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Clinical significance of Abolished PTEN expression to differentiate between malignant and hyperplastic endometrium as a diagnostic marker.

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ABSTRACT

Aim : Clinical significance of Abolished PTEN expression to differentiate between malignant and hyperplastic endometrium - A diagnostic marker.

Material and Methods : We used immunohistochemistry (IHC) to compare changes in PTEN expression in 50 endometrial biopsies that were diagnosed as endometrial hyperplasia without atypia, endometrial hyperplasia with atypia, and endometrial carcinoma and the control used was PTEN IHC for carcinoma prostate. The study uses PTEN as a marker to distinguish hyperplastic non-atypical, atypical versus malignant endometrium in 50 endometrial biopsy and results were graded according to immunoreactivity scoring system (IRS).

Results : PTEN was shown to be differentially expressed in atypical hyperplasia and EC in scores of 1+ and 0 or negative, while it was shown to be well expressed in hyperplasia without atypia in a score of 2+. Thirty of the fifty endometrial biopsy samples showed a score of 2+, seventeen showed 1+, and three showed 0 or negative.

Conclusions : According to the current study PTEN expression is decreased in precancerous hyperplasia. PTEN IHC proved beneficial in screening precancerous hyperplasia lesions and diagnosing the earliest stage of endometrial carcinoma.

KEYWORDS:
EC- Endometrial Carcinoma, EH- Endometrial Hyperplasia, PTEN- Phosphatase and Tensin Homolog, IHC- Immunohistochemistry.

INTRODUCTION:

In Western countries, endometrial carcinoma is a highly prevalent malignant disease of the female genital tract. It manifests in several histomorphological variants, the most frequent of which is endometrioid carcinoma [1]. The basis of endometrial carcinoma's emergence is a hyperestrogenic state that frequently follows endometrial hyperplasia in women in the perimenopausal age range [2, 3]. The condition known as endometrial hyperplasia, which is characterized by hyperplastic elongated and coiled endometrial glands in a compact oedematous endometrial stroma, occurs when endometrial tissue becomes hyperplastic. Two types of endometrial hyperplasia—simple endometrial hyperplasia (SH) and complex endometrial hyperplasia (CH)—are distinguished by morphological changes in the stroma and endometrial glands as well as by the overcrowding and branching pattern of the glands [4]. Exogenous and endogenous sources of oestrogen may be the cause of endometrial hyperplasia, which typically occurs in a hyperestrogenic state. The primary sources of endogenous oestrogens include increasing body fat storage and ovarian tumors that produce oestrogen. On the other hand, exogenous oestrogens are mostly obtained from hormone replacement treatment and contraceptive pills.

Nuclear stratification, focal areas of cellular and nuclear overpopulation, and proliferation of endometrial cells are further characteristics of endometrial hyperplasia. Endometrial cells have been known to exhibit a range of nuclear abnormalities, such as nuclear pleomorphism and varied degrees of nuclear atypia, which can manifest as loss of polarity, nuclear overcrowding, nucleomegaly, and nucleolus in some nuclei. In addition to relying on simple glandular hyperplasia and complicated branching hyperplasia, endometrial hyperplasia can be classified as simple or complex

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depending on whether nuclear atypia is present or absent. In contrast, endometrial hyperplasia with nuclear atypia is classified as either simple or complex endometrial hyperplasia with atypia [3,4]. Endometrial glands exhibiting hyperplastic changes without observable nuclear atypia as previously mentioned are classified as simple or complex hyperplasia with no atypia. As previously mentioned endometrial carcinoma is the most prevalent cancer among women in affluent western nations, with a comparatively lower incidence observed in Asian and other emerging regions. Endocrine hyperplasia or a persistently hyperestraemic state typically precedes endometrial cancer. Women between the ages of 40 and 60 are more likely to get endometrial cancer. White women are more likely to experience it than Black women. Asian nations have relatively low rates of endometrial carcinoma. Oestrogen-dependent (Type I) and Oestrogen-independent (Type II) endometrial carcinomas are classified into two groups. Type II endometrial carcinoma develops without the influence of estrogen stimulation, whereas type I endometriod carcinoma is the result of prolonged oestrogen stimulation. Endometriod carcinoma, or type I, is a type of malignant tumor that is usually low-grade and does not frequently invade the myometrium[5]. Despite being more common, type I endometrial carcinoma's pathophysiology is not well understood, with prolonged oestrogenic stimulation playing a significant part. It is unclear what the molecular causes of endometrial carcinogenesis are. On chromosome 10q23 lies the tumor suppressor gene PTEN (Phosphatase and Tensin Homologue). It has somatic mutations in a variety of tumor kinds[2].

