IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL ADENOCARCINOMA

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IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL ADENOCARCINOMA (Abstract)  
Aim: Endometrial hyperplasia is a proliferation of endometrial glands due to the prolonged stimulation with estrogens of the endometrium that occurs in women receiving exogenous estrogens, with anovulatory cycles, or in patients with ovarian tumours with estrogen secretion. 

Material and methods: The study performed by the authors included 575 patients with endometrial hyperplasia and 163 patients with endometrial adenocarcinoma admitted to the “Cuza-Vodă” Obstetrics and Gynæcology Clinical Hospital of Iasi, between 2005-2007. 

Results: There were selected, for these immunohistochemistry reactions, 22 cases of simple hyperplasia without atypia, 26 cases of complex hyperplasia without atypia, 23 cases of endometrial adenocarcinoma of endometrioid type, well differentiated, 22 cases of endometrial adenocarcinoma of endometrioid type, moderately differentiated, and 19 cases of non-endometrioid adenocarcinomas represented by nine clear cells and 10 serous endometrial adenocarcinomas. 

Estrogen receptors have been positive in about 85-90% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the estrogen receptors were positive in approximately 70-85% of the tumour cells. 

Conclusions: Endometrial hyperplasia, especially complex endometrial hyperplasia with atypia, increase the risk for endometrial adenocarcinoma, and their early detection becomes mandatory under cancer prevention. Well-differentiated endometrioid endometrial adenocarcinomas were ER and PR-positive, so that the ER expression correlated with the PR expression. Well-differentiated endometrioid endometrial adenocarcinomas (GI) in the studied group also showed a higher content of ER and PR compared to the endometrial moderately-differentiated endometrioid endometrial adenocarcinomas (GII). In non-endometrioid adenocarcinomas, represented by clear-cell endometrial adenocarcinomas, the ER content was reduced and the PR expression was negative. Serous adenocarcinomas failed to show an immunohistochemically expression for ER and PR.

Keywords: IMMUNOHISTOCHEMISTRY, ADENOCARCINOMA, ENDOMETRIUM.

The link between endometrial hyperplasia and endometrial carcinoma was mentioned in several studies. Some of them have shown a development of the endometrial carcinoma in 2% of the complex endometrial hyperplasia without atypia and
20-30% of the complex atypical hyperplasia, therefore the complex atypical hyperplasia is a precursor of endometrial carcinoma (1).

Pesce and al. (1) describe two subtypes of endometrioid adenocarcinoma, represented by adenocarcinoma with trophoblastic differentiation and adenocarcinoma with Sertoli form differentiation. The endometrioid adenocarcinoma with trophoblastic differentiation has giant multinucleated cells, of the syncytiotrophoblastic type, that are immunoreactive to HGG, while the cells of the endometrioid adenocarcinoma with Sertoli form differentiation are similar to the ovarian tumours with the same differentiation type.

The expression of PTEN (Phosphatase and tensin homologue deleted on chromosome ten) is an oncosupressor gene of the same class of p53, rbp and APC recently identified in the Cz 10q23 (LI) region, which encodes a protein with 403 amino acids, of the multifunctional phosphatase type, with a very wide tissue nuclear expression, which is involved in the regulation of the cell cycle and apoptosis. PTEN's tumour suppressor gene mutation has been reported in approximately 50-83% of endometrial adenocarcinoma.

PTEN is expressed in most human tissues. PTEN deletions and mutations occur not only in endometrial carcinomas but also in glioblastoma, prostate carcinoma, lung carcinoma and mammary carcinoma, and it is mostly associated with invasiveness.

PTEN inactivation in endometrioid adenocarcinomas and in many other types of tumors cannot be explained only on the basis of the observed mutations. This observation suggests that the expression of PTEN is suppressed at transcriptional and translational level through other mechanisms (12).

With the help of the immunohistochemically evaluation of the estrogens (ER) and progesterone (PR) it is better appreciated their distribution in tumour tissues and normal peritumoral tissues. In endometrial carcinoma, the ER/PR status, in particular PR, correlate with histological differentiation and survival. In well-differentiated carcinoma, the ER/PR positivity is more common and it is associated with a better prognosis. It is believed that an ER/PR positive endometrial carcinoma responds better to hormonal therapy (9).

