

Histopathological, Immunohistochemical and Therapeutical Assessment of Premalignant Endometrial Lesions in a Hospital Based Series of Cases

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ABSTRACT

Introduction: Endometrial hyperplasia represents a diversified set of disorders which has challenged pathologists for decades. Due to its high likelihood of progression to carcinoma, endometrial intraepithelial neoplasia (EIN) demands prompt and specialized intervention.

Materials and methods: This 15-month (September 2014 – November 2015) retrospective analysis involved 258 cases of female patients with atypical and non-atypical endometrial hyperplasia investigated and treated at the University Emergency Hospital in Bucharest, Romania. Our purpose was to evaluate the histopathological, immunohistochemical and therapeutical aspects of premalignant endometrial lesions as well as their concurrence with endometrial carcinoma.

Results: Our findings indicate that 23% of the women preoperatively diagnosed with atypical hyperplasia were found with endometrial carcinoma on the hysterectomy specimen. Surprisingly, we identified two cases of atypical hyperplasia with focal p53 expression. Mutation of p53 is a late genetic event seen in endometrial carcinoma which does not usually occur in EIN. Interestingly, these cases did not present endometrial carcinoma on the hysterectomy specimen.

Conclusions: All female patients diagnosed with EIN have an increased risk of developing endometrial carcinoma, as there are no histologic subdivisions or grades of atypical hyperplasia to further stratify risk for malignancy. Therefore, we emphasize the importance of accurate detection of premalignant endometrial lesions and exclusion of a coexisting endometrial carcinoma as mandatory prerequisites for proper medical management.

Keywords: non-atypical endometrial hyperplasia, atypical endometrial hyperplasia, EIN, endometrial carcinoma

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Type of lesion	Hysterectomy	Endometrial Biopsy	Total
Endometrial Hyperplasia	42 (16.3%)	11.5 (83.7%)	0.13 (100%)
• Non-atypical Hyperplasia	33	199	232 (89.9%)
• Atypical Hyperplasia	9	17	26 (10.1%)

TABLE 1. Frequency of the histopathological diagnoses in the studied population.

INTRODUCTION

Endometrial hyperplasia represents a diversified set of disorders which has challenged pathologists for decades. Long envisioned as an endless variety of morphologic changes of increasing severity, endometrial hyperplasia actually embodies only two separate entities, which must be accurately distinguished from endometrial carcinoma (1). Until recently, pathologists were using two different systems for reporting endometrial precancerous lesions: the four-class *World Health Organization* (WHO94) classification and the Endometrial Intraepithelial Neoplasia (EIN) schema introduced by Mutter and the International Endometrial Collaborative Group in 2000. After more than a decade of debates, the *World Health Organization* has established a consensus with the introduction of EIN in the newly updated classification system of endometrial hyperplastic lesions (2014)(2). □

MATERIALS AND METHODS

The purpose of this retrospective analysis was to investigate the histopathological, im-

munohistochemical and therapeutical aspects of premalignant endometrial lesions as well as their concurrence with endometrial carcinoma in a series of cases investigated and treated at the University Emergency Hospital in Bucharest, Romania. The study has been developed as a descriptive-quantitative evaluation, performed over a period of 15 months (September 2014 – November 2015). It includes a total of 258 cases of female patients diagnosed with histopathological features of endometrial hyperplasia and associated lesions. All biological samples obtained either from endometrial biopsies or hysterectomy specimens were processed using the conventional method of paraffin embedding and hematoxylin and eosin staining. In addition, we used immunohistochemistry to evaluate the most challenging cases. Data collection and processing was performed using Microsoft Office Excel 2010. □

RESULTS

This 15-month retrospective analysis involved 258 cases of female patients with histopathological features of atypical and non-atypical endometrial hyperplasia and associated lesions. The frequency of each type of lesion detected in the studied population is presented in Table 1.