The PTEN gene exhibits normal regulation, with the highest physiological expression in endometrial glands in environments rich in estrogen. Therefore, there is a direct correlation between the risk of cancer and decreased PTEN tumor suppressor function, especially in high estrogenic situations. PTEN expression immunohistochemistry analysis is a useful screening technique for endometrial cancer, regardless of the underlying cause. Atypical hyperplasia is a precursor lesion, but endometrial hyperplasia is the ideal benign counterpart for endometrioid carcinoma. Although it can occasionally arise in premenopausal women, especially in the presence of hyperestrogenism, endometrial cancer mostly affects perimenopausal and postmenopausal women[6].

It's interesting to note that research has also linked PTEN to the prognosis of a number of malignancies, including endometrial carcinoma. In order to assess the potential for early detection of endometrial premalignant lesions, the current study was designed to examine the expression of normal, hyperplastic, and neoplastic endometrial glands using PTEN as a marker and distinguish between hyperplastic and malignant endometrial glands, particularly in small biopsies.

MATERIAL AND METHODS:

The PTEN immunohistochemical marker on endometrial biopsy specimens is the subject of the current prospective study. The study was carried out in the Department of Pathology at MGM Medical College in Navi Mumbai, India, and involved a thorough analysis of an endometrial biopsy sample. Following the formalin fixation, the tissues were processed for standard histopathological and further PTEN immunohistochemical staining.

Cases: The Department of Gynaecology provided 50 samples of endometrial tissues via dilatation and curettage (D&C). Further routine histopathological examination was done and endometrial biopsies that showed features of endometrial hyperplasia with atypia, endometrial biopsy with atypia, endometrial intraepithelial neoplasm/endometrial carcinoma were taken for the PTEN IHC study.

Control: In this study, we used PTEN IHC staining on a prostate specimen with histopathological proven cancer, which on IHC revealed total loss of PTEN expression.

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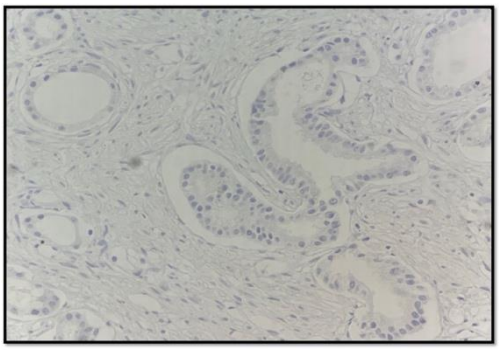

Complete loss of PTEN IHC staining in case of carcinoma prostate.

Procedure:

Histopathology technique: The specimens was inspected grossly, and representative sections were taken before the standard processing steps of dehydration, clearing, and embedding. Paraffin-embedded tissue was sliced to a thickness of 4 µm, and H&E stain was used to stain it. Additionally, 3 µm thick slices of the paraffin embedded blocks were prepared and smeared on slides coated with Poly L Lysine for use in immunohistochemistry (IHC) procedures. Immunohistochemistry: Bio SBg, USA provided a mouse anti-PTEN monoclonal primary antibody, which was used in the IHC technique for 3 µm blocks. The detection system used was the Avidin Biotin Complex (ABC). We used a microwave oven to retrieve antigens. Overnight at 600˚C, the slides were incubated. Two xylene changes lasting 15 minutes each were used for section deparaffinisation, and graded alcohols were used for hydration.

After 20 minutes of primary blocking with 3% H2O2 in methanol, the mixture was rinsed with distilled water for three minutes. Two changes of the TBST buffer wash, lasting five minutes each, came next. For 20 minutes, secondary blocking was carried out with 3% BSA. 30 minutes at room temperature were spent incubating the main antibody, and five minutes each were spent washing in two changes of TBST buffer. After covering the tissue pieces with Poly Excel Target Binder, they were allowed to sit at room temperature for ten minutes.

three times, each lasting two minutes, in the TBST buffer. For ten minutes at room temperature, tissue slices coated with Poly Excel Poly HRP were incubated. three times, each lasting two minutes, in the TBST buffer. Parts coated in Stunn DAB chromogen solution were allowed to stand at room temperature for five minutes. (Preparation of the solution: 1 ml of Stunn DAB buffer plus 1 drop of Stunn DAB chromogen; thoroughly mixed and kept in a dark, stable environment for a week at 2-80 ˚C). Wash in two changes of TBST buffer for five minutes each, and then counterstain for ten minutes at room temperature with Harri's Hematoxylin.

