Original Paper

Immunohistochemical Aspects of Endometrium Hyperplasias in Perimenopause

DANIELA ILIE[(1)],CLAUDIA VALENTINA GEORGESCU[(2)], CRISTIANA SIMIONESCU[(3)], ANCA DANIELA BRAILA[(4)], M. BRAILA[(4)]

[(1)] Department of Obstetrics and-Gynecology, Emergency County Hospital Slatina, [(2)] Department of Pathology, Emergency University Hospital, Craiova, [(3)] Department of Pathology, University of Medicine and Pharmacy, Craiova; [(4)] Department of Obstetrics and-Gynecology, University of Medicine and Pharmacy, Craiova

ABSTRACT The present study presents a comparative study of 30 diagnosed cases of endometrial hyperplasia at patients with menopause. The study group was compared with a group with well-differentiated endometrial carcinoma (G1) and a group with normal endometrium. The antibodies studied were represented by markers for hormone receptors (estrogen receptors-ER and progesterone receptor-PR), proliferation marker (Ki-67), epithelial membrane antigen (EMA) and p53 oncoprotein. ER and PGR values in hyperplasia have intermediate values, between the values of these receptors in normal proliferative endometrium and the ones of typical G1 endometrial carcinoma. Mitotic activity decreased with the increasing degree of hyperplasia, likely because the estrogen receptors have been repressed by a number of cofactors. The EMA immune-marking pattern seems to be useful in differentiating hyperplastic aspects without atypical markings. However, EMA cannot distinguish between complex atypical hyperplasia and endometrial carcinoma (G1). The correlation of results, obtained at p53 marking with the ones obtained for cell proliferation, suggests that the presence of wild type p53 protein accounts for the decrease in cellular proliferative activity with the increase of endometrial hyperplasia. Immunohistochemistry accounts for the same response to hormonal therapy in cases of endometrial hyperplasia but does not allow selection of cases presenting atypical hyperplasia, which are likely to develop subsequently into endometrial carcinoma.

KEY WORDS *endometrial hyperplasia, menopause, immunohistochemistry*

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).

Material and methods

The biological material of the study group is represented by 30 cases of endometrial hyperplasia. 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 hyperplasic 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 hyperplazia, 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 (estrogen 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.

Table 1 Index to ER positivity in the glandular epithelium of normal endometrium, hyperplasic endometrium and endometrial carcinoma

Lesion type		PI-ER
Normal endometrium	Proliferative phase	94,4%
	Secretory phase	10,6%
Endometrial hyperplasia	Simple hyperplasia	41,5%
	Complex hyperplasia	72,3%
	Atypical hyperplasia	57%
Endometrial carcinoma	Endometroid carcinoma G1	28,5%

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 hyperplasic 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.

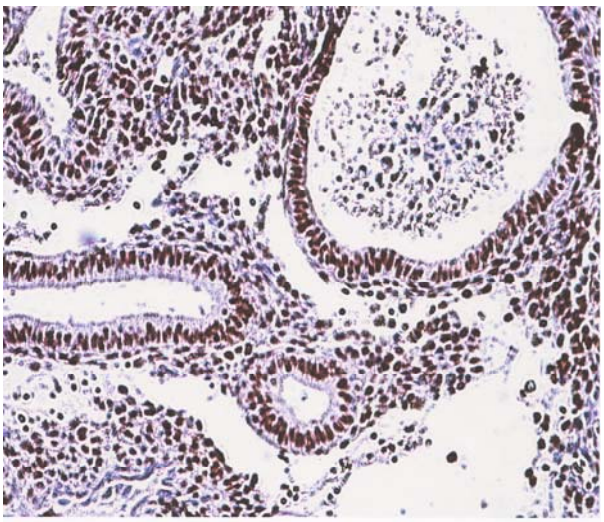

Fig.nr.1. Hiperplazie simplă fără atipii (glandulo – chistică), imunomarcaj pentru ER X 100