Demographic breakdown revealed that the vast majority of patients (77.04%) came from urban areas. Most women were postmenopausal and the average age at presentation was 47.75 years, ranging from 23 to 79 years for patients with non-atypical endometrial hyperplasia and 54.19 years, ranging from 39 to 73 years for patients with endometrial intraepithelial neoplasia. Distribution of cases according to age groups is presented in Figure 1.

Most patients addressed the Department of Obstetrics-Gynecology of the University Emergency Hospital in Bucharest for postmenopausal vaginal bleeding. Few patients presented with unspecific or no clinical background and an even lower number of women who were of childbearing age accused uncharacteristically prolonged or excessive bleeding at irregular intervals.

Endometrial biopsy was the predominant method of diagnosis for all hyperplastic lesions with a total of 216 (83.7%) cases evaluated within this 15-month follow-up. There were 42 (16.3%) incidental findings of endometrial hy-

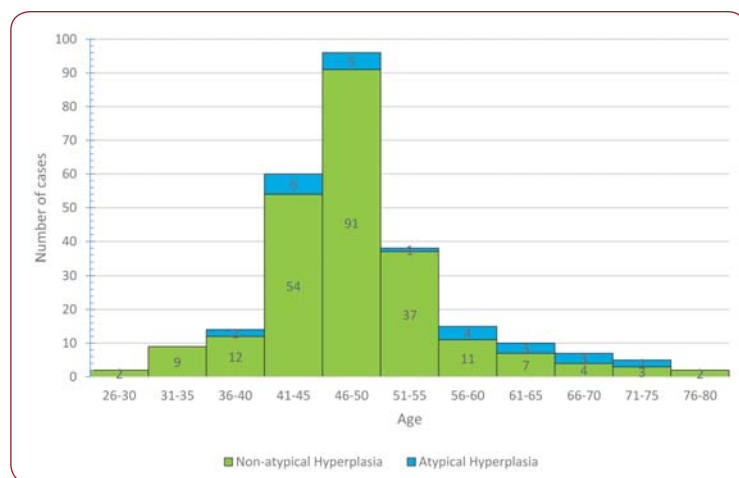


FIGURE 1. Distribution of cases according to age groups.

perplasia diagnosed directly on the hysterectomy specimen.

All endometrial hyperplastic lesions have been classified according to the latest *World Health Organization* classification system (2014). Out of the 258 females diagnosed with endometrial hyperplasia on both hysterectomy and biopsy specimens, 232 (89.9%) had non-atypical hyperplasia and 26 (10.1%) had atypical hyperplasia or endometrial intraepithelial neoplasia (EIN). Among them, 45 (17.5%) cases of benign hyperplasia were associated or developed on endometrial polyps. All patients diagnosed with atypical hyperplasia on endometrial biopsy undergone radical hysterectomy in our clinic. Their hysterectomy findings are presented in Table 2.

Upon histopathological examination, non-atypical hyperplasia presented an irregular glandular remodeling pattern with little intervening stroma, vascular thrombi, architectural breakdown and randomly scattered cytologic changes. EIN revealed crowded aggregates of cytologically altered tubular and branching glands with increased gland to stroma ratio and little intervening stroma. The main distinction from non-atypical hyperplasia was based on atypia, pleomorphism, enlargement, rounding, loss of polarity and pseudostratification of nuclei. Various types of endometrial metaplasia have also been identified. Ciliated (tubal) metaplasia was the most common type of epithelial cytoplasmic change encountered predominantly in association with non-atypical endo-

metrial hyperplasia. Other types of cytoplasmic changes encountered were: eosinophilic change, squamous metaplasia and clear cell change (secretory metaplasia).