Cyclooxygenases COX-1 and COX-2 are enzymes which play an important role in the synthesis of prostaglandins from the arachidonic acid. COX-2 expression has been related to carcinogenesis and the COX-2 specific inhibitors that have anti-tumour effects. The immunohistochemically expression of COX-2 has been reported in a variety of normal and neoplastic tissues.

Her-2/neu /cerbB2 oncogene is part of the EGFR genes family. The detection of Her-2/neu in gynaecological tumours is important, both as a prognostic marker and also to qualify the Herceptin therapy. The normal endometrial glands are weakly to moderately positive for Her-2/neu. Compared with normal endometrium, 9% of the endometrial adenocarcinoma demonstrate a stronger positivity for Her-2/neu.

Ki-67 is a nuclear factor of the cellular proliferation, with protein structure with two isoforms.

P53 is a tumour suppressor gene, located on the short arm of chromosome 17. P53 is most frequently positive in the papillary serous adenocarcinoma and also in other carcinomas with poor prognosis, with a high degree of malignancy and high prolif-
K-ras oncogene mutations have been detected in 10-25% of endometrial carcinomas and 17% of the atypical hyperplasia (3). K-ras activation is an early event in the carcinogenesis of endometrial carcinoma. Studies have shown that K-ras oncogene is an independent negative prognostic factor (4).

MATERIAL AND METHODS

The study included 575 patients with endometrial hyperplasia and 163 patients with endometrial adenocarcinoma admitted to the “Cuza-Vodă” Obstetrics and Gynaecology Clinical Hospital of Iasi, between 2005-2007.

The collection, processing and interpretation of the information required for the clinical-statistical study on knowing the peculiarities of the cases were performed using data recorded in the case report forms and in the computer system.

The immunohistochemistry reactions performed on the cases in the hyperplasia and endometrial adenocarcinoma group in our study had in view the assessment of the hormone receptors for estrogen (ER) and progesterone (PR), the PTEN gene protein, the Her2/neu (cerb-2)/ gene protein, the nuclear factor of Ki-67 cell proliferation, cyclooxygenase 2 (COX-2), metalloproteinases (MMP-2 and MMP-9).

The data used as study material from the patient charts refer to the division of cases into age groups, their distribution according to their residence environments, according to their profession, to their admission reasons, to personal physiological history, to personal pathological history, and to family history relevant for endometrial carcinoma.

The age of patients with endometrial carcinoma is an important prognosis factor independent from other parameters. In our study, the patients with endometrial carcinoma were aged 30 to 70, with an average age of 58.94 years. The highest percent – 29.3% - are patients over 65 years old. The percent of patients younger than 50 was 6.84% of the cases. The socioeconomic status can be perceived as a risk in developing endometrial adenocarcinoma, as well as a condition for the increase in the rate of women who submit to specialty consultation, due to a high education level enabled precisely by their high standard of living. Moreover, the low socioeconomic level is a factor affecting negatively the subsequent monitoring of patients who can develop endometrial adenocarcinomas.

RESULTS

There were selected, for these immunohistochemistry reactions, 22 (3,82%) of simple hyperplasia without atypia, 26 (4,52%) of complex hyperplasia without atypia, 23 (4%) of endometrial adenocarcinoma of endometrioid type, well differentiated, 22 (3,82) of endometrial adenocarcinoma of endometrioid type, moderately differentiated, and 19 (3,3%) of non-endometrioid adenocarcinomas represented by nine clear cells and 10 (1,74%) serous endometrial adenocarcinomas.

Estrogen receptors were present in different percentages in the studied hyperplasia and endometrial adenocarcinomas (table I). The marking recognized amount is represented by the nuclear marking.