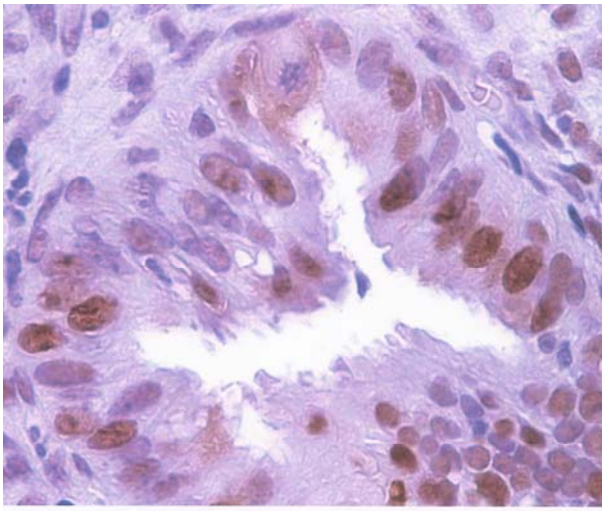

Fig nr.2. Hiperplazie cu atipii, imunomarcaj pentru ER X 400

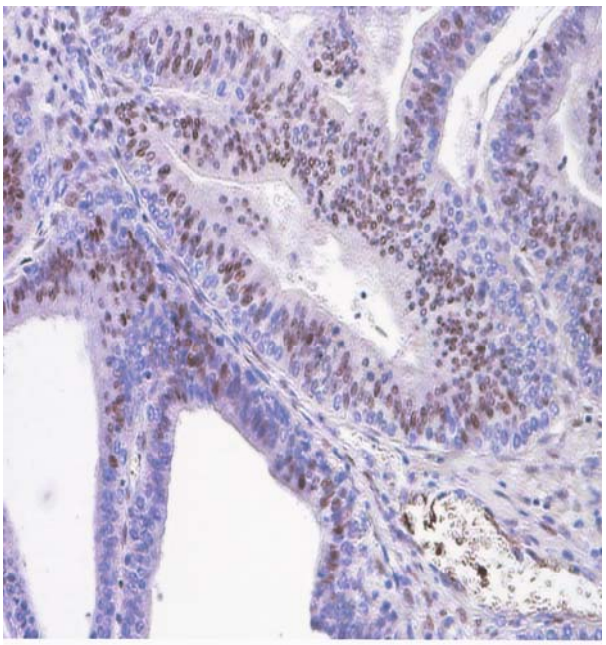

Fig nr.3. Carcinomul endometrial endometroid G1, imunomarcaj pentru ER X 100

Immunomarking analysis at PGR

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

Table 2 Index of PGR positivity in glandular epithelium of normal endometrium, hyperplasic endometrium and endometrial carcinoma

Lesion type		PI-ER
Normal endometrium	Proliferative phase	97,6%
	Secretory phase	32,5%
Endometrial hyperplasia	Simple hyperplasia	43,8%
	Complex hyperplasia	78,5%
	Atypical hyperplasia	75,4%
Endometrial carcinoma	Endometroid carcinoma G1	29,5%

As shown in the table above, the PGR expression in normal endometrium was observed predominantly in the proliferative phase (PI = 97.6%) compared with secretory phase (PI = 32.5%) (fig. no. 2). Furthermore, the PR expression decreases in hyperplasic 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 PGR 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 PGR were higher compared with average values of ER for similar injuries. We noticed a higher expression of ER / PGR report for proliferative endometrium compared with secretory endometrium and a small change of this report for the three studied types of hyperplasia.

Analyzing the PGR expression of various types of endometrial hyperplasia, we found that complex non-atypical hyperplasia has the highest level of PGR 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 immunomarking, but with slightly higher retention of PI for the PGR.

The three cases of endometrial carcinoma showed the lowest values of PI for PGR (mean PI was 29.5%), these values were even slightly reduced compared with the values of PI for PGR 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.

Table 3 Ki-67 index in glandular epithelium of normal endometrium, hyperplasic endometrium and endometrial carcinoma

Lesion type		PI-Ki 67
Normal endometrium	Proliferative phase	22,5%
	Secretory phase	2%
Endometrial hyperplasia	Simple hyperplasia	8%
	Complex hyperplasia	5%
	Atypical hyperplasia	3%
Endometrial carcinoma	Endometroid carcinoma G1	12%

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 hyperplasic 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.

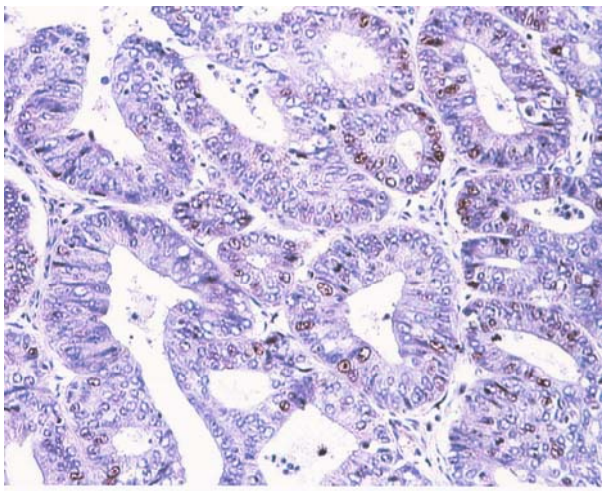

Fig. nr. 4 Carcinom endometrial endometroid G1,imunomarcaj pentru ki-67, x100

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 (+ +). The immunomarking pattern was predominantly luminal membrane type (with a higher intensity at the apical pole) for cases of proliferative and secretory endometrium as well as for simple and complex non-atypical hyperplasia.

