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The expression analysis of the estrogen and progesterone receptors in endometrial carcinomas

Endometrial carcinoma is currently the most frequently occurring malignant neoplasm of female genital organs in highly developed countries. Considering the background in which the neoplasm develops, there are two types of endometrial carcinoma. The first type, hormone-dependent, generates on the basis of endometrial hyperplasia. It is associated with the stimulation of the endometrium by estrogens, which are unbalanced by progesterone (14). It occurs in women before menopause, and usually has a high degree of differentiation and favourable prognosis. The second type of carcinoma with bad prognosis occurs most often in women after menopause. It develops *de novo* on the basis of atrophic endometrium, independent of estrogen stimulation. From the clinical viewpoint, the receptor state of endometrial carcinomas, especially the progesterone receptor, is not only the prognostic factor but also the factor of sensitivity and susceptibility of these carcinomas to hormonal therapy applied in non-operative cases. About 20% to 40% of all recurrent and metastatic lesions react to hormonal gestagen therapy (13).

The aim of the study was the expression analysis of the estrogen receptor (ER) and the progesterone receptor (PR) in endometrial carcinomas. The expression of each of the receptors was examined with reference to such parameters as: patients' age, the relation to menopause, histological type and grading, clinical staging, the depth of myometrial infiltration and the presence of endometrial hyperplasia adjacent to the tumour.

MATERIAL AND METHODS

The study group included 42 females operated on for endometrial carcinoma. The women were 47 to 81 years old (mean age 61.6 years). In all cases surgical material was obtained after total hysterectomy with adnexis. Material was fixed in 10% buffered formaline. After gross examination tissue samples were embedded in paraffin and stained using H+E. A tumour typing and grading according to: WHO as well as staging according to FIGO classification were assessed. For immunohistochemistry two paraffin blocks from each case were chosen containing neoplastic tissue

and neighbouring endometrium. Immunohistochemical staining was performed on 3μm thick sections using ABComplex/HRP (DAKO) method. The following primary antibodies were used: monoclonal mouse anti-human Estrogen Receptor, M7047, clone: 1D5 (DAKO) (dilution 1:50) and polyclonal rabbit anti-human Progesterone Receptor, A 0098 (DAKO) (dilution 1:25). After dewaxing and dehydrating the sections were placed in citrate buffer at pH 6.0 and heated 2x5 min in microwave oven (750W). The endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution for 20 min. Then they were incubated 20 min with normal rabbit serum for estrogen receptor or swine serum for progesterone receptor. The primary antibodies and ABComplex/HRP was incubated at room temperature for 30 min each. For colour staining 3,3' diaminobenzidine (DAB) was used. Sections were counterstained with Mayer's hematoxylin. In all reactions negative control was performed replacing primary antibody with non-immunised mouse or rabbit serum.

Positive tumour cells were counted per 1,000 tumour cells in randomly selected fields under 250x magnification. We used a computer-aided image analysis system using the program Multiscan v.5.1.(CSS, Poland). In all cases the expression of ER and PR was evaluated semiquantitatively using H score index. The significance of differences between ER and PR H-scores was tested using Mann–Whitney and Kruskal-Wallis tests.

RESULTS

In the histopathological examination, 23 cases (55%) of adenocarcinoma (Ad) were stated: [5 cases (38%) before menopause, 18 (62%) after menopause], 17 (40%) of adenosquamous carcinoma (Ad-Sq) [8 cases (62%) before menopause and 9 cases (31%) after menopause] and 2 cases (5%) of the clear cell carcinoma (Cl-C) of endometrium after menopause. Among the neoplasms there prevailed moderately differentiated carcinomas G2 (52%) of all cases. Well differentiated carcinomas G1 were 26% and poorly differentiated G3 were 22%. In Table 1, the degrees of differentiation in individual histological types of carcinomas were presented with their correlation with menopause. The presence of endometrial hyperplasia in the neoplasm surroundings was stated in 15 cases (36%): in 9 women before menopause and in 6 women after menopause. In the remaining 27 cases (64%) the presence of endometrial hyperplasia in the neoplasm surroundings was not stated. In the group of 15 neoplasms with the presence of endometrial hyperplasia, 6 cases (40%) were well differentiated, 8 cases (53%) moderately, and 1 case (7%) was poorly differentiated. In the remaining group of 27 cases, 5 cases (19%) were well, 14 cases (52%) moderately, and 8 cases (29%) poorly differentiated carcinomas.