In simple endometrial hyperplasia without atypia, estrogen receptors were positive in 85-95% of the glandular epithelial cells. In complex endometrial hyperplasia without atypia, the estrogen receptors were positive in 70-90% of the glandular epithe-
Estrogen receptors have been positive in about 85-90% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the estrogen receptors were positive in approximately 70-85% of the tumour cells. The estrogen receptors in clear-cell endometrial adenocarcinomas were positive in 40-45% of the tumour cells. In serous endometrial adenocarcinomas, the estrogenic receptors were negative in the tumour cells and they showed positivity only in the remaining glandular epithelium (table I).

<table>
<thead>
<tr>
<th>Histological type</th>
<th>No. Cases</th>
<th>ER %</th>
<th>PR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple hyperplasia without atypia</td>
<td>22</td>
<td>85-95</td>
<td>85-95</td>
</tr>
<tr>
<td>Complex hyperplasia without atypia</td>
<td>26</td>
<td>70-90</td>
<td>80-90</td>
</tr>
<tr>
<td>Well-differentiated endometrioid ADK (GI)</td>
<td>23</td>
<td>85-90</td>
<td>90-95</td>
</tr>
<tr>
<td>Moderately-differentiated endometrioid ADK (GII)</td>
<td>22</td>
<td>70-85</td>
<td>80-85</td>
</tr>
<tr>
<td>Clear-cell endometrial ADK</td>
<td>9</td>
<td>40-45</td>
<td>0</td>
</tr>
<tr>
<td>Serous cells endometrial ADK</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The cases of simple endometrial hyperplasia without atypia expressed progesterone receptors in 85-95% of the glandular epithelial cells. In complex endometrial hyperplasia without atypia, the progesterone receptors were positive in 80-90% of the glandular epithelial cells.

Progesterone receptors were positive in 90-95% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI).

In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the progesterone receptors were positive in 80-85% of the tumour cells.

In serous endometrial adenocarcinomas, the progesterone receptors were negative in the tumour cells and they showed positivity only in the remaining glandular epithelium.

The progesterone receptors were negative in clear-cell endometrial adenocarcinomas and they were positive only in the remaining glandular epithelium (table I).

In simple hyperplasia and complex hyperplasia without atypia, PTEN showed regional positivity in the glandular epithelial cells. The reaction was negative in a single case of complex hyperplasia without atypia. PTEN showed regional positivity in the tumour cells of the endometrial adenocarcinoma tumor of endometrioid type, well differentiated (GI) and moderately differentiated (GII).

In serous endometrial adenocarcinomas, PTEN gene protein was positive in rare tumor cells and diffusely positive in the remaining glandular epithelium.

The PTEN gene protein was regionally positive in the tumour cells of the clear-cell endometrial adenocarcinomas.
In terms of the immunohistochemical expression of C-erbB2 (Her2/neu/cerb-2 gene protein), in endometrial adenocarcinomas of endometrioid type and in clear-cell endometrial adenocarcinomas of our lot, the reaction to cerb-2 was negative. In serous endometrial adenocarcinomas, cerb-2 showed regional positivity in tumor cells, scored with 2+.

The positivity of Ki-67 reaction was approximately 2-15% of the glandular epithelial cells in the simple endometrial hyperplasia without atypia. In complex endometrial hyperplasia without atypia, Ki-67 was positive in about 3-10% of the glandular cells. Ki-67 was positive in about 25-30% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), Ki-67 was positive in approximately 20-40% of the tumor cells. Ki-67 was positive in about 60-65% of the tumor cells in the clear-cell adenocarcinomas. In serous endometrial adenocarcinomas, Ki-67 was positive in about 50-60% of the tumour cells.

In simple endometrial hyperplasia without atypia, COX-2 is variably expressed in glandular epithelial cells from weak regional positivity to diffuse positivity. COX-2 shows a weakly positive regional reaction in the glandular epithelial cells of the complex endometrial hyperplasia without atypia. In the well-differentiated endometrial adenocarcinoma of the endometrioid type (GI) and moderately differentiated ones (GII), COX-2 is expressed diffusely in all the tumor cells. COX-2 is diffusely present in the cytoplasm of the tumor cells of the serous endometrial adenocarcinomas.