Atypical hyperplasia and endometrial carcinoma presented a more frequent cytoplasmic pattern sometimes associated with the luminal type.

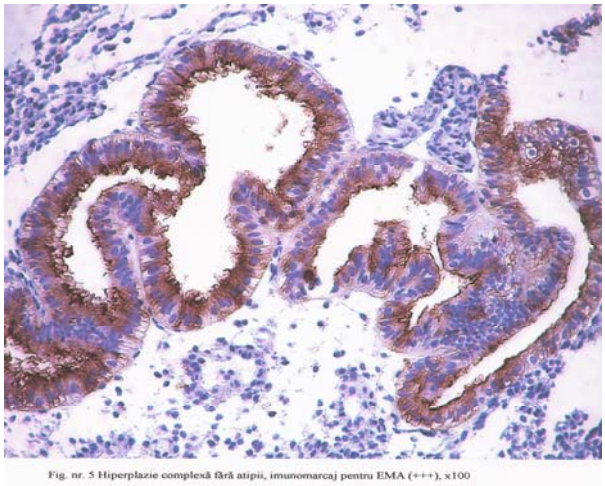

Fig. nr. 5 Hiperplazie complexă fără atipii, imunomarcaj pentru EMA (+++), x100

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.

Table 4 Distribution of positive cases at p53 in study

Lesion type		No.cases	Percentage
Normal endometrium	Proliferative phase	0	0%
	Secretory phase	0	0%
Endometrial hyperplasia	Simple hyperplasia	0	0%
	Complex hyperplasia	3	30%
	Atypical hyperplasia	6	60%
Endometrial carcinoma	Endometroid carcinoma G1	3	100%

Cases that showed positive immunoreactivity to p53 belonged to complex hyperplasia endometria (3 cases, 30%) and atypical hyperplasia endometria (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.

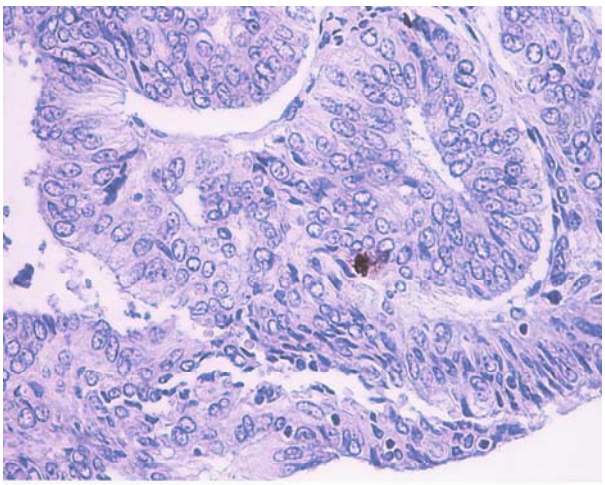

Fig. nr. 6 Carcinom endometrial endometroid G1, imunomarcaj pentru p53 (+) pattern nuclear, x200

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%).

Discusions

ER induces PR formation, thus the co-expression of both hormones in the same cells reflect the estrogen-progestin cell axis (Azumi N, Czernobilsky B, 2002).The monoclonal antibody for anti-estrogen receptor (clone 1D5) used in this study react with N-terminal domain (region A / B) of the estrogen alpha receptor.

In this study, all cases of endometrial hyperplasia presented estrogen receptors both at proliferate glands and endometrial stroma. Studies on a larger number of cases also described negative cases at ER in endometrial hyperplasia glands (96.1% of cases present ER receptors at this level), while stromal immunostaining was observed in all cases of hyperplasic-type lesions. (Bozdogan O et al., 2002).

The selected and studied cases of normal endometrium and the ones of endometrial carcinoma were all ER positive, however, the consulted references mention that 94.7% of normal endometriums, respectively 86.3% of endometrial carcinomas are ER positive at glandular level, all cases presenting these receptors stromal level (Bozdogan O et al., 2002).

