# Assessment of ER-α and ER-β expression levels in malicious tumors of the uterine corpus

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**Summary**

*Purpose:* The purpose of this article is to present an assessment of the expression levels of estrogen receptors ER-α and ER-β in malicious tumors of the uterine corpus.

*Material and Methods:* Estrogen receptor expression levels were tested using semiquantitative immunohistochemical methods. Paraffin-embedded sections of tissue from the corpus of the uterus from 171 patients were used in the research.

*Results:* Analysis of the relation between ER-β expression levels and the clinical grade of disease (based on FIGO classification) showed that these parameters are significantly related: p = 0.0099. There were no statistically significant relations between ER-α expression levels in tumors or clinical stages of tumors based on the FIGO criteria. The presence of high estrogen receptor beta expression levels is often accompanied by a low estrogen receptor alpha expression level and such arrangements allow the overt biological function of a dominant receptor.

*Conclusion:* The differences in tissue distribution of both estrogen receptors could indicate their different biological roles.

*Key words:* ER-α expression; ER-β expression; Malicious tumours; Uterine corpus.

## Introduction

In physiological conditions, three natural estrogens exist in a woman's system: estron/E1/estradiol/E2 and estriol/E3, which can be distinguished by the intensity of their influence [1]. There are some circumstances which may point out possible relations between their action and influence on processes of carcinogenesis. Particularly it concerns cases where the proliferative action of estrogen is not balanced by the effect of progesterone [2, 3].

Metabolites of estrogens may also exert a strong mutagenic effect; 3,4 semiquinons and 3,4 quinons could join to the DNA chain by covalent bonding, leading to the production of DNA adducts which disturb the DNA-3D structure. If the mutation occurs in genome stability related genes, for example in the proto-oncogene or in cancer transformation suppressor genes, it could initiate the process of carcinogenesis [4-8].

Estrogens exert their biological effect mostly through genomic action, using receptor proteins. Currently two types of estrogen receptors are known – ER-α and ER-β. The activated estrogen receptor attaches to specific places in DNA called the estrogen responsive element, located in the gene's promoter region and initiates specific genomic action [9, 10]. Also activated estrogen receptors can react with DNA using the gene expression amplified element – AP1. In such case, binding to DNA occurs with the participation of the transcription factors – the proteins Fos and Jun. Depending on the type of receptor, interaction with DNA through AP1 can cause different biological effects [11, 12].

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So far the physiological presence of different types of estrogen receptors in one cell has not been unambiguously established. It is supposed that they are components of a mechanism which decides on the tissue-specific regulation of a this hormone's action. Different transcription effects, as the result of activation by the same ligand connected to different abilities of the ER-α and ER-β receptors, the difference in their distribution in tissues as well as the modulation of the AF-1 and AF-2 activation domains by formation of the homodimers and heterodimers by both receptors types, could explain the enormous variety of the estrogen and anti-estrogen actions in target tissues [9, 13, 14]. The discovery of receptors which have a similar structure to the ER-α and β estrogen receptors, defined as estrogen-related receptors, indicates the additional regulation abilities of tissuespecific response to estrogen action. Furthermore, the isoforms of the alternative estrogen receptor's splicing could have a significant influence on the regulation of gene expression levels [9, 15]. They are defective forms of receptor proteins, which in a fundamental way change the biological function of the receptor. The proteins arise during the transcriptional process of the receptor's mRNA [16, 17]. Changes in the receptor's structure could influence its affinity for a ligand, preventing the receptor from binding to DNA, or affect the proper transcription process [16]. Their presence in a cell modifies the final biological effect of estrogen action in the tissue and could influence tissue specificity. The purpose of this work was to assess ER-α and ER-β expressions in malicious tumor tissue of the uterine corpus compared to normal tissue and to tissue which undergoes hyperplasia processes. This work is the continuation of prior research

concerning the expression of estrogen receptors in endometrial cancer tissues [18].