Graded alcohols were used to dry the sections. Following two 5-minute Xylene changes for clearing, the corresponding sections were sealed with DPX mounting media. Soheila Sarmadi et al. [1] and Kapucuoglu et al. [5] state that when brown staining was found in the glandular cells' cytoplasm or nuclei, the PTEN immunoreactivity was considered positive for each specimen. Scores for cell staining were as follows: negative for less than 10%, 1+ for 10%–50%, and 2+ for more than 50% of the slide stained positively. Immunohistochemistry microscopy pictures of endometrioid-type endometrial cancer, proliferative, secretory, simple hyperplasia, and atypical complex hyperplasia.

OBSERVATIONS AND RESULTS: Histopathology Results :

Variably sized glands that were well divided by cellular stroma were observed in EH without atypia. The ratio of cystically dilated and tortuous glands was not significantly altered, and there was pseudo stratification with round, monotonous nucleoli and fine chromatin.

EH with Atypia showed back to back arrangement, increased gland to stroma ratio, variable sized glands showing frequent outpouching and complex branching pattern with significant overcrowding with less stroma along with epithelium showing true stratification with loss of polarity. The cell show enlarged round nuclei with moderate pleomorphism having irregular membrane and coarse granular chromatin with frequent mitotic activity.

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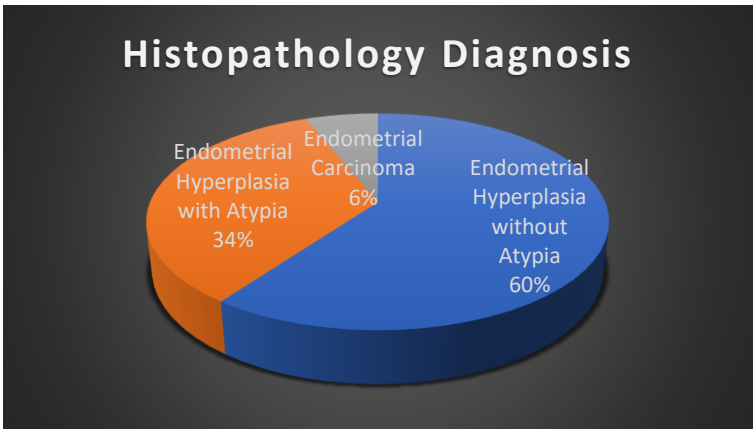
EIN showed extensive overcrowding, with back to back arrangement, irregular shape and size with branching with very less or no stroma at places. Cells show loss of polarity, nuclear enlargement, pleomorphism, high nuclear to cytoplasmic ration and prominent nucleoli with frequent mitotic activity.

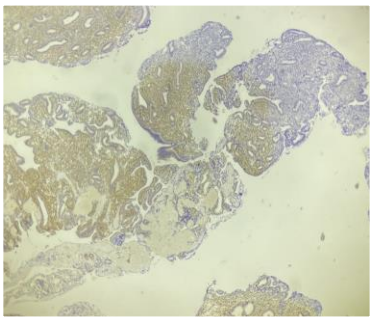
Endometrial Carcinoma showed multiple glands arranged in back to back arrangement, complex branching pattern, with no stroma. Lining cells of glands are enlarged with round pleomorphic nuclei having prominent nuclei and coarse chromatin along with frequent mitotic activity.

HISTOLOGY DIAGNOSIS		
EH without Atypia	EH with Atypia	EIN/Endometrial Carcinoma
30 cases	17 cases	3 Cases



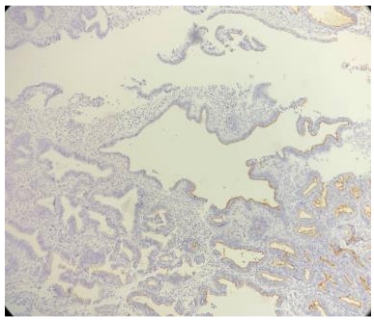
PTEN IHC SCORING

Based on a well-established immunoreactivity scoring system (IRS), the endometrial specimens were graded. The following score was used to evaluate the degree of PTEN immune-expression: staining values: 0 (negative), 1+ (moderate), and 2+ (strong). We decided the percentage of positive cells as follows: 0/negative (less than 10%), 1+ (10–50%), and 2+ (more than 50%).