In simple endometrial hyperplasia without atypia, MMP-2 showed only in one case a weak positive reaction in the epithelial cells and a positive reaction in the stromal cells. In complex endometrial hyperplasia without atypia, MMP-2 showed only in one case a regional positive reaction in the epithelial cells and a regional positive reaction in the stromal cells. In well-differentiated endometrioid endometrial adenocarcinomas (GI) MMP-2 showed positive reaction in rare tumor cells and negative reaction in stromal cells. In moderately-differentiated endometrioid endometrial adenocarcinomas (GII), MMP-2 showed weak regional positive reaction in tumor cells only in one case and negative reaction in the stroma in all the cases. In serous endometrial adenocarcinomas, MMP-2 showed weak positive reaction in tumor cells and stromal cells. In clear-cell endometrial adenocarcinomas, MMP-2 has shown a negative reaction in both tumor cells and stroma.

MMP-9 showed a regional positive reaction in the epithelial cells and a regional positive reaction in stromal cells in simple endometrial hyperplasia without atypia. In complex endometrial hyperplasia without atypia, MMP-9 showed regional positive reaction in the epithelial cells and regional positive reaction in the stromal cells. In well-differentiated endometrioid endometrial adenocarcinomas (GI), MMP-9 showed regional positive reaction in the tumor cells and negative reaction in stromal cells. In moderately-differentiated endometrioid endometrial adenocarcinomas (GII), MMP-9 showed, in 2 cases, regional positive reaction in tumor cells and negative reaction in stromal cells in all cases. In one case it showed diffusely positive reaction in tumor cells and regional positive in stromal cells. MMP-9 showed a diffuse
positive reaction in tumour cells and stromal cells of serous endometrial adenocarcinomas. In clear-cell endometrial adenocarcinomas, MMP-9 showed weak positive reaction in tumor cells and stromal cells.

**DISCUSSION**

The completion of the histopathologic diagnosis in endometrial hyperplasias and adenocarcinomas with the immunohistochemical study has unquestionable value in assessing the prognosis of these lesions (13).

The results of the immunohistochemically study of ER and PR, PTEN, MMP-2, MMP-9, C-erbB-2, COX-2 and Ki 67 showed the value of these markers in differentiating the complex endometrial hyperplasia from the endometrioid adenocarcinomas.

The benign squamous metaplasia adenocarcinoma presents immunohistochemically positivity for ER and PR and it is rarely positive at Ki-67 and p53.

The malignant squamous metaplasia adenocarcinoma presents rare positivity for ER and PR and it is positive at Ki-67 and p53. No aspects of stromal invasion are identified at the serous intraepithelial carcinoma. In terms of immunohistochemistry, tumour cells are positive for Ki-67 and p53 and with focal positivity for ER and PR.

In our study, in simple and complex endometrial hyperplasia with no atypia, PTEN showed regional positivity in glandular epithelial cells, and in only one case of complex hyperplasia with no atypia the reaction was negative.

Both in the well-differentiated endometrioid endometrial adenocarcinomas (GI) and in the moderately differentiated adenocarcinomas (GII) in our cases, the expression of PTEN was regionally positive in tumour epithelial cells.

In weakly differentiated adenocarcinomas, represented by serous adenocarcinomas and clear-cell adenocarcinoma, the PTEN expression was weak.

In a study on the development of endometrial carcinomas, show that after various molecular alterations, represented by microsatellite instability and mutations of PTEN, K-ras and beta-catenin, the endometrial cells are transformed in endometrioid carcinoma, and the modification of the expression of p53 protein and the loss of LOH (loss of heterozygosity) on different chromosomes, leads to the development of endometrial non-endometrioid tumours (5).

PTEN is an early change that occurs in the pathogenesis of the endometrioid endometrial adenocarcinoma (6). These studies demonstrate that the immunohistochemically identification of PTEN protein is an important marker for endometrial premalignant lesions.