All cases of endometrial hyperplasia were PR positive, the results are similar to those in medical literature. 100% of endometrial hyperplasias were positive to stromal and epithelial PGR (Bozdogan O et al., 2002), although some studies have reported that progesterone receptors (PR) are frequently more often positive in non-atypical endometrial hyperplasia compared with atypical hyperplasia (Bergerson C et al., 1988).

As ER immunomarking, the PR immunomarking was consistently positive at epithelial and stromal level, both in normal endometriums as well as in endometroid carcinoma.

It is noted that, after a comparative analysis of ER and PR expression, progesterone receptors are better expressed than estrogen ones. Thus, for every morphological aspect of the endometrium examined in this study, the mean PI values for PGR were higher compared with average values of ER for similar injuries. We noticed higher expression of ER / PR report in proliferative endometrium compared with secretory endometrium and a small variation of this report in the three studied types of hyperplasia.

ER and PR values in hyperplasia were intermediate, between the receptors in normal proliferative endometrium and endometrial carcinoma, thus suggesting the reason why the response to hormonal therapy for endometrial hyperplasia is maintained compared with the one for endometrial carcinoma. Similar observations were made by Ukikawa et al. in a study conducted in 2003.

The Ki-67 antigen, a non-histone protein useful for the identification of proliferating cells that has no specific phase, is expressed in all active phases of cell cycle (Ki-67 is not expressed in G0 phase) (Keating JT, Ince T, Crum PA, 2001). An increase of Ki-67 expression shows an increased mitotic activity and cell proliferation (Taylor RJ et al., 2003). Ki-67 expression is normally increased at tendometrium level during the proliferative phase of the menstrual cycle (Taylor RJ et al., 2003).

We completed the study of cell proliferation with this antibody precisely because it is a more accurately marker of mitotic activity and is not expressed in cells when DNA repair occurs.

Low values of mitotic activity in endometrial hyperplasia, highlighted by Ki-67 index, are due to an increase in the expression of NCoR (co-repressor of steroids receptors), which blocks estrogen-dependent growth (Ukikawa et al., 2003).

Thus, it could be explained why mitotic activity within atypical hyperplasia was slightly lower compared with non-atypical hyperplasia from this study, although estrogen receptors were better represented in the first, but probably repressed.

Monoclonal anti-EMA antibody (clone E29, subclass IgG2, kappa) recognizes the antigen epitopes of epithelial membrane (Cordell J et al., 1985).

Every normal, hyperplastic and neoplastic endometrium that was analyzed showed a positive marking to anti-EMA antibody.

The EMA immunomarking pattern was predominantly of luminal membrane (with a higher intensity at the apical pole) in cases of proliferative and secretory endometriums as well as for simple and complex hyperplasia without atypia.Atypical hyperplasia and endometrial carcinoma had, more frequently, a cytoplasmic pattern, sometimes associated with the luminal type.Also, the results showed that the EMA cannot distinguish between complex atypical hyperplasia and endometrial carcinoma.

Oncoprotein p53 is a Kd 53 phosphoprotein, encoded by p53 gene located on the short arm of chromosome 17 (Ardeleanu C et al., 1999). Monoclonal antibody used in this study recognizes both wild type and mutant type of protein p53, by binding to a distortion-resistant epitope located in the protein extreme N-terminal.

The immunohistochemical methods of detecting P53 are based on the accumulation of

p53 protein in cells.The wild type of p53 may accumulate including on cellular hypoxia or DNA alterations. In addition, not all abnormal mutations of p53 cause accumulation of p53 and may be a cause of false negative results (Bostwich DG et al., 2002).

Cases that showed positive immunoreactivity to p53 belonged to the endometria with complex (3 cases, 30%) and atypical hyperplasia (6 cases, or 60%). Also, all cases of endometrial adenocarcinoma were p53 positive. The positive imnunomarking to p53 was observed in some isolated cells in cases of endometrial hyperplasia and low grade squamous endometrial G1 (Maia H et al., 2001).

We considered, after correlating the results obtained at p53 immunomarking with the ones obtained by cell proliferation, that the cell "arrest" in phase S due to wild p 53 explains why the average Ki-67 index decreased with the hyperplasia increase, because the number of cases of p53 positive, in which cell division was blocked, increased with the increase of hyperplasias. Thus, Ki-67 index, which detects mitotic cells, decreases.

Conclusions

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.

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 imunomarking, 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.

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Correspondence Adress: *Daniela Ilie MD, PhD student, Department of Obstetrics and-Gynecology, Emergency County Hospital Slatina, email:dr_danailie@yahoo.com*