Immunohistochemical studies have been performed on all 9 hysterectomy specimens with atypical hyperplasia and 10 randomly selected cases of non-atypical hyperplasia. The most prominent aspect was the loss of PTEN expression in all cases of endometrial intraepithelial neoplasia. Estrogen receptor (ER) and progesterone receptor (PR) were consistently positive in both atypical and non-atypical endometrial hyperplasia. Comparatively, PR was better expressed than ER across all examined slides and both ER and PR were slightly better expressed in non-atypical hyperplasia than in atypical hyperplasia. Two cases of atypical hyperplasia revealed weak focal p53 expression. Ki-67 proliferation index ranged from 30% to 80%, higher values being associated with architectural complexity. As expected, mitotic activity in endometrial intraepithelial neoplasia was low in comparison with proliferative endometrium and high in comparison with secretory endometrium. □

DISCUSSION

The classification of endometrial hyperplasia introduced by the International Society of Gynecological Pathologists (ISGP) and defined by the *World Health Organization* in 1994 was dividing hyperplastic lesions in four subtypes,

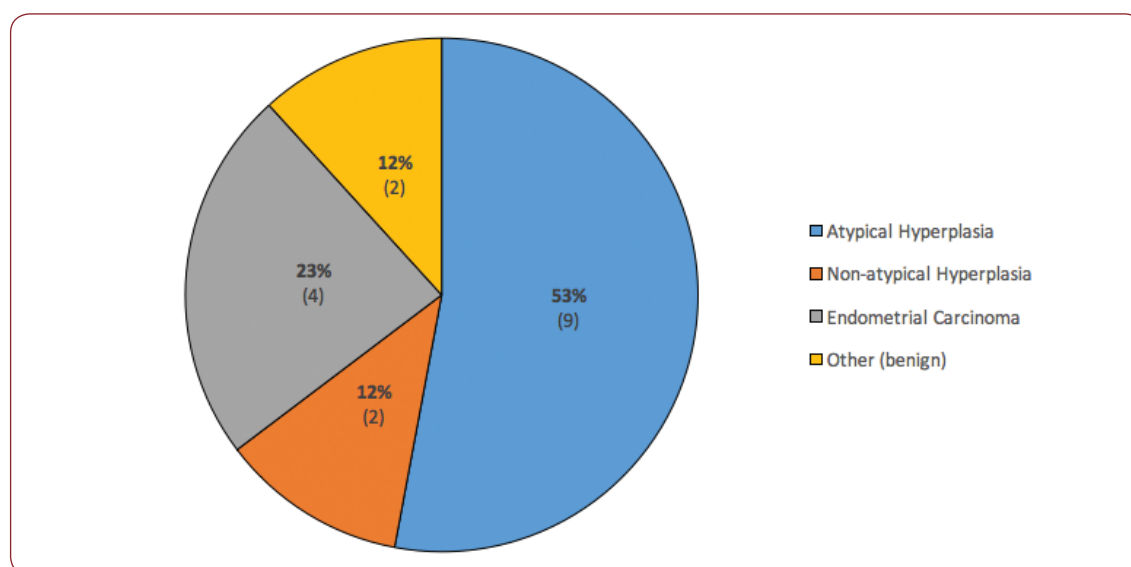


TABLE 2. Hysterectomy findings in patients diagnosed with atypical hyperplasia on a previous endometrial biopsy.

