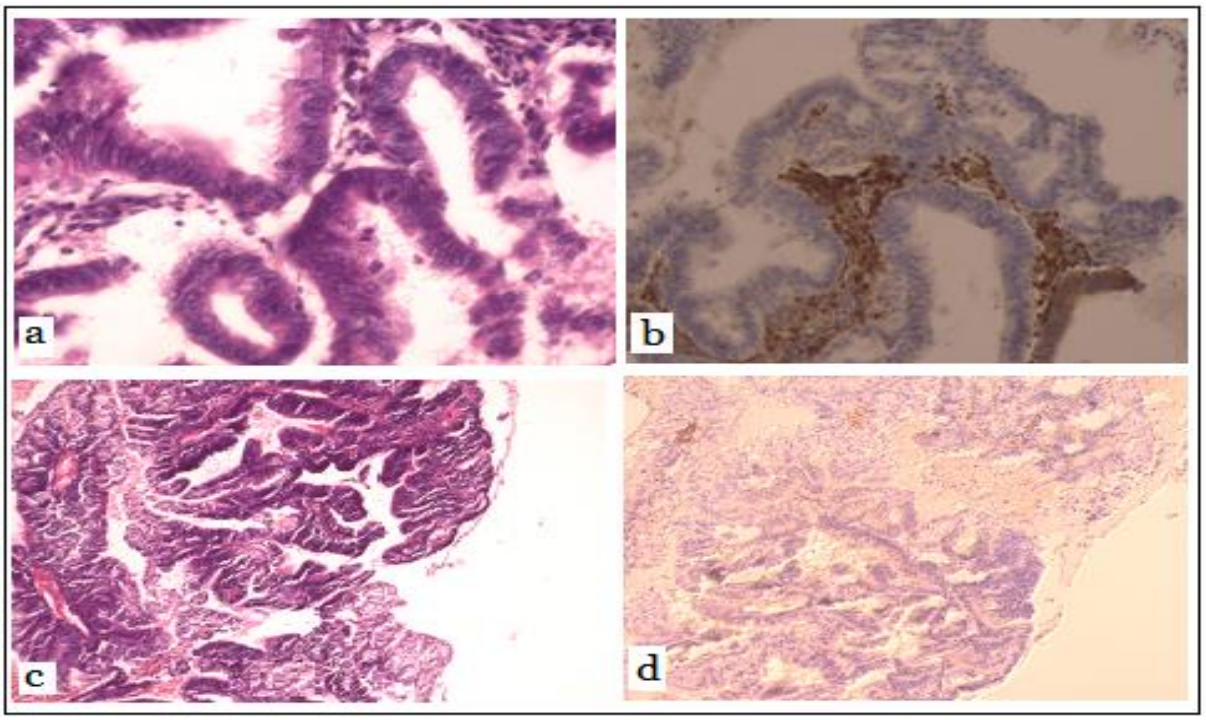

Fig. 3. (a) Complex hyperplasia with atypia (H&E;40x); (b) PTEN immunohistochemistry showing focal and weak reactivity in complex hyperplasia with atypia (40x); (c) Type I endometrial carcinoma (H&E;10x) and (d) PTEN immunohistochemistry showing complete loss of reactivity in Type I endometrial carcinoma (10x).

The negative expression was observed more commonly in Type I endometrial carcinoma than Type II endometrial carcinoma while positive expression was noted more commonly in Type II endometrial carcinoma. Garg *et al.* (2012) supported it. Mutter *et al.* (2000) also reported similar findings. In present study, a complete loss of expression was found in 4 out of 5 cases (80%) of Type1 endometrial carcinoma and 4 out of 12 cases (33%) of atypical complex hyperplasia (Table 1). These findings were in contrast to results of Orbo *et al.* (2014) who detected the loss of PTEN expression in 55% cases of Type I endometrial carcinoma. This may be due to difference in expressivity in our female population. But were somewhat similar to the observations of Kapucuoglu *et al.* (2007) who reported that loss of expression was observed in 20% cases of complex hyperplasia with atypia. Highly significant statistical difference was found in PTEN expression between normal endometrium and Type I endometrial carcinoma and also between simple hyperplasia and atypical complex hyperplasia. But there was no statistically significant difference in PTEN expression between atypical complex hyperplasia and Type I endometrial carcinoma (Table 1). That was highly consistent with the results of study carried out by Sarmadi *et al.* (2009).

