Ph.D. THESIS

ENDOMETRITAL HYPERPLASIAS IN
PERIMENOPAUSE

SUMMARY

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**INTRODUCTION**

Hyperplasia is a non-physiological and noninvasive proliferation at the endometrium level whose results consist in the growth of various forms and shapes of glands. (Marsden DE, 2003). The term of endometrial hyperplasia refers to an abnormality characterized by the increase of the endometrium quantity (volume), alteration of glandular architecture and change of glands / stroma ratio (M. Sezgin, 2006).

There are two forms of hyperplasia: the atypical form, representing a precursor lesion with certain characteristics found in relation to endometrial adenocarcinoma, and the non-atypical form, which is a self-limiting increase which do not seem to lead to cancer. (Kurman RJ, 1985, Horn LC, 2004).

**GENERAL SECTION**

Endometrial hyperplasia are the pathological situations which consist in proliferation of glandular component, either the endometrial stromal determined by hyperestrogenism of exogenous or endogenous origin, with deficiency or absence of progesterone stimulus.

Tumorigenicity estrogen appears to be driven by accelerating cell multiplication, may actually explained by three assumptions:

- estrogen stimulates DNA - polymerase resulting in cell proliferation;
- estrogen stimulates estromedine synthesis, cell growth and multiplication;
- estrogen derepresse genetic control of cell growth repression.

Recently WHO glandular endometrial hyperplasia divided into four groups:

1. Simple hyperplasia without atypia:
   - Gladulo cystic hyperplasia
   - Simple adenomatous hyperplasia

2. Simple hyperplasia with atypia, plus the presence of epithelial stratifications, metaplasia and nuclear changes of this group, requires further research in relation to the assessment of the precancerous state.

3. Complex hyperplasia without atypia includes the classic form of aggravated adenomatous hyperplasia, don’t have precancerous significance.
4. Complex hyperplasia with atypia in the form described above aspects is added the
presence of stratification, the nuclear atypia, and has a metaplasia precancerous lesion
significance.

Atypical cell is represented by: this nuclear hypertrophy (cell nucleus have round-
ovular shaped) presence of nuclear hyperchromasia and disorderly arrangement of nucleus,
disruption of nuclear chromatin, the nucleus increase in volume, increased mitotic activity.

Caused by lack of progesterone, endometrial proliferative lesions occur in most of
cases in premenopausal, being manifested in abnormal bleeding (menorrhagia, metrorrhagia, polimenoree). Often coexist with other proliferative lesions (atypical and / or
adenocarcinoma), fibromyomas, ovarian tumors, fibrocystic mastosis. Clinical examination
is usually normal.

Diagnosis of endometrial hyperplasia, clinical and paraclinical was performed by
the most recent methods (endoscopics and imagistics: conventional transabdominal and
transvaginal sonography, hysteroigraphy, MRI, hysteroscopy, vaginal smears and methods
by endometrial cytology, histological methods, biochemical methods / hormonal,
cytometry flow).

**SPECIAL SECTION**

**MATERIAL AND METHODS**

The study was a prospective, with retrospective component to achieve that I used:

- records of the case law of the Laboratory of Pathology
- clinical observation sheets (from the archives of hospital) of patients,
- sheets accompanying parts surgical excision,
- histological preparations in histotech Pathology Laboratory.

In this histopathological study I used paraffin inclusion technique and standard
hematoxylin-eosin staining to classify lesions into categories and diagnostic special stains
highlighting certain issues, particularly for differential diagnosis.

For immunohistochemical study I used a method based on immunoenzymatic
soluble complex, called LSAB / HRP (labeled streptavidin biotin), one of the methods
called ABC (avidin-biotin complex), and the grout used was DAKO LSAB 2 System HRP
(DAKO Universal Streptavidin Biotin Labbeled Horseradish Peroxide System 2).

The biological material of the study group is represented by 30 cases of
endometrial hyperplasias. All fragments that were immunohistochemically analyzed were
harvested by curettage biopsy from patients admitted to Obstetrics and Gynecology or
Surgery department at Emergency County Hospital Craiova. They corresponded to the following: simple endometrial hyperplasia (10 cases), complex endometrial hyperplasia (10 cases) and atypical endometrial hyperplasia (10 cases).