## Material and Methods

Paraffin-embedded sections of tissues from the uterine corpus from 171 patients were used in the research. Samples of the tissues were obtained by diagnostic dilation and curettage of the uterine cavity. In 149 cases indications for the dilation and curettage were present or previous abnormal bleeding from the uterine cavity.

The tissues were fixed in phosphate buffered with 10% paraformaldehyde and embedded in paraffin. In 96 cases adenocarcinoma of the endometrium of various grades was diagnosed. In nine cases malignant tumors were diagnosed as sarcomas and were classified as homologous mixed mesodermal tumors. Forty-three cases were classified as endometrial hyperplasia of which 24 cases were diagnosed as simplex endometrial hyperplasia and 19 cases as atypical hyperplasia (Figure 1).

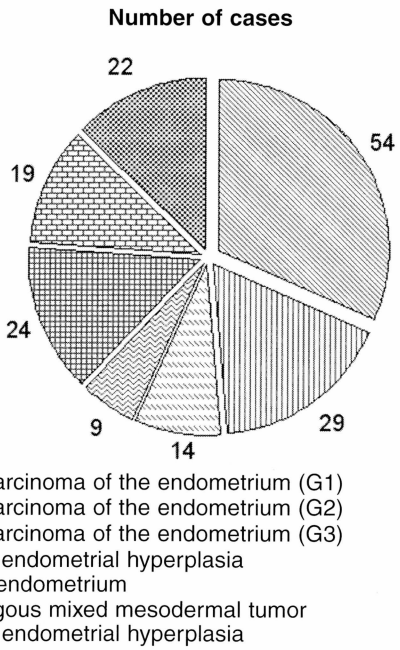


Figure 1. — Characteristics of cases.

The control group consisted of normal endometrial tissues scraped from healthy women before a planned surgical procedure.

Every patient who had a uterine malignant tumor diagnosed underwent a surgical procedure. Based on the surgical report the final clinical stages of cancers were estimated based on FIGO criteria and are shown in Figure 2.

Estrogen receptor expression levels were tested using semi-quantitative immunohistochemical methods. The research was performed at the Laboratory of Animal Endocrinology and Tissue Culture Institute of the Zoology Department of Jagiellonian University, Krakow, Poland.

Estrogen receptor expression levels were estimated in histological specimens which were obtained from tissues embedded in paraffin and individually verified by a histologist. Sections were cut into 5 μm thick slices using microtomes and then mounted on Super Frost/Plus glasses coated by APES (Sigma) in steam baths at a temperature of 45°C. Sections were dewaxed by incubation at 56°C for one hour. Afterwards sections were

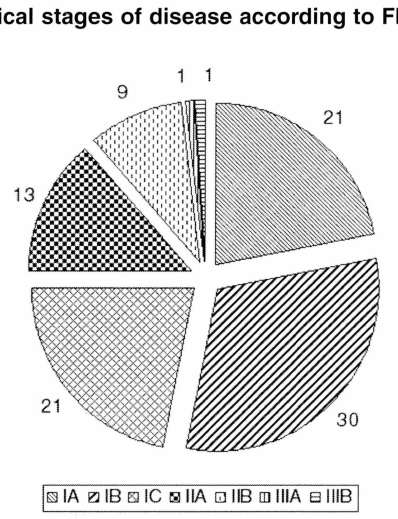


Figure 2. — Clinical stages of disease according to FIGO - number of cases.

repeatedly washed in a series of alcohol: Xylene I, Xylene II, absolute alcohol I, absolute alcohol II, ethanol 96% I, ethanol 96% II, ethanol 70%, ethanol 50% and distilled water for 5-10 minutes in each liquid.

The dewaxed tissues were heated with 0.01 M citrate buffer, pH 6.0, three times for 5 min in a microwave oven. After cooling sections were incubated in 0.3% $H_2O_2$ in PBS for 30 min to quench endogenous peroxidase activity.

Sections in which the ER-α expression level was estimated were incubated with horse serum for 20 minutes. Samples were then incubated for 12 hours at 4°C with monoclonal antibody against ER-α (Dako) at a dilution of 1:100. Afterwards specimens were washed three times for 5 min with TBST and incubated with biotinylated anti-mouse antibody at a dilution of 1:400 for 1.5 h at room temperature.

