Expression of estrogen receptors (ER) and p53 in endometrial carcinoma and correlated with of histological type, grade and stage

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Abstract
Objective. The differences in immunohistochemical expression of estrogen receptors (ER) and p53 in endometrial carcinoma and correlated with of histological type, grade and stage are crucial in the planning of further monitoring and treatment of patients. Materials and methods. This paper deals with data of the patients treated for endometrial carcinoma in Public Hospitals in Travnik, gynecological department in the period from 01.01.2007. to 01.01.2013 the sample consisted of 97 women with endometrial carcinoma, with ages ranging from 42 to 90 years (mean of 64 years). 72 cases (74.2%) were of endometrioid and 25 (25.8%) nonendometrioid carcinoma. Results. The 72 endometrioid carcinoma cases were final disease stage as follows: stage I = 29/72 (40%) and stages II, III et IV = 43/72 (60%). Markers and histological types: p53 expression was found in 10 patients (13.8%) of the endometrioid and 17 patients (68%) of the nonendometrioid endometrial carcinoma. The estrogen receptors were more frequent in the endometrioid type 44 patients (61%) with regard to 7 patients of the nonendometrioid type endometrial carcinoma. Markers and histological grade: In the 72 cases of endometrioid carcinoma, those with grade I expressed estrogen (22 out of 36 cases = 61.1%) more frequently than those with grades II and III. p53 was expressed in only 3.8% of endometrioid carcinomas. Conclusion. The different immunohistochemical profiles of endometrioid and nonendometrioid carcinomas confirm different molecular pathways in their development. The correlation of immunohistochemical findings with histological grade and clinical stage could help in predicting biologic behavior and planning treatment in patients who are diagnosed as having these tumors.
1. Introduction

In developed countries, endometrial carcinoma is the most common malignant neoplasm of the female genital tract. Peak incidence of serous and clear cell endometrial adenocarcinoma around 68th year. Only 1-8% of endometrial carcinoma occurs before the 40th year. The differences in the epidemiology, presentation and biological behavior of endometrial carcinoma suggest that there are two types of pathogenic diseases- type I and type II. Type I endometrial carcinoma known as estrogen dependent or endometrioid type, and the type II estrogen independent or non endometrioid type. Type I accounts for two thirds of endometrial carcinoma and occur in younger perimenopausal women with hyperlipidemia, overweight, hyperestrogenism, anovulatory bleeding, infertility, late menopause, ovarian stromal hyperplasia and endometrial hyperplasia. In contrast, type II endometrial carcinoma occurs in older postmenopausal women when absent exogenous or endogenous hyperestrogenism and endometrial hyperplasia (1, 2, 3).

In type I exist PTEN mutation, in type II over expression of p53. Mutations or increased expression of the p53 tumor suppressor gene occurs in 10-48% of endometrial carcinoma, in poorly differentiated carcinoma with no steroid receptor for advanced stage with poor prognosis (4, 5). Given that a mutation of the p53 gene is absent in endometrial hyperplasia, it is believed that it represents a late event in the pathogenesis of endometrial carcinoma of endometrioid type (6,7). Anti oncogenes p53 mutations could be found in 45-85% of serous endometrial cancer, even in early stages (8).

Carcinoma of endometrioid type contain receptors for estrogen and progesterone. Endometrial carcinoma non endometrioid type usually does not contain these receptors. Levels of estrogen and progesterone receptors are high in hyperplastic endometrium and higher in endometrial hyperplasia without nuclear atypia than in atypical hyperplasia (9, 10).

Known are two separate types of estrogen receptors: ERα with 595 amino acids and ERβ with 530 amino acids. ERα gene is located on chromosome 6, ERβ gene on chromosome 14, which indicates that there are two different receptors (11). The presence of estrogen receptors ERα are associated with low grade and early stage of the disease (12). Determining ER status may also help doctors to plan treatment when adjuvant therapy is controversial (8, 15).

The last revision of the classification of tumors of the uterus has been carried out in 2003. The World Health Organization (WHO) defines a stepped system in determining the grade of endometrioid adenocarcinoma where squamous carcinoma component of the tumor has no effect. Histological grade of endometrial cancer was determined according to FIGO criteria, which are defined as follows: grade 1-less than 5% of tumors are solid fields, grade 2- 6-50% of tumors are solid fields and grade 3-more than 50% of tumors are solid fields (16, 17). After staging the team of deciding decides about the most optimal form of treatment. For localized disease the treatment of choice is surgical intervention, total hysterectomy with bilateral adnexectomy. After the surgical intervention implemented postoperative radiotherapy to the stage of the disease. Radiotherapy is carried combined-through the skin (external, transcutaneous) and vaginal (internal, intracavitarily). Can be combined with chemohormonal therapy (based on progesterone) (5, 18).

The aim of the this study was to determine the expression of p53 and ER and histological types, histological grades and determine the correlation between the expression of p53 and ER and pTNM stage of the disease.