Conclusion

In the light of the results of the present study, it is concluded that loss of PTEN expression is highly associated with malignant transformation. There is a statistically significant difference of PTEN expression among different groups of normal, hyperplasic and

neoplastic endometrium. On the basis of PTEN expression in endometrial biopsy of a patient with atypical complex hyperplasia, it can be predicted that neoplastic change can occur in future. This can guide the clinicians to plan follow-up/ treatment. Therefore, it can be said with pertinence that PTEN expression assessed by immunohistochemistry (IHC) may be a new effective tool for screening of premalignant endometrial lesions.

References

Ayala EG, Mastrascusa LC, Martínez DS, Anaya HM. 2010. Hiperplasia endometrial: analisis de serie de casos diagnosticados en biopsia endometrial. Revista Chilena De Obstetricia Y Ginecolgia **75(3),** 146-152. http://dx.doi.org/10.4067/S071775262010000300002

Cristofano AD, Ellenson LH. 2007. Endometrial carcinoma. Annual Review of Pathology **2,** 57-85. https://dx.doi.org/10.1146/annurev.pathol.2.010506.091905

Djordjevic B, Henessy BT, Li J, Barkoh BA, Luthra R, Mills GB, Broaddus RR. 2012. Clinical assessment of PTEN loss in endometrial carcinoma: immunohistochemistry outperforms gene sequencing. Modern Pathology **25(5),** 699-708. https://dx.doi.org/10.1038/modpathol.2011.208

Gamble M. 2008. The hemtoxylins and eosin. In: Theory and practice of histological techniques (Eds. Bancroft JD and Gamble M.). 6th Edition. Elsevier P 121-134. http://dx.doi.org/10.1016/B978-0-443-10279-0.50016-6

Garg K, Broaddus RR, Soslow RA, Urbauer DL, Levine DA, Djordjevic B. 2012. Pathologic scoring of PTEN immunohistochemistry in endometrial carcinoma is highly reproducible. International Journal of Gynecological Pathology **31(1),** 48-56. https://dx.doi.org/10.1097/PGP.0b013e3182230d00

Garg K, Soslow RA. 2014. Endometrial carcinoma in women aged 40 years and younger. Archives of Pathology and Laboratory Medicine **138(3),** 335-342. https://dx.doi.org/10.5858/arpa.2012-0654-RA

Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N, Ciriş M. 2007. Immunohistochemical expression of PTEN in normal, hypeplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathology, Research and Practice **203(3),** 153-162. https://dx.doi.org/10.1016/j.prp.2007.01.003

Kiechle M, Hinrichs M, Jacobsen A, Lüttges **J,** Pfisterer **J,** Kommoss F, Arnold N. 2000. Genetic imbalances in precursor lesions of endometrial cancer detected by comparative genomic hybridization. The American Journal of Pathology **156(6),** 1827-1833. https://dx.doi.org/10.1016/S0002-9440(10)65055-9

Kurman RJ, Mazur MT. 1994. Benign diseases of the endometrium. In: Blaustein's pathology of the female genital tract (Ed. Kurman RJ.). 4th Edition. Springer-Verlag New York. P 367-410. https://dx.doi.org/10.1007/978-1-4757-3889-6_10

Lee H, Choi HJ, Kang CS, Lee HJ, Lee WS, Park CS. 2012. Expression of miRNAs and PTEN in endometrial specimens ranging from histologically normal to hyperplasia and endometrial adenocarcinoma. Modern Pathology **25(11),** 1508-1515. https://dx.doi.org/10.1038/modpathol.2012.111

Mutter GL, Baak JP, Crum CP, Richart RM, Ferenczy A, Faquin WC. 2000. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. The Journal of Pathology **190(4),** 462–469. https://dx.doi.org/10.1002/(sici)1096-9896(200003)190:4<462::aid-path590>3.0.co;2-d

Mutter GL. 2002. Diagnosis of premalignant endometrial disease. Journal of Clinical Pathology

55(5), 326-331.