Sections in which the ERβ expression level was estimated were incubated with 5% goat serum for 20 minutes and incubated overnight at 4°C with polyclonal antibody against ER-β (Affing Bioreagents) at a dilution of 1:100. Afterwards specimens were washed three times for 5 min with TBST and incubated with biotinylated anti-rabbit antibody at a dilution of 1:400 for 1.5 h at room temperature.

Further processing was the same for all specimens:

– washing three times for 5 minutes with TBST;

– using ABC complex at a dilution of 1:100 (Strep ABC complex-HRP, DAKO A/S, Denmark);

– incubation in a humid chamber at room temperature for 40 min;

– washing two times for 5 min with TBST and once for 5 min in TBS.

The color reaction developed in a solution containing TBS with the addition of imidazole (68 mg/100 ml) DAB (50 mg/10 0 ml) and 30% $H_2O_2$ (70 μl/100 ml) for eight minutes. After a color reaction in specimens they were washed in distilled water for five minutes to inhibit the reaction. Then the specimens were dehydrated in a set of alcohols in the following sequence: 50% ethanol, 70% ethanol, 96% ethanol I, 96% ethanol II, absolute alcohol I, absolute alcohol II, xylene I and xylene II for 5 min in each alcohol. Finally, the specimens were mounted in DPX, covered with glass, and estimated under an Olympus microscope taking into consideration the extent and intensity of the immunohistochemical reaction. Estrogen receptor expression was estimated using semi-quantities methods (Table 1).

Table 1. — *Semi-quantities immunohistochemical method assessment pattern.*

| Tissue immunohistochemical reactions | Points |
|---|---|
| Lack of reaction (negative reaction) – absence of color reaction in cells | 1 |
| A very weak reaction – color reaction in less than 5% of all cells | 2 |
| A positive reaction – color reaction in more than 5% and less than 10% of all cells | 3 |
| A strong reaction – color reaction in more than 10% and less than 25% of all cells | 4 |
| A very strong reaction – positive color reaction in more than 25% of all cells | 5 |

## Results

The results of ER-α expression levels are presented in Figure 3. The ER-α expression level in tumor tissue was estimated in relation to the clinical stage of tumors based on the FIGO criteria. Statistically significant relations between examined parameters were not established (Figure 4).

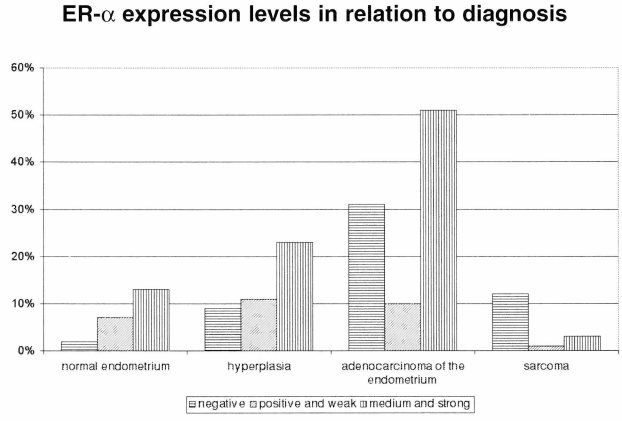


Figure 3. — ER-α expression levels in examined tissues.

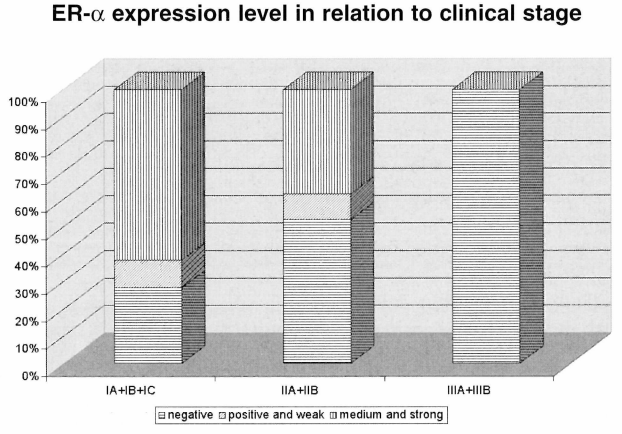


Figure 4. — ER-α expression levels in various clinical stages of endometrial cancer based on FIGO criteria.