Three diagnosed cases with atypical endometrial hyperplasia on curettage have been presented subsequently, on the fragments of hysterectomy after immunohistochemical analysis, a well-differentiated endometrioid type of endometrial carcinoma.

The study of immunohistochemical profile of hyperplastic endometrium was conducted in comparison with the immunohistochemical profile of the normal endometrium, thus 6 normal endometrial tissue specimens were introduced. These six cases of normal endometrium can be divided further: three cases, which at morphological level corresponded to the endometrium in the proliferative phase and three cases, which corresponded morphologically to the endometrium in the secretory phase.

As a result, the comparative immunohistochemical analysis of endometrial hyperplasias, normal endometrium and endometrial carcinomas amounted to a total of 39 cases.

The immunohistochemical method used was LSAB/HRP, and the studied antibodies were markers for hormone receptors (estradiol receptors-ER and progesterone receptor-PR), proliferation marker (Ki-67), epithelial membrane antigen (EMA) and p53 oncoprotein.

For every antibody we analyzed the immunomarking at the level of pad epithelial cells of the endometrial glands but not at the stromal level.

RESULTS
IMMUNOMARKING ANALYSIS AT ER

In this study, all analyzed cases presented receptors for estrogen at the level of proliferate glands as well as endometrial stroma.

It is noted that the ER expression in normal endometrium was observed predominantly in the proliferative phase rather than secretory phase, the medium values of PI for ER were 94.4% for cases with endometrium in the proliferative phase and only 10.6% for cases for endometrium with secretory aspect.

It was noted that ER expression decreases also in hyperplastic and neoplastic endometrium compared with the proliferative phase of normal endometrium, but the values are higher than those of secretory phase endometrium. Thus, the highest values of PI for estrogen
receptors were found in case of complex endometrial hyperplasia, the mean PI values for ER were 72.3%, followed by atypical hyperplasia with an average PI of 57% and simple non-atypical endometrial hyperplasia, with an average PI of 41.5%. The lowest values were present for the three endometrial carcinomas, where the average PI was 28.5% but the ER level was approximately three times higher compared to the secretory phase endometrium.

**IMMUNOMARKING ANALYSIS AT PR**

As for immunomarking with ER, PR immunomarking was consistently positive at the epithelium level for all cases included in the study.

The PR expression in normal endometrium was observed predominantly in the proliferative phase (PI = 97.6%) compared with secretory phase (PI = 32.5%).

Furthermore, the PR expression decreases in hyperplastic and neoplastic endometrium compared with proliferative phase of normal endometrium, but the values are higher than those of secretory phase endometrium.

A comparative analysis of ER and PR expression reveals that progesterone receptors are better expressed than the estrogen ones. Thus, for every analyzed aspect of endometrial morphology in this study, the mean PI valued for PR were higher compared with average values of ER for similar injuries. We noticed a higher expression of ER / PR report for proliferative endometrium compared with secretory endometrium and a small change of this report for the three studied types of hyperplasia.

Analyzing the PR expression of various types of endometrial hyperplasia, we found that complex non-atypical hyperplasia has the highest level of PR hormone receptor (PI average was 78.5%), followed by atypical hyperplasia (PI average, 75.4%) and then simple non-atypical hyperplasia (PI average, 43.8%).

These results were similar to those obtained with ER immunomarkung, but with slightly higher retention of PI for the PR.

The three cases of endometrial carcinoma showed the lowest values of PI for PR (mean PI was 29.5%), these values were even slightly reduced compared with the values of PI for PR of normal endometrial glands in secretory phase (PI = 32.5%).

**IMMUNOMARKING ANALYSIS FOR Ki—67**

We noted, after all 30 cases were immuno-histochemically tested, that the presence of cell proliferation and mitotic activity in both endometrial glandular epithelium and endometrium stroma, demonstrated by nuclear Ki-67 positive immunomarking.
The presence of mitotic activity in normal endometrium was observed predominantly in the proliferative phase (PI = 22.5%) compared with secretory phase (PI = 2%). Also, mitotic activity in neoplastic and hyperplastic endometrium was low compared with proliferative phase of normal endometrium, but increased compared with the endometrium in secretive phase.