Table 1. Histological grading in pre- and postmenopausal women, and histological type of carcinomas

Grading - G	Menopause		Histological type		
	before	after	Ad	Ad-Sq	Cl-C
G 1 11 (26%)	4 (30%)	7 (24%)	10 (42%)	1 (6%)	-
G 2 22 (52%)	8 (60%)	14 (48%)	10 (42%)	12 (70%)	-
G 3 9 (22%)	1 (10%)	8 (28%)	3 (16%)	4 (24%)	2 (100%)

The grouping of all cases considering clinical staging of the carcinoma in the whole investigated group, in groups before and after menopause, in individual histological types and in the group with and without the presence of hyperplastic endometrium was shown in Table 2. The grouping considering the depth of myometrial infiltration was shown in Table 3.

Table 2. Correlation of clinical stage with menopause, histological types
of carcinomas and presence of endometrial hyperplasia

Clinical	Menopause		Histological type			Endometrial hyperplasia	
Stage	before	after	Ad	Ad-Sq	Cl-C	present	absent
Stage I 26 (62%)	9 69%	17 58%	18 52%	8 41%	-	11 74%	15 56%
Stage II 9 (22%)	1 8%	8 28%	3 13%	6 35%	-	2 13%	7 24%
Stage III 6 (14%)	2 15%	4 14%	2 9%	2 11%	2 100%	2 13%	4 16%
Stage IV 1 (2%)	1 8%	-	-	1 7%	-	-	1 4%

Table 3. Correlation of depth of myometrial infiltration with menopause,
histological type and grading of carcinomas

Depth of myometrial	Menopause		Histological type			Grading		
infiltration	before	after	Ad	Ad-Sq	Cl-C	G1	G2	G3
1^0 - to 1/3 of muscle 19 (45%)	8 61%	11 42%	12 52%	7 41%	-	9 72%	7 32%	3 33%
2^0 - to 1/3 - 2/3 of muscle 14 (33%)	4 30%	10 34%	4 18%	6 35%	1 50%	2 18%	10 45%	2 22%
3^0 - ↑ 2/3 of muscle 9 (22%)	1 9%	8 24%	7 30%	4 24%	1 50%	-	5 23%	4 45%
total 42 (100%)	13 100%	29 100%	23 100%	17 100%	2 100%	11 100%	22 100%	9 100%

The ER expression was found in 28 cases (67%) of endometrial carcinomas. In 4 cases (10%) the expression was very strong (+++), in 14 cases (33%) moderate (++), in 10 cases (24%) weak (+). In 14 cases the presence of ER was not stated. The PR expression was stated in 24 cases of carcinomas (57%). In 3 cases (7%), the expression was very strong (+++), in 16 cases (38%) moderate (++), and in 5 cases (12%) it was weak (+). In 18 cases (43%), the presence of PR was not stated. The coexpression of both receptors was revealed in 24 cases (57%) of the endometrium carcinomas. In 4 cases (10%), only the presence of ER was stated and in 14 cases (33%), the expression of none of the receptors was stated. The squamous component of the adenosqamous carcinoma and clear cell carcinomas did not

reveal the receptor expression.

The degree of differentiation and clinical phase correlated with the expression of both receptors. Additionally, three other parameters (the depth of infiltration, the presence of endometrial hyperplasia and the age of patients) correlated solely with the expression of PR. In patients before menopause the H-score ER values correlated only with the degree of histological grading (p=0.014). This correlation also occurred for the H-score PR (p=0.023). Values of the histological index PR correlated with the depth of infiltration (p=0.005) and the presence of hyperplastic endometrium in tumour surroundings as well (p=0.029).

In cases with the presence of hyperplastic endometrium in carcinoma surroundings significant relations occurred between the H-score PR values and the depth of infiltration (p=0.001) and the histological grading (p=0.008). These relations also occurred between the histological grading and the H-score ER values (p=0.037).