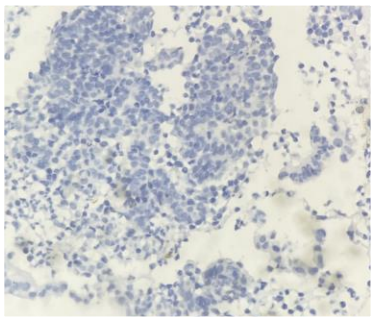
> 50 % Positive Ptaining

SCORE 2+



10-50 % Positive Staining

SCORE 1+



< 10 % Positive Staining

SCORE 0 / NEGATIVE

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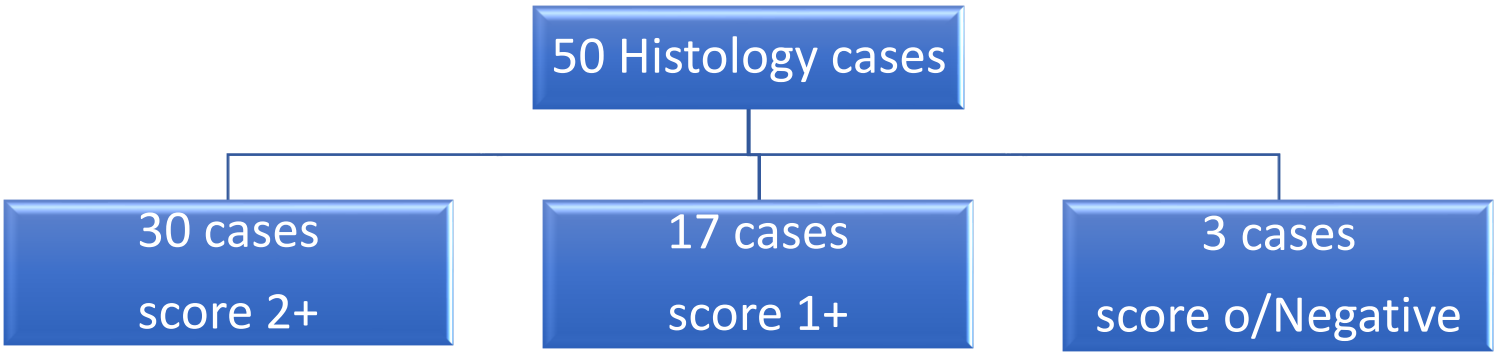
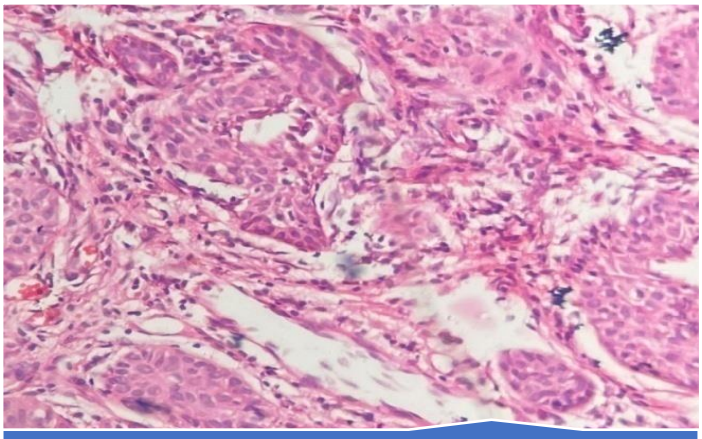
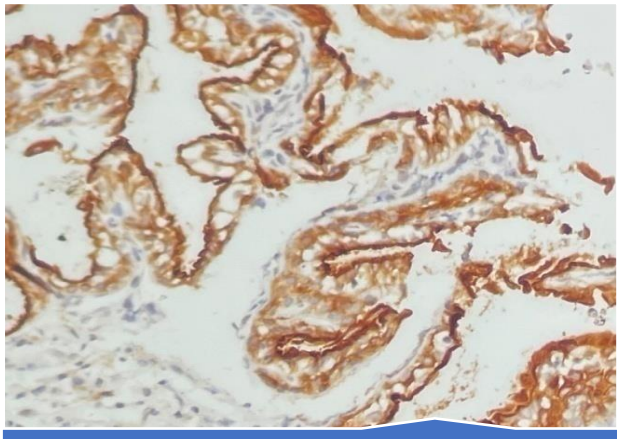