Orbo A, Vereide A, Arnes M, Pettersen I, Straume B. 2014. Levonorgestrel-impregnated intrauterine device as treatment for endometrial hyperplasia: a national multicentre randomised trial. BJOG-An International Journal of Obstetrics and Gynaecology **121(4),** 477-486. http://dx.doi.org/10.1111/1471-0528.12499

Pallares J, Bussaglia E, Martínez-Guitarte JL, Dolcet X, Llobet D, Rue M, Sanchez-Verde L, Palacios J, Prat J, Matias-Guiu X. 2005. Immunohistochemical analysis of PTEN in endometrial carcinoma: a tissue microarray study with a comparison of four commercial antibodies in correlation with molecular abnormalities. Modern Pathology **18,** 719-727. https://dx.doi.org/10.1038/modpathol.3800347

Risinger JI, Hayes K, Maxwell GL, Carney ME, Dodge RK, Barrett JC, Berchuck A. 1998. PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. Clinical Cancer Research **4,** 3005-3010.

Romano C, Schepis C. 2012. PTEN gene: A model for genetic diseases in dermatology. The Scientific World Journal 2012, 1-8. https://dx.doi.org/10.1100/2012/252457

Rosai J, Ackerman LV. 2011. Gross techniques in surgical pathology. In: PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics (Eds. Housten M and Scott J.). 10th Edition. Edinburg. Elsevier p 25-30.

Salvesen HB, Carter SL, Mannelqvist M, Dutt A, Getz G, Stefansson IM, Raeder MB, Sos ML, Engelsen IB, Trovik J, Wik E, Greulich H, Bo TH, Jonassen I, Thomas RK, Zander T, Garraway LA, Oyan AM, Sellers WR, Kallan KH, Meyerson M, Akslen LA, Beroukhim R. 2009. Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. Proceedings of the National Academy of Sciences of the United States of America **106(12),** 4834-4839. https://dx.doi.org/10.1073/pnas.0806514106

Samulak D, Grosman-Dziewiszek P, Michalska MM, Mojs E, Samulak K, Romanowicz H, Smolarz B. 2013. Evaluation of expression of the PTEN gene, oestrogen and progesterone receptors as diagnostic and predictive factors in endometrial cancer. Pathology Oncology Research **20(1),** 191-196. http://dx.doi.org/10.1007/s12253-013-9684-3

Sarmadi S, Izadi-Mood N, Sotoudeh K, Tavangar SM. 2009. Altered PTEN expression; a diagnostic marker for differentiating normal, hyperplastic and neoplastic endometrium. Diagnostic Pathology **4,** 41-46. https://dx.doi.org/10.1186/1746-1596-4-41

Shawana S, Kehar SI, Shaikh F. 2014. Differential expression of phosphatase and tensin homologue in normal, hyperplastic and neoplastic endometrium. The Journal of the Pakistan Medical Association **64(10),** 1103-1108.

Spenceer LT, Bancroft JD. 2008. Tissue processing. In: Theory and practice of histological techniques (Eds. Bancroft JD and Gamble M.). 6th Edition. Elsevier, p 90-92.

Sun H, Enomoto T, Shroyer KR, Ozaki K, Fujita M, Ueda Y, Nakashima R, Kuragaki C, Ueda G, Murata Y. 2002. Clonal analysis and mutations in the PTEN and the K-ras genes in endometrial hyperplasia. Diagnostic Molecular Pathology **11(4),** 204-211. http://dx.doi.org/10.1097/00019606-200212000-00003

Tantbirojn P, Triratanachat S, Trivijitsilp P, Niruthisard S. 2008. Detection of PTEN immunoreactivity in endometrial hyperplasia and adenocarcinoma. Journal of the Medical Association

of Thailand **91(8),** 1161-1165.

Yevgeniya J, Chiappinelli KB, Mutch DG, Zighelboim I, Goodfellow PJ. 2012. Phosphatase and tensin homolog (PTEN) pseudogene expression in endometrial cancer: a conserved regulatory mechanism important in tumorigenesis? Gynecologic Oncology **124(2),** 340-346. https://dx.doi.org/10.1016/j.ygyno.2011.10.011