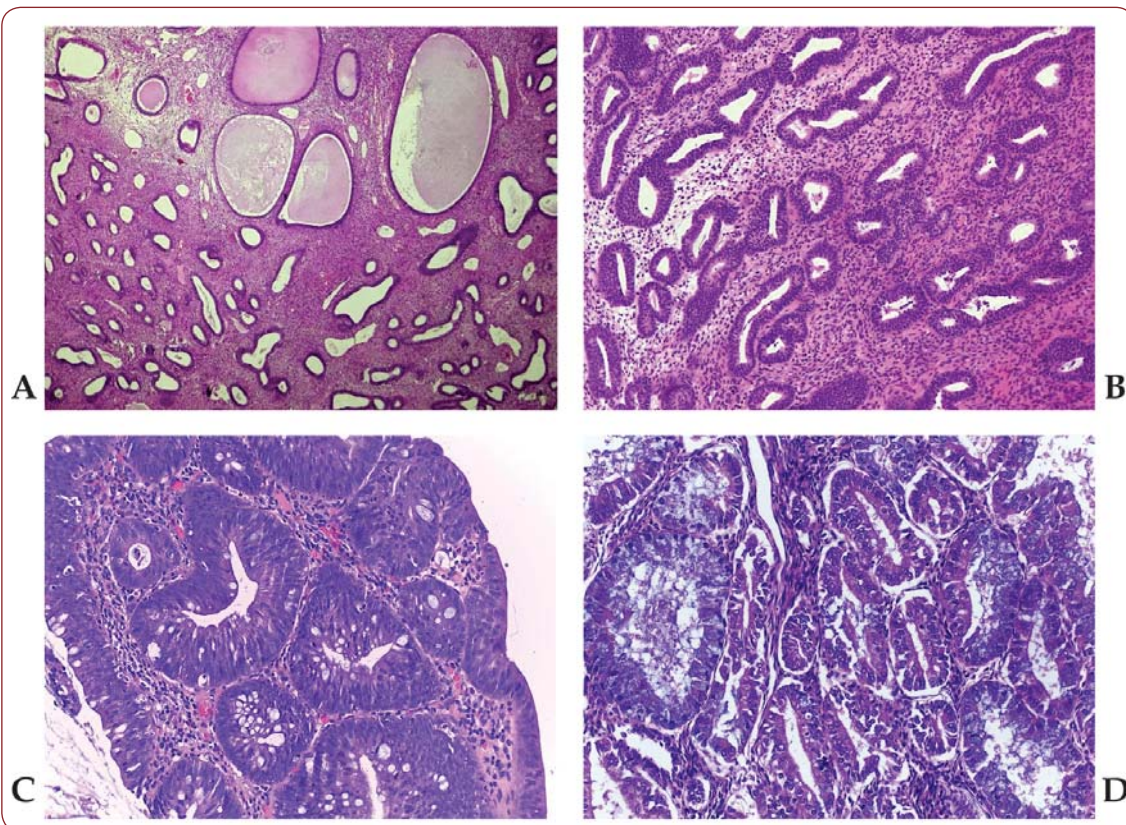


FIGURE 2. Histopathological aspects of atypical and non-atypical endometrial hyperplasia.

A. Endometrial polyp with irregular interspersed cystic and tubular glands in a fibrous and focally edematous stroma (H.E., 40x). **B.** Non-atypical hyperplasia with closely packed endometrial glands and no cytologic atypia (H.E., 100x). **C.** High power view of endometrial intraepithelial neoplasia with increased glandular density and cytologic atypia (H.E., 200x). **D.** Considerable architectural complexity and cytologic atypia in endometrial intraepithelial neoplasia (H.E., 200x).

according to the degree of architectural complexity and the presence or absence of nuclear atypia: (1) simple hyperplasia without atypia (2) complex hyperplasia without atypia (3) simple hyperplasia with atypia and (4) complex hyperplasia with atypia (3). Although the usage of this classification system for reporting endometrial hyperplastic lesions seems very didactic and intuitive, routine practice over the past decade demonstrated poor reproducibility among pathologists (3). While categories (1), (2) and (4) were generally acknowledged, experts began to dispute the clinical importance of simple hyperplasia with atypia. Moreover, this system was missing several key diagnostic elements which have been discovered and/or better understood in recent years. The search for an alternative classification system has led Mutter and the Endometrial Collaborative Group to the introduction of Endometrial Intraepithelial Neoplasia (EIN) – a new diagnostic scheme which is the result of cautious correlation

between genetically ascertained premalignant lesions, histopathologic features and clinical outcomes (4).