Total cases on histopathology (50)	PTEN IHC Positivity Percentage
30 EH WITHOUT ATYPIA	10 cases – 80% 13 cases – 70% 05 cases – 65% 02 cases – 60%
17 EH WITH ATYPIA	09 cases – 40% 04 cases – 30% 04 cases – 25%
03 EIN / ENDOMETRIAL CARCINOMA	02 cases – <10% 01 case – 0%



ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA



SCORE : 2 +

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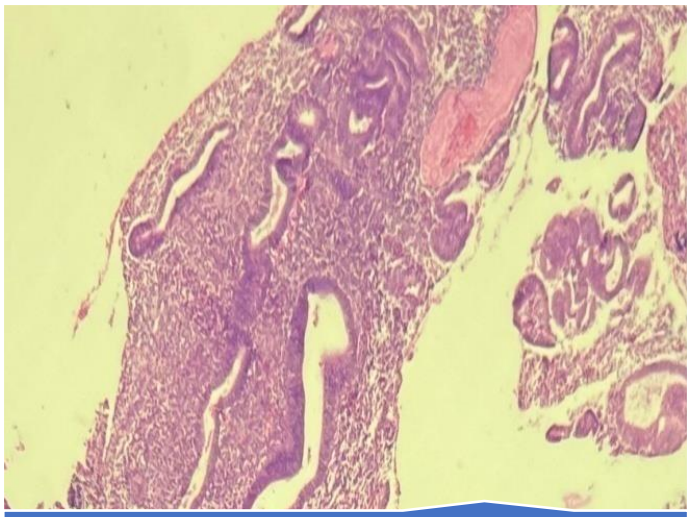
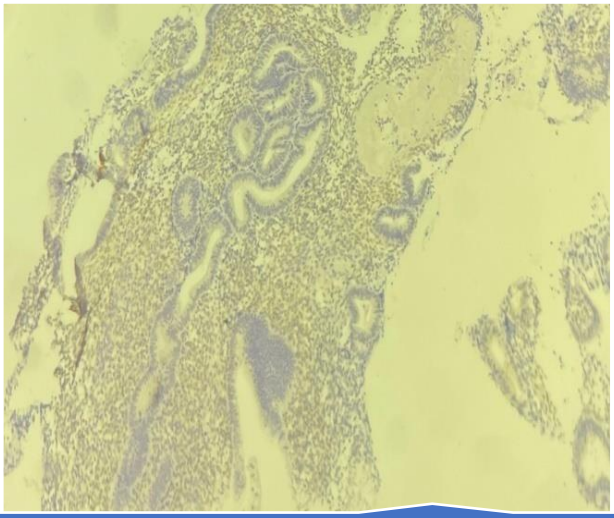
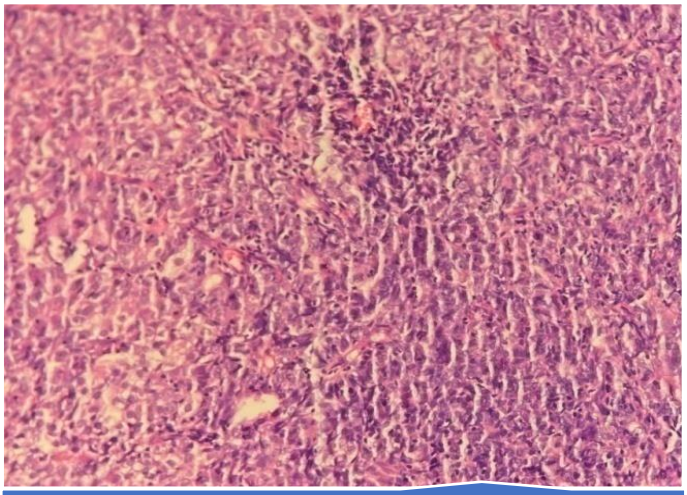