In cases without endometrial hyperplasia, it was stated that there exists a very distinct relation between the histological grading and the H-score values of both receptors (p<0.0001). The H-score PR values and the clinical progression of the disease mutually correlated as well (p=0.034). A very high correlation occurred between the degree of histological grading and the H-score values of both receptors (p<0.0001). The H-score values of both receptors also correlated with the depth of infiltration (for ER p=0.008; for PR p=0.003). The H-score PR values correlated with two more parameters: age of patients (p=0.034) and the presence of hyperplastic endometrium in carcinoma surroundings (p=0.005).

While analysing the depth of myometrial infiltration it was stated that in the cases of infiltration to 1/3 of the muscle thickness, the H-score PR values correlated with histological grading (p=0.001), presence of endometrial hyperplasia (p=0.001) and the age of patients (p=0.019). The H-score ER values revealed the relation in terms of the degree of differentiation (p=0.001). In the cases of infiltration, ranging from 1/3 to 2/3 of the muscle thickness, correlations occurred between the clinical progression the H-score ER values (p=0.001), and the H-score PR values (p= 0.001). There were also correlations between the histological grading, the H-score ER values (p=0.019), and the H-score PR values (p=0.008). In the group of cases with infiltration above 2/3 of the muscle thickness, more than half of the cases in this group did not reveal expression of any of the receptors.

DISCUSSION

Numerous researchers tried to find the relation between the condition of steroid hormone receptors in endometrial carcinoma and various clinical and morphological features. It seems interesting whether in the neoplastic cells there exists a relationship between the expressions of both receptors, as in the normal endometrium.

In our material of the group of 42 endometrial carcinomas, in 14 cases (33%) no expression of the examined receptors was stated while in 4 cases (10%) only the ER revealed the expression but this expression was weak. However, in more than half of carcinomas, 24 cases (57%) ER and PR were present. It was not stated in any of the cases that the expression of PR was revealed without the presence of ER. According to M c G u i r e (8) in about 5% of tumours the PR is detected without the presence of an ER. It seems surprising, since the PR is one of several albumins and the expression of which depends on ER. Also according to P e r t s c h u k et al. (12), the active ER is simply indispensable for the synthesis of PR.

The PR-positive patients are one of the youngest in the group, whereas after the mean age of 63 years, the majority of cases (14/20) was PR-negative. This relation did not occur for the estrogen

receptor, which on the contrary, revealed a very strong expression in patients between the ages of 63 to 75. Similar relations were shown by C a r c a n g i u et al. (1) who obtained statistically essential, converse age correlation, also for PR only. Again, P a l m e r et al. (11) obtained the converse correlation with age for both receptors. C h a m b e r s et al. (2) stated that the age is related neither to the ER nor to PR level. If the patients were divided into 2 groups regarding the presence of endometrial hyperplasia in neoplasm surroundings, the relation of the PR value towards the infiltration occurred in none of the groups. It can be concluded that when endometrial carcinoma is generated in the background of endometrial hyperplasia caused by hyperestrogenism, the PR level in the tumour is not connected with age of the patient. In the pre-menopausal period the hormonal activity of the ovary begins to subside and the peripheral tissues (and first of all fatty tissues) become the source of estrogens circulating in the blood. In these tissues, extra-glandular aromatization of adrenal androgens to estrogens takes place (9).

The correlation between female menopause and the expression of one of the receptors was not stated. It means that the relation to menopause is not a differentiating feature regarding estrogen or progesterone receptors. P a l m e r et al. attained similar results (11).

In conclusion, it can be suggested that the presence of endometrial hyperplasia is a parameter, which in a statistically essential way differentiates neoplasms into two groups in respect of progesterone expression. It is known that the PR amount in the target cells is regulated by the level of estrogens. Therefore, the authors perceive the hyperestrogenism not only as the reason of the development of the hyperplastic lesions in endometrium (5), but also in high expression of the PR in carcinoma formed in the hyperplastic area. To summarize the literature data and the results of our own study, we can state that the morphological picture of endometrium in carcinoma surroundings has the influence on the receptor level in the neoplasm, especially on the PR. Many researchers consider the amount of PR in endometrial carcinoma to be the tumour behavioural exponent, its susceptibility to hormone therapy and the most essential survival rate (3, 6).