The World Health Organization has clarified the matter in its latest classification system, distinguishing between only 2 categories of endometrial hyperplasia: (1) non-atypical hyperplasia and (2) atypical hyperplasia or endometrial intraepithelial neoplasia (2). This new classification system is not simply a cutback of the previous ones. It is rather a reflection of the new understanding of what molecular changes occur in premalignant endometrial lesions. Non-atypical hyperplasia does not involve any relevant genetic changes, representing a benign alteration which will regress once the endocrine milieu has normalized. However, there have been described cases (1–3%) in which progression to invasive disease could occur if the endocrine disorder persists over longer periods of time (5). Atypical hyperplasia, on the other hand, exhibits several mutations charac-

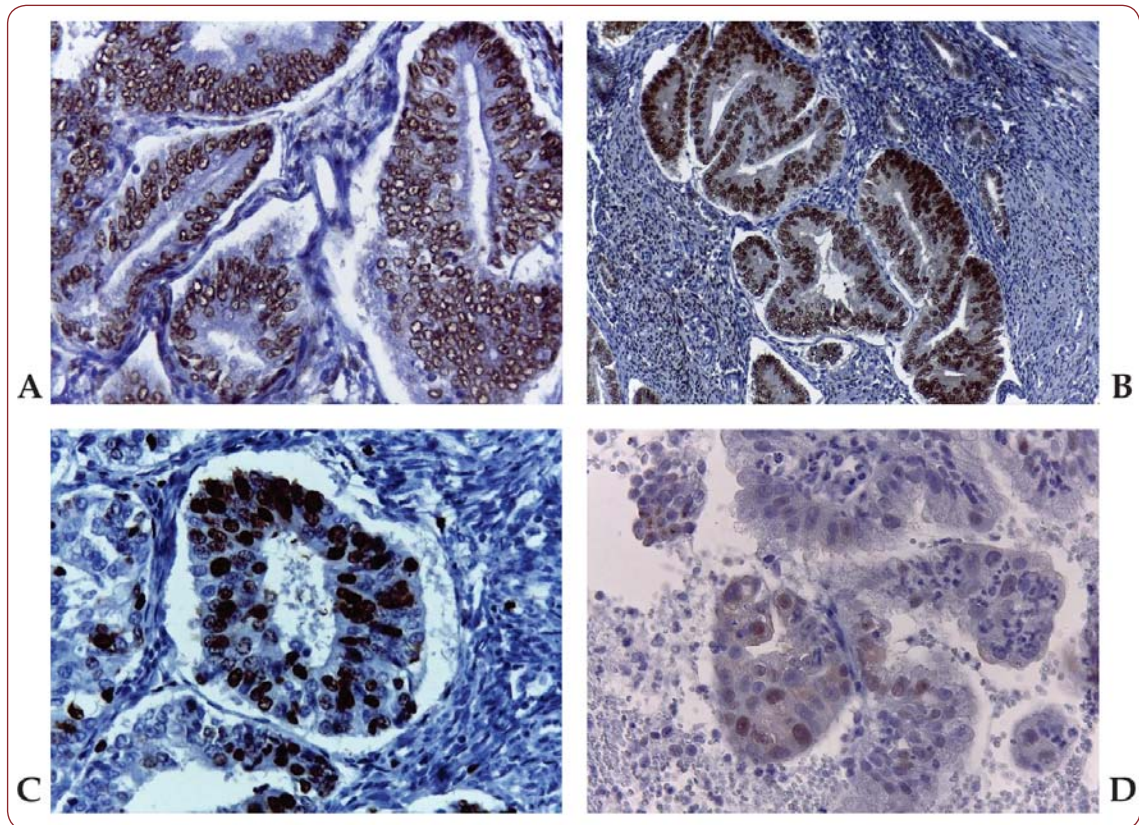


FIGURE 3. Immunohistochemical findings in atypical endometrial hyperplasia. **A.** Nuclear estrogen receptor (ER) staining in atypical endometrial hyperplasia (IHC staining with DAB chromogen, 400x). **B.** Strong nuclear progesterone receptor (PR) positivity in atypical hyperplasia (IHC staining with DAB chromogen, 200x). **C.** Moderately-high Ki-67 proliferation index in atypical endometrial hyperplasia (IHC staining with DAB chromogen, 400x). **D.** Weak focal p53 positivity in atypical endometrial hyperplasia (IHC staining with DAB chromogen, 400x).