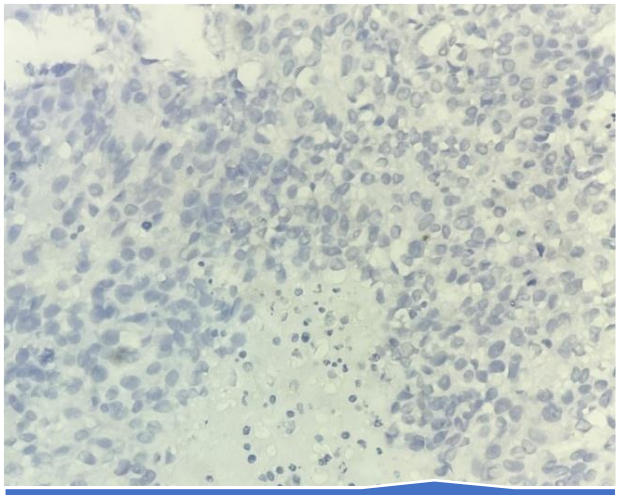
ENDOMETRIAL HYPERPLASIA WITH ATYPIA



SCORE 1 +



EIN/ENDOMETRIAL CARCINOMA



SCORE 0/NEGATIVE

When PTEN expression in endometrial carcinoma, atypical EH, and EH without cytological atypia were compared, it was found that EC had complete loss of PTEN expression, while most atypical EH specimens had decreased PTEN expression. In contrast, EH without cytological atypia showed strong glandular and cytoplasmic PTEN expression.

CONCLUSION:

Endometrioid endometrial carcinoma has PTEN loss, suggesting that PTEN is downregulated in both endometrioid endometrial cancer and atypical hyperplasia. Endometrioid endometrial carcinoma has PTEN loss, suggesting that PTEN is downregulated in both endometrioid endometrial cancer and atypical hyperplasia. With a substantial statistical difference in PTEN immunoreactivity across groups of normal, hyperplasia, and carcinoma, we concluded that PTEN expression is related with premalignant endometrial malignancies and decreased PTEN expression is associated with atypical endometrium features.

PTEN IHC can aid in an early and precise diagnosis, which is crucial for diagnosing premalignant endometrium, when combined with histology. As a result, PTEN and H and E staining should be utilized often. Using the PTEN IHC marker to diagnose EIN early can potentially save lives. Also, PTEN staining in EIN/EH will help to identify lesions that H and E staining may have missed.

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A risk factor for EC is PTEN loss in EH, yet this risk cannot be accurately predicted. A probability of concurrent EC over 50% in slide is linked to PTEN reduction in atypical EH. When it comes to treatment decisions (hysterectomy vs. conservative), this information may incorporate the patient's informed consent, particularly in circumstances where there is ambiguity.

REFERENCES:

1. Dr. Shanmugapriya M, Dr. Sudha M, Dr.Geetha P. A study of PTEN expression in endometrial hyperplasia and endometrioid type of endometrial carcinoma. Trop J Path Micro 2017;3(1):39-45.doi: 10.17511/jopm.2017.i1.07.
2. H. Gbelcová, P. Bakeš, P. Priščáková, V. Šišovský, I. Hojsíková, Ľ. Straka, M. Konečný, J. Markus, C. W. D'Acunto, T. Ruml, D. Böhmer, Ľ. Danihel, V. Repiská, "*PTEN* Sequence Analysis in Endometrial Hyperplasia and Endometrial Carcinoma in Slovak Women", *Analytical Cellular Pathology*, vol. 2015, Article ID 746856, 7 pages, 2015. https://doi.org/10.1155/2015/746856
3. Stacey Mills. Sternberg's diagnostic surgical pathology.. LWW. Philadelphia, 5th Ed. 2004
4. Travaglino A, Raffone A, Saccone G, Mascolo M, Pignatiello S, Mollo A, De Placido G, Insabato L, Zullo F. PTEN immunohistochemistry in endometrial hyperplasia: which are the optimal criteria for the diagnosis of precancer? APMIS. 2019 Apr;127(4):161-169. doi: 10.1111/apm.12938. PMID: 30803040.
5. Histopathology of endometrial hyperplasia and endometrial carcinoma: An update LarsChristianHornMD,PhD[a]AlexandraMeinelMSc[a]RomyHandzelMSc[b] JensEinenkelMD[b] Annals of Diagnostic Pathology Volume 11, Issue 4, August 2007, Pages 297-311
6. Robert J. Kurman et al .Blaustein's Pathology of female genital tract, Springer Cham. Switzerland , 7th ed 2019