The relation between patient age and ER-α expression levels were checked by the Kruskall-Wallis test. The relation between examined parameters was proved. A high ER-α expression level, estimated as ≥ 4, was related to lower patient age – about 53.4 years. However, the lack of ER-α expression was often significantly related to higher patient age – about 60.15 years ($p = 0.0104$). The results of ER-β expression levels are presented in Figure 5.

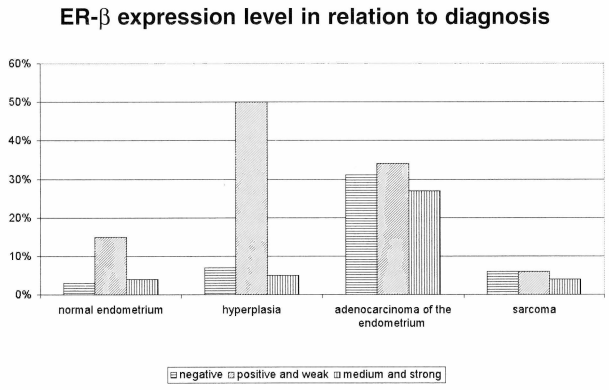


Figure 5. — ER-β expression levels in examined tissues.

No relation between histological diagnosis and ER-β expression level was found in the research.

It has been stated that the lack of ER-α expression occurs most rarely in clinical grade 1 and most often in clinical grade 3. The difference was statistically significant ($p = 0.08$), however it must be pointed out that unfortunately the count of examined cases in clinical grade 3 was very small. The analysis of relations between ER-β expression levels and clinical grade based on the FIGO classification using the Kruskall-Wallis test showed that these parameters are significantly related ($p = 0.0099$). It was characteristic that the highest expression levels of ER-β occurred in the highest clinical stages of disease (Figure 6).

The mutual relations between these two forms of estrogen receptor expression levels were also estimated in examined tissues (Figure 7).

It was proved that there are statistically significant relations between the expressions of the two forms of the estrogen receptor. The lack of ER-β expression statisti-

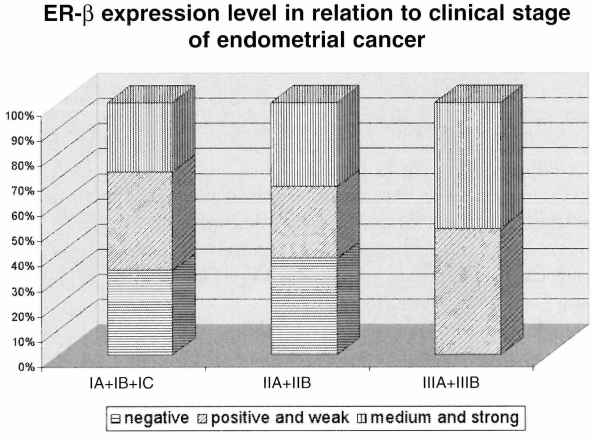


Figure 6. — ER-β expression levels in relation to clinical stage of endometrial cancer.