teristic for invasive endometrial carcinoma, and patients either present with coexisting invasive cancer or are at extremely high risk of developing invasive endometrial cancer (6). These include microsatellite instability, PAX2 inactivation and PTEN, KRAS and CTNNB1 mutations (7-10). Non-atypical endometrial hyperplasia or benign hyperplasia is defined as a functionally normal endometrium which has suffered morphological alterations due to an abnormal hormonal setting, frequently represented by unopposed estrogenic stimulation. It usually occurs in women around menopause but it may occur in young women and adolescents as well. Atypical endometrial hyperplasia or endometrial intraepithelial neoplasia (EIN) is a monoclonal proliferation of architecturally and cytologically altered glands, associated with increased occurrence of endometrial carcinoma through malignant transformation of its constituent cells (1). Due to its high likelihood of progression to carcinoma, EIN demands

prompt and specialized intervention. Studies reveal that the rate of progression to carcinoma is 1% for simple hyperplasia without atypia, 8% for simple hyperplasia with atypia, 3% for complex hyperplasia without atypia and 29% for complex hyperplasia with atypia (11).

Besides morphometric image analysis, specialized biomarkers play a crucial role in evaluating endometrial intraepithelial neoplasia (1). We were surprised to find two cases with focal p53 expression. Mutation of p53 is a late genetic event seen in endometrial carcinoma which does not usually occur in EIN precursor lesions. Interestingly, these cases did not present endometrial carcinoma on the hysterectomy specimen, diagnosis of atypical hyperplasia being confirmed on several samples.

Accurate diagnosis of endometrial hyperplasia is of very important clinical and prognostic significance because it represents the precursor lesion for endometrial endometrioid carcinoma. For patients with non-atypical hy-

perplasia, the underlying cause of unopposed estrogenic stimulation will establish appropriate clinical management. In young and perimenopausal women, endogenous estrogen sources are commonly represented by failed or delayed ovulation. Anovulatory females of reproductive age must be evaluated by a reproductive endocrinologist in order to establish whether there is a primary endocrine abnormality, or if anovulation is secondary to other treatable factors such as stress or low body weight. In postmenopausal women, non-atypical hyperplasia with no history of exogenous (pharmacologic) estrogen use demands further investigation. Estrogen production by hormonally active ovarian tumors should be considered, especially in this group, but may also occur in the younger females (1).

It is extremely important to note that diagnostic accuracy may be severely compromised by progestin-based hormonal therapies. Due to this aspect, primary diagnosis or follow-up surveillance of a suspected atypical hyperplasia should be based, whenever possible, on a sample obtained while the patient is not on therapeutic hormones. For women on progesterone therapy, biopsy should be performed 2-4 weeks after the exogenous hormone administration has been discontinued.

The primary objectives in patients newly diagnosed with endometrial intraepithelial neoplasia are exclusion of a coexisting carcinoma (the management of which would supersede that of the atypical hyperplasia itself) and ablation of the atypical endometrium as a cancer preventive strategy. When clinically appropriate, radical hysterectomy is the most effective treatment method, fulfilling both of these goals and providing definitive assessment of a potential concurrent carcinoma.

In Romania, women diagnosed with endometrial intraepithelial neoplastic lesion, are usually treated by radical hysterectomy, especially when in perimenopause. In our study, approximately one third of the women treated by radical hysterectomy presented endometrial carcinoma on the hysterectomy specimen. We consider these cases concomitant occurrences of endometrial carcinoma. The malignancy was most likely already present when the diagnosis of atypical hyperplasia was made but it was

probably missed due to sampling error. EIN is a disorder which is usually detected by routine sampling of random areas of the endometrium. Therefore, careful sampling is a crucial element in effective discovery of atypical hyperplasia, especially if the lesions are small or confined to a limited surface.