After analyzing the activity of cell proliferation for various types of endometrial hyperplasia, we found that it decreased with the hyperplasia advancement. Thus, for simple hyperplasia, we obtained the highest values of PI for Ki-67 (8%), followed by complex hyperplasia (PI = 5%) and atypical hyperplasia (PI = 3%).

The three cases of endometrial carcinoma showed lower PI values for Ki-67 (12%) compared with proliferative endometrium, but higher compared with secretory endometrium hyperplasia, indicating mitotic activity at endometrial carcinomas compared with endometrial hyperplasia.

Mitotic activity in various types of analyzed endometrial hyperplasia decreased with the increasing of hyperplasia, but was higher compared with secretory endometrium and lower with proliferative endometrium and endometrial carcinoma.

**IMMUNOMARKING ANALYSIS AT EMA**

All analyzed endometriums whether normal, hyperplastic or neoplastic showed positive marking for anti-EMA antibody. The immunomarking intensity was maximal (+++) in most cases except a case of atypical hyperplasia, which submitted a low mark (+) and a case of secretory endometrium with moderate immunomarking (+ +).

**IMMUNOMARKING ANALYSIS AT P53**

The result of the analysis was that no positive reaction to mark anti-p53 antibody clone DO-7 was found in normal endometrium during the proliferative and secretory phase. Also, p53 showed no cases of simple endometrial hyperplasia.

Cases that showed positive immunoreactivity to p53 belonged to complex hyperplasia endometrial (3 cases, 30%) and atypical hyperplasia endometrial (6 cases, or 60%). Also, all cases of endometrial adenocarcinoma were p53 positive.

All analyzed cases had weak (+) immunomarking intensity at p53. Thus, both cases of hyperplasia and endometrial adenocarcinoma had a low intensity immunomarking at p53, although isolated cells showed nuclei with moderate immunomarking.
The immunomarking distribution at p53 was focal for all cases that showed positive reaction. The immunomarking pattern was nuclear in most cases except one of endometrial adenocarcinoma, which was also accompanied by a weak cytoplasmic pattern.

The percentage of positive cells at p53 positive ranged between 2% and 5% with an average of 3.5% ± 1% in all analyzed cases, the endometrial carcinoma case usually presents it at the upper limit value (5%).

CONCLUSIONS

- The study was conducted over a period of five years, between 2005-2009 and included a total of 452 diagnosed endometrial hyperplasia during menopause, which represented 46.12% of casuistry analyzed.
- The number of cases was growing, peaking in 2009 when they recorded 22.4% of lesions.
- The fifth decade of life has included most of the lesions examined (65.2%).
- Endometrial hyperplasia is more common at women in urban areas.
- Personal pathological history: obesity, diabetes mellitus, hypertension, heart disease, thyroid disease have value and can be used in determining risk groups in endometrial hyperplasia and cancer endometrial.
- Endometrial biopsy, transvaginal ultrasound and hysteroscopy can be used as diagnostic scale to detect precursor lesions of endometrial cancer, both in the medical service and hospital unit.
- Pathogenesis of endometrial hyperplasia involving prolonged hyperestrogenism non-antagonistic progesterone, and the diagnosis of these lesions remains mainly at histology.
- Of the types of endometrial hyperplasia, atypical common hyperplasia was most frequently (74.7%), followed by complex hyperplasia without atypical (16.8%) and atypical hyperplasias (8.5%). Simple atypical hyperplasia was present in only 4 cases.
- Histopathological analysis of cases showed the presence of particular aspects of endometrial hyperplasias, like the xanthomatous cells, glandulo-cystic appearance of some simple non-atypical hyperplasia or glands placed “back to back”, presents in complex hyperplasias.
- Metaplasia associated with endometrial hyperplasia was present in 204 cases, which represented 45.14% of the cases analyzed.
- The most common form of metaplasia was the eosinophilia, present in 48% of cases, followed by ciliated metaplasia in 36.3% of cases, squamous metaplasia and clear cells in 9.3%, respectively 5.5% and mucinous metaplasia in 0.9% of cases, although the presence of metaplasia in the absence of cytologic atypia is not considered a factor worsening the prognosis, may increase the complexity of glandular architecture, with the risk of over-diagnosis.
- The importance to diagnose endometrial metaplasia associated with endometrial hyperplasia derives from its ability to induce resistance at progestogen therapy and treatment failure to hormone receptors change at the target cell, epithelium metaplasia was not so one responsive to hormonal treatment.
Eosinophilia and ciliated metaplasia were associated in most cases with simple hyperplasia without atypia and squamous metaplasia, with clear cells and mucinous metaplasia were most closely associated with complex hyperplasias with or without atypias.