2. Patients and Method

This paper deals with data of the patients treated for endometrial carcinoma in Public Hospitals in Travnik, gynecological department in the period from 01.01.2007. to 01.01.2013th the sample consisted of 97 women with endometrial carcinoma, with ages ranging from 42 to 90 years (mean of 64 years).

The study include 97 patients with a diagnosis of endometrial carcinoma, according to the current TNM classification, in accordance with the classification of the International Federation of Gynecologists and Obstetricians (FIGO). For the study was use the data from the medical records of patients archived histopathological reports. For research and display of endometrial carcinoma were used paraffin blocks of tissue samples diagnosed adenocarcinoma who were initially fixed in 10% formalin and then embedded in paraffin. On histological sections of the most representative parts of the tumor thickness 3μm underwent immunohistochemical analysis.

Immunohistochemical staining: Individual preparations are fixed in paraffin and cut at 3μm routinely deparaffinized methanol with 0.3% hydroperoxic acid for 30 minutes at room temperature to block endogenous peroxidase activity. After that cause estrogen α antibody and p53. If the antibodies bind to specific proteins on the cell surface of tumor finding is positive, if the antibodies do not bind to specific proteins on the cell surface of the tumor findings were negative. The results were confirmed by microscopic examination.

For each patient, it was made a separate questionnaire was administered remanded following data: age, general condition, blood type, associated diseases (anemia, hypertension, diabetes, obesity, other diseases), the time of occurrence first symptoms, macropathological findings in the abdomen, the presence of malignant cells in ascites, macropathological findings on the uterus, macroscopically enlarged lymph nodes, type of surgery, tumor diameter in mm, stage endometrial carcinoma, microscopic appearance, histological tumor grade.

Testing should show the frequency of endometrial
carcinoma type I and type II endometrial carcinoma. Up regulation of p53 occurs in type II endometrial carcinoma, and the poorly differentiated carcinoma without steroid receptors, and advanced stage with poor prognosis. Estrogen α antibody is extensively bound to receptors on the cell surface of tumors in carcinoma type I.

Immunohistochemical studies are highly sensitive detection method and type of the tumor with the help of specific antibodies bind to receptors on the cell surface of the tumor.

Two-thirds of all endometrial carcinoma accounted for type I endometrial carcinoma, which was detected by binding estrogen α antibody and p53 antibody and verified by microscopic examination. Determining ER status and expression of the p 53 can help doctors in the treatment of patients when adjuvant therapy is controversial.

3. Statistical Analysis

Statistical analysis results were used descriptive statistics methods such as total sample size for the data set. To test the statistical significance of differences among samples were used parametric and non-parametric tests of significance (X2 test, Student's t-test, Kruskal-Wallis test), and the method of linear correlation (Pearson's correlation coefficient and Spearman's rank correlation coefficient). x2 statistics were used to test for correlations. Statistical significance was considered achieved when the p value was less than or equal to 0.05. All statistical analyses were performed using the statistical program SPSS 20 for Windows.

4. Results

The sample consisted of 97 women with endometrial carcinoma, with ages ranging from 42 to 90 years (mean of 64 years). 72 cases (74.2%) were of endometrioid and 25 (25.8%) nonendometrioid carcinoma (Figures 1 and 2).

According to the stage of the disease the majority of the patients with endometrioid (60%) and nonendometrioid (52%) carcinoma were diagnosed in stage II, III et IV (Table 1).

Markers and histological types: p53 expression was found in 10 patients (13.8%) of the endometrioid and 17 patients (68%) of the nonendometrioid endometrial carcinoma (Figure 3). The estrogen receptors were more frequent in the endometrioid type 44 patients (61%) with regard to 7 patients (28%) of the nonendometrioid type endometrial carcinoma (Figure 4) and (Table 2).

Markers and histological grade: In the 72 cases of endometrioid carcinoma, those with grade I expressed estrogen receptors (26 out of 36 cases = 72%), more frequently than those with grades II and III. p53 was expressed in only 13,8% of endometrioid carcinomas (Table 3).

Markers and final disease stage: 8 patients in stage I had p53 expression and 17 patients had expression p53 in stage II, III and IV. We observed increase p53 positivity in the advanced stages.
The presence of estrogen receptors had in 21 patients (51.2%) in stage I and 28 patients in stage II, II and IV (Table 4).

![Fig 4. Nuclear estrogen receptor immunostaining in endometrioid adenocarcinoma.](image)

**Table 1. Stages and histological types of endometrial carcinoma specimens.**

<table>
<thead>
<tr>
<th>Stage of disease</th>
<th>Endometrioid carcinoma n (%)</th>
<th>Nonendometrioid carcinoma n (%)</th>
<th>Total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>29 (40)</td>
<td>12 (48)</td>
<td>41</td>
</tr>
<tr>
<td>II, III, IV</td>
<td>43 (60)</td>
<td>13 (52)</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>72 (100)</td>
<td>25 (100)</td>
<td>97</td>
</tr>
</tbody>
</table>

**Table 2. p53 and estrogen receptor expression and histological endometrial carcinoma.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Endometrioid carcinoma n (%)</th>
<th>Nonendometrioid carcinoma n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>10 (13.8)</td>
<td>17 (68)</td>
<td>0.005</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>44 (61)</td>
<td>7 (28)</td>
<td>0.326</td>
</tr>
</tbody>
</table>

p= significance by Fisher Exact test.