The PTEN mutations are important in the pathogenesis of the endometrioid endometrial adenocarcinoma. These mutations are shown in the normal mucosa, in postmenopausal women and in complex endometrial hyperplasia, aspects which suggest that PTEN mutations show an early change that occurs in the pathogenesis of the endometrioid endometrial adenocarcinoma (6). These studies demonstrate that the immunohistochemically identification of PTEN protein is an important marker for endometrial premalignant lesions (10).

Some authors (7) have demonstrated that the number of the progesterone receptors is dependent on the concentrations of estradiol and the progesterone in the serum. It was found that PR is more often positive in endometrial hyperplasia without atypia compared to the hyperplasia with atypia.
PR is expressed at a lower level in the endometrial carcinoma compared to the endometrial hyperplasia.

In endometrial carcinomas, the Her-2/neu positivity is correlated with ER negativity and it is associated more commonly with the existence of metastasis. This suggests that Her-2/neu positivity is associated with a much more aggressive evolution (8).

In our study, regional positivity scoring 2+ was found only in the serous endometrial adenocarcinoma, which is considered a weakly-differentiated carcinoma. In the clear-cell carcinomas and the endometrioid adenocarcinomas under study, the C-erb-2 reaction was negative.

In our cases, in endometrial hyperplasia, the MMP-2 and MMP-9 expressions were different, with a better expression of the MMP-9 than that of the MMP-2. MMP-9 showed regional expression in glandular epithelial cells and in stromal endometrial cells, both in simple hyperplasia with no atypia and in complex hyperplasia with no atypia. MMP-2 showed regional positivity in glandular epithelial cells only in 50% of simple and complex hyperplasia with no atypia, which was accompanied by a positive reaction in the endometrium stroma.

The antigenic profile of endometrial adenocarcinomas is different. The endometrioid adenocarcinoma is positive to ER and PR, negative to C-erb B-2 and it shows low positivity for Ki67. The non-endometrioid adenocarcinoma is negative to ER and PR, positive to C-erb B-2 and it shows a high Ki67 proliferation risk. The endometrioid adenocarcinoma is associated more frequently with PTEN mutations, compared to the non-endometrioid adenocarcinoma. In endometrial adenocarcinomas, MMP-9 is expressed more intensely than MMP-2 and it correlates with a low degree of differentiation of adenocarcinomas. In our study, COX-2 shows a positive diffuse reaction in most endometrial adenocarcinomas and in non-endometrioid adenocarcinomas (11).

In the endometrial hyperplasia of the cases under study, the expression of COX-2 showed a weak positive reaction regionally in glandular epithelial cells. An exception was a case of simple glandular hyperplasia with no atypia, in which the COX-2 was positive diffusively in glandular epithelial cells; this reaction can be explained by the patient’s young age, correlated to her hormonal status (active genital period).

In all endometrial adenocarcinomas in our study, the COX-2 showed a positive reaction diffused in the tumoral cells, except for a case of endometrioid endometrial carcinoma GII, which showed a weak positive regional reaction.

In our cases, we noticed a positive correlation between the level of the Her-2/neu signal and the COX-2 expression in the serous endometrial carcinoma.

**CONCLUSIONS**

In our case studies, well-differentiated endometrioid endometrial adenocarcinomas (GI) and moderately differentiated (GII) ones had a relatively low content in the ER and PR of the tumor cells compared to the simple and complex hyperplasia without atypia. Well-differentiated endometrioid endometrial adenocarcinomas were ER and PR-positive, so that the ER expression correlated with the PR expression. Well-differentiated endometrioid endometrial adenocarcinomas (GI) in the studied group also showed a higher content of ER and PR compared to the endometrial moderately-differentiated endometrioid endometrial adenocarcinomas (GII).

In non-endometrioid adenocarcinomas,
represented by clear-cell endometrial adenocarcinomas, the ER content was reduced and the PR expression was negative. Serous adenocarcinomas failed to show an immunohistochemically expression for ER and PR.

The loss of alleles and mutations in p53 have been detected not only in endometrial carcinoma but also in atypical hyperplasia. These findings suggest that p53 inactivity is a late event in the endometrial carcinogenicity.

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