Immunohistochemical study of proliferation markers and adhesion molecules, demonstrate endometrial intraepithelial lesions, those with increased risk of progression to carcinoma and evaluation of estrogen and progesterone receptors status, in these lesions has therapeutic value.

The expression of estrogen and progesterone receptors, within various types of endometrial hyperplasia, was maximal for cases of complex hyperplasia without atypia, followed by atypical hyperplasia and simple hyperplasia without atypia; all types of hyperplasia present a smaller number of receptors compared to the endometrium in proliferative phase, but higher compared with secretory endometrium and endometrial carcinoma.

Progesterone receptors density was higher than that of estrogen receptors in all cases analyzed.

The ER and PGR values in hyperplasias were intermediate, between the values of these receptors in normal proliferative endometrium and endometrial carcinoma G1, suggesting why the response to hormonal therapy for endometrial hyperplasia is maintained compared with endometrial carcinoma.

Mitotic activity for various types of endometrial hyperplasia, analyzed with anti-Ki-67 antibody, decreased with the increasing of hyperplasia, likely because the estrogen receptors upon which cells proliferation depend on, although IHC highlighted, were repressed by a series of cofactors.

The EMA immunomarking pattern may be useful in differentiating aspects of atypical hyperplasia, given that normal endometria and hyperplasia without atypia had predominantly a luminal immunomarking, atypical hyperplasia and endometrial carcinoma are almost always associated with a cytoplasmic pattern. However, EMA can’t distinguish between complex atypical hyperplasia and well differentiated endometrial carcinoma.

The p53 immunoexpression was present in many cases of complex hyperplasia and atypical hyperplasia as well as in all cases of well-differentiated endometrial adenocarcinoma.

The weak and focal marking at p53, different from the intense and diffuse marking of serum papillary carcinoma of the endometrium, is the consequence of increased intracellular levels of wild p53 protein type, with the purpose of stopping cell division and correcting DNA errors.

Correlating the results obtained at p53 marking with the ones obtained for cellular proliferation markers, we consider that the “arrest” of cells in S phase, due to the presence of wild type p53 protein, explains the decrease of the cellular proliferative activity due to the increasing degree of endometrial hyperplasia.

Immunohistochemistry explains the same response to hormonal therapy in cases of endometrial hyperplasia and suggest that, in these cases, there are a number of alterations of cellular DNA, but does not allow selection of cases presenting atypical hyperplasia which will subsequently develop into endometrial carcinoma. This selection remains the prerogative of molecular genetics; a method that shows the clinician the cases in which drug and surgical therapy is indicated.

Electron microscopy (EM) as opposed to immunohistochemistry (IHC), does not explain the preservation of response to hormone therapy. Neither electron microscopy can not directly indicate inconclusive or suggestive of specific changes to some development of
hyperplasia. However immunohistochemistry can establish some evolutionary patterns, this type of study being made too early.

- **Pseudoviral type genital infections (Ureaplasma, Mycoplasma),** the epithet tropic cylindrical can generate clinical and histopathological aspects of endometrial hyperplasia. Early diagnosis of their specific anti-infective treatment reduces the risk of aggravation of endometrial hyperplasia.

- **Hysteroscopy and uterine curettage is required in cases of atypical hyperplasia in premenopausal and in all cases of hyperplasia with or without atypia in postmenopausal women.**

- **Curettage biopsy is the method of choice in the diagnosis of endometrial pathology.**

- **Transvaginal ultrasound and Doppler are useful in assessing the risk of endometrial hyperplasia or endometrial cancer at womens in postmenopausal without / with continuous combined substitution therapy, but can not replace endometrial biopsy to exclude endometrial cancer.**

- **Medical treatment mainly with progesterone preparations is addressed of endometrial hyperplasia without atypia.** Surgery is recommended to patients in premenopausal with atypical hyperplasia and adenocarcinoma.

The risk of progression to malignancy found in 5-10% require clinical and histopathological follow-up to avoid insufficient treatment of lesions with evolving risk and of aggressive treatment of lesions without risk.