Endometrial ablation with thermal or cautery-mediated devices applied directly to the endometrial lining is not recommended as treatment method for endometrial intraepithelial neoplasia because it may leave residual islands of neoplastic tissue behind or it may generate intrauterine adhesions which could prevent post-treatment surveillance and further biopsy (1). This proposal is consistent with the recommendation of the American College of Obstetricians and Gynecologists (ACOG) that women with endometrial hyperplasia or uterine cancer should not undergo endometrial ablation (12). Although in Romania hysterectomy is currently the most frequently used therapeutic method for treating endometrial hyperplasia in women past childbearing age, there is an active interest and continuous development regarding uterus-sparing alternatives for younger women who wish to conserve their fertility as well as for those with elevated surgical risks (13-14). Nonsurgical management options are represented by progestin-based hormonal treatments capable of reversing atypical hyperplastic lesions, but the optimal procedures, preferred delivery method (oral, injected, impregnated intrauterine device), expected clinical response and attendant risks are yet to be defined (13). □

CONCLUSION

All female patients diagnosed with EIN have an increased risk of developing endometrial carcinoma, as there are no histologic subdivisions or grades of atypical hyperplasia to further stratify risk for malignancy. Our findings indicate that 23% of the women preoperatively diagnosed with atypical hyperplasia on endometrial biopsy were found with endometrial carcinoma on the hysterectomy specimen.

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REFERENCES

1. **Mutter GL, Prat J** – Endometrial Hyperplasia without Atypia and EIN. In: Pathology of the Female Reproductive Tract, Churchill Livingstone Elsevier, 2014:349-69, ISBN 978-0-7020-4497-7.
2. **Zaino RJ, et al.** – Tumours of the uterine corpus: epithelial tumours and precursor lesions. In: Kurman RJ, Carcangiu ML, Herrington S, Young RH, editors. WHO classification of tumours of the female reproductive organs. Lyon, France: IARC Press; 2014.
3. **Nucci MR, Esther O** – Gynecologic Pathology, Elsevier Churchill Livingstone, 2009:233-59, ISBN: 978-0-443-06920-8.
4. **Owings RA, Quick CM** – Endometrial Intraepithelial Neoplasia, *Arch Pathol Lab Med*—2014;138.
5. **Emons G, Beckmann MW, Schmidt D, Mallmann P and for the Uterus commission of the Gynecological Oncology Working Group (AGO)** – New WHO Classification of Endometrial Hyperplasias. *GeburtshilfeFrauenheilkd.* 2015;75:135–136.
6. **Kandoth C, Schultz N, Cherniack A D, et al.** – Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013;497:67–73.
7. **Jovanovic AS, Boynton KA, Mutter GL** – Uteri of women with endometrial carcinoma contain a histopathological spectrum of monoclonal putative precancers, some with microsatellite instability. *Cancer Res.* 1996;56:1917-1921.
8. **Matias-Guiu X, Catusas L, Bussaglia E, et al.** – Molecular pathology of endometrial hyperplasia and carcinoma. *Hum Pathol* 2001;32: 569-577.
9. **Monte NM, Webster KA, Neuberger D, et al.** – Joint loss of PAX2 and PTEN expression in endometrial precancers and cancer. *Cancer Res* 2010;70:6225-6232.
10. **Moreno-Bueno G, Hardisson D, Sarrio D, et al.** – Abnormalities of E- and P-cadherin and catenin (beta-, gamma-catenin, and p120ctn) expression in endometrial cancer and endometrial atypical hyperplasia. *J Pathol* 2003;199:471-478.
11. **Kurman RJ, Kaminski PF, Norris HJ** – The behavior of endometrial hyperplasia. A long-term study of “untreated” hyperplasia in 170 patients. *Cancer* 1985;56:403-12.
12. **ACOG Committee on Practice Bulletins** – ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. Number 81, May 2007, *Obstet Gynecol.* 2007;109:1233-48.
13. **Trimble CL, Method M, Leitao M, et al.** – Management of endometrial precancers. *ObstetGynecol* 2012;120:1160–75.
14. **Minig L, Franchi D, Boveri S, et al.** – Progestin intrauterine device and GnRH analogue for uterus-sparing treatment of endometrial precancers and well-differentiated early endometrial carcinoma in young women. *Ann Oncol* 2011;22:643–9.