**Table 3. p53 and estrogen receptor expression and histological grade of endometrioid carcinoma.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Endometrioid carcinoma grade I n (%)</th>
<th>Endometrioid carcinoma grade II n (%)</th>
<th>Endometrioid carcinoma grade III n (%)</th>
<th>Total n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>2/36 (5.5)</td>
<td>3/29 (10.3)</td>
<td>5/7 (71.4)</td>
<td>10/72 (13.8)</td>
<td>0.098</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>26/36 (72.2)</td>
<td>14/29 (48.2)</td>
<td>4/7 (57.1)</td>
<td>44/72 (61)</td>
<td>0.679</td>
</tr>
</tbody>
</table>

p= significance by Fisher Exact test.

**Table 4. p53 and estrogen receptor expression and endometrial carcinoma stages.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Stage I n (%)</th>
<th>Stages II, III, IV n (%)</th>
<th>Total n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>8/41 (19.5)</td>
<td>17/56 (30.3)</td>
<td>25/97 (25.7)</td>
<td>0.03</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>21/41 (51.2)</td>
<td>28/56 (50)</td>
<td>49/97 (50.5)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

p= significance by Fisher Exact test.

5. Discussion

Current concepts of endometrial carcinoma successfully integrate traditional histopathology with pathogenetic mechanisms. Endometrial carcinoma have long been classified into two major divisions (types I and II) based on light microscopic appearance, clinical behavior, and epidemiology. Type I, those with endometrioid histology, comprise 70% to 80% of newly diagnosed cases of endometrial carcinoma in the United States. They are associated with unopposed estrogen exposure and are often preceded by premalignant disease. In contrast, type II endometrial carcinoma have nonendometrioid histology (usually papillary serous or clear cell) with an aggressive clinical course.

Our results agree with the literature with regard to the higher frequencies of the endometrioid type and higher histological grade 40 vs. 60% carcinoma. While most nonendometrioid (type II) carcinoma contain mutations of p53, endometrioid (type I) carcinoma demonstrate larger numbers of genetic changes in which the temporal sequence of mutation, and the final combination of defects differ substantially between individual examples.

According to the stage of the disease the majority of the patients with endometrioid (60%) and nonendometrioid (52%) carcinoma were diagnosed in stage II, III et IV. The overall rate of p53 positivity in our study was found in 10 patients (13.8%) of the endometrioid and 17 patients (68%) of the nonendometrioid carcinoma. The difference in p53 over expression between the histological grades of endometrioid carcinoma grade I=5.5%, grade II=10.3% and grade III=71.4%.

This indicates possible mutation of p53 as an early event, which is typical of the carcinogenic pathway for nonendometrioid carcinoma (10). Conversely, estrogen receptors are more frequently positive in endometrioid carcinoma, particularly if well-differentiated, as reported by others (14). We were able to confirm that p53 expression is inversely related to that of estrogen receptors, thus indicating a dual theory of carcinogenesis in the endometrium (15).

p53 and estrogen receptor expression and endometrial carcinoma stages we found the following p53 was present in 19,5% patients in stage I and in stage II, III, and IV was present in 30.3%. Estrogen receptor was present in 51.2% patients in stage I and 50% in stage II, III and IV. It has
been reported that p53 expression correlates with more advanced stages. Although we found slightly greater p53 expression in stages II and III, this was not statistically significant.

This finding is in agreement with the study of Doll et al. (16) as well as with similar correlations of p53 positivity with high nuclear grade in infiltrating duct carcinoma of the breast. The estrogen receptors were more frequent in the endometrioid type 44 patients (61%) with regard to 7 patients of the nonendometrioid type endometrial carcinoma. Hormonal risk factors have not been identified, and there is no readily observed premalignant phase. The morphologic and clinical differences are paralleled by genetic distinctions, in that type I and II cancers carry mutations of independent sets of genes.

6. Limitation of Study

Criteria for non-inclusion in the study: Patients who do not wish to participate in the study and criteria for exclusion from the study: Patients who have a different malignant gynecological disease.

7. Conclusion

The result is an enhanced understanding of early genetic events, reinforcement of the clinicopathologic subgroups originally defined by histological and clinical features, and development of biomarkers informative in identification of previously unknown or poorly described precursor lesions. Nonendometrioid carcinoma predominated in elderly women, as expected, although the frequency of 14% was higher than found in other studies (5,6). This difference may be attributable to the relatively small size of the population studied and the fact that our service is a reference center in which complex cases are concentrated.

Authors’ Contribution

Conception and design: MB and SM; Acquisition, analysis and interpretation of data: MB and SM; Drafting the article: SM and AS; Revising it critically for important intellectual content: SM and AS.

Conflict of Interest

The authors declare that they have no conflict of interest.

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