According to various authors, one of the most essential clinical and morphological prognostic parameters in the endometrial carcinoma is its histological differentiation (4). It was found in our own material that there exist very significant correlations between the histological grading and the expression of both receptors. This means that the histological grading was the parameter differentiating in the highest degree endometrial carcinomas in respect of expression of the steroid receptors, and especially the PR. In the majority of published papers, there exists the agreement that the level of receptors in tumours decreases with the decline of histological grading. The G3 carcinomas were more often receptor-negative in comparison to groups G1 and G2 (2, 6), however statistically, no significant difference between the two last groups was noted (11).

In all cases, statistically significant correlations between the depth of myometrial infiltration and receptors expression occurred only for the PR. This dependence occurred in both groups, divided according to the relation to menopause. On the other hand, when dividing the cases according to the presence of hyperplastic endometrium, this dependence occurred only for the neoplasms, which generated on the basis of endometrial hyperplasia. This means that in the case of neoplasms which were generated *de novo*, the depth of infiltration does not reflect the level of PR in the tumour.

On the basis of the obtained results, it can be stated that the depth of myometrial infiltration is not the parameter which differentiates endometrium carcinomas in respect of the expression of ER. However, it possesses the differentiating value in relation to the expression of ER, especially in the cases when the infiltration exceeds 1/3 of the myometrium thickness. There are various opinions in the literature on the correlation between the state of receptors and the depth of myometrial infiltration. In some papers, in which the biochemical method was employed, the correlation for the PR and ER was statistically essential (11) while other papers denied it (3, 6). The authors examining receptors

with the use of immunohistochemical method also presented divergent results. For some authors, this correlation was statistically significant for both receptors (10), for others it was not (1). Individual reports can be encountered in which, from the two examined receptors, only ER (2) and PR (7) revealed essential dependence in relation to the depth of infiltration.

In all cases, a very high degree of correlation was obtained between the values of the histological index ER and PR. The lack of correlation in the group of poorly differentiated carcinomas may be possibly justified by the existence of mutant forms of ER. Probably because of that, some percentage of the ER-positive carcinomas does not react to hormonal therapy.

The receptor state of endometrial carcinomas reflects their clinical course and their susceptibility to hormone therapy. It seems indispensable to define the levels of ER and PR expressions. As this type of examination is costly, we must take into account the fact that it will not be included into the routine histopathological diagnosis in the near future. Therefore, these morphological parameters should be most accurately examined, which in a statistically essential way, differentiate endometrium carcinomas in respect of the expression of steroid hormone receptors. According to the results of our own research, these parameters are: the degree of the tumour histological differentiation, and in the case of PR, also the presence of hyperplastic endometrium in the neoplasm surroundings. If there are possibilities of the examination of receptors, one can restrict to the analysis of only one of them, especially in tumours restricted to the myometrium, because in these tumours there exists mutual correlation between the ER and PR expressions. In carcinomas exceeding beyond the uterus the receptor state of the neoplasm can be foreseen. Therefore, especially in these cases, as the literature data show and the results of the performed analysis confirm, the expression of both receptors should always be examined simultaneously.

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SUMMARY

The aim of the study was the analysis of expression of estrogen (ER) and progesterone (PR) receptors in endometrial carcinomas. The expression of each of the receptors was examined with reference to such parameters as: patients' age, the relation to menopause, histological type and grading, the depth of myometrial infiltration and the presence of endometrial hyperplasia adjacent to the tumour. There were found very significant correlations between the degree of histological grading and expression of ER and PR and between values of index ER and PR. There was obtained correlation between the expression and endometrial hyperplasia only for PR.

Analiza ekspresji receptora estrogenowego i progesteronowego
w rakach błony śluzowej jamy macicy

Celem pracy była analiza ekspresji receptorów estrogenowego (ER) i progesteronowego (PR) w rakach błony śluzowej jamy macicy. Ekspresję każdego z receptorów badano w odniesieniu do takich parametrów, jak: wiek pacjentek, stosunek do menopauzy, typ histologiczny i stopień histologicznego zróżnicowania, głębokość naciekania mięśnia trzonu macicy oraz obecność rozrostu gruczołowego endometrium w sąsiedztwie guza. Stwierdziliśmy bardzo znaczącą korelację pomiędzy stopniem histologicznego zróżnicowania i ekspresją ER i PR oraz statystycznie istotne zależności pomiędzy badanymi receptorami. Stwierdziliśmy też, że występuje korelacja pomiędzy rozrostem gruczołowym endometrium i ekspresją jedynie dla PR.