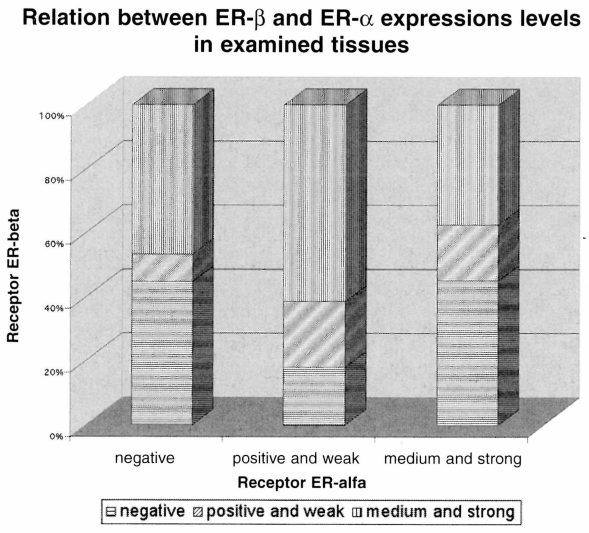


Figure 7. — Relations between ER-β and ER-α expressions levels in examined tissues.

cally significantly coexists with high levels of ER-α expression ($p < 0.05$). Simultaneously, high expression levels of ER-β – statistically significant – coexist with low expression levels of ER-α ($p < 0.01$).

## Discussion

The research of steroid receptor expression levels in cancerous tissues could point out possible changes which may influence one of the most important mechanisms of cell growth. One of the biological features of tumor cells is uncontrolled and excessive proliferation and the loss of individual features essential for cell maturity and specialization [19]. One of its characteristics could be changes in the receptor expression levels of steroid hormones.

In the group of patients with endometrial hyperplasia the absence of estrogen receptor alpha expression levels occurred in nine cases among 42 (21.4%). In the cancer group in 33.7% of examined cases receptor expression levels were not detected. Therefore, the cases without ER-α receptor expression were mostly detected in endometrial cancer tissues, and extremely rarely in normal endometrium.

The highest receptor expression levels were detected in normal endometrium with an incidence frequency of 60%. Receptor expression in endometrial hyperplasia and adenocancers occurred with similar frequency – 55.4% for cancers and 52.3% for endometrial hyperplasia. Using the chi-square test observed differences were confirmed to be statistically significant ($p = 0.038$). The ER-β expression level was also different depending on the histological diagnosis. In normal endometria, cases with weak receptor expression (68%) were detected. In endometrial hyperplasia cases weak receptor expression levels predominated (71.4%). Lack of receptor expression level was mostly related to a diagnosis of endometrial cancer (33.7%). Lack of receptor expression was rarely detected in endometrial hyperplasia or in normal endometria – 16.6% for hyperplasia and 13.6% for normal endometria. The discovered differences turned out to be statistically significant ($p = 0.003$). In examined tissues beta receptor expression levels clearly differed from those of alpha receptors. The detected differences were probably the result of the different biological roles of these receptors in tissues – the specific response to estrogen action. The proportion between the types of receptors and the interaction of the activated receptors and DNA determine the final tissue response to hormonal action [20]. In cases with homologous mixed mesodermal tumors, ER-β receptor expression occurred in 77.8% of cases. At the same time, positive expression of an alpha receptor was detected in only 44.5% of examined tissues. The obtained result could indicate, a role of estrogen receptor distribution disorders in the appearance of these types of tumors. The presence of estrogen receptors in sarcoma tissues could indicate hormonal dependence and legitimatize using hormonal therapy in mixed mesodermal tumor cases.

In examined tissues, a relation between clinical stages and estrogen receptor alpha expression levels was not established. The obtained result points out to the dependence of receptor expression and the maturity and specialization of cells which form tumors. It is expected that the loss of histological maturity of a tumor could be accompanied by a decreased frequency of positive expression of this receptor. It is assumed that the expression would be higher in grade G1, lower in G2, and the lowest in G3. Histological grade decides on the aggressiveness of the disease and the length of the disease course; however, it has no influence on the clinical stage of the disease at present. It has been stated that high expression level of estrogen receptor beta was more often detected in higher clinical stages of endometrial cancer – Stage II according to FIGO – than in lower clinical stages – Stage I. The number of cases in clinical Stage III was too small to determine if this tendency may concern higher clinical stages of disease. The total number of cases was not very high, thus the differences may have been accidental. However it should not be precluded that it might be the result of a different regulative role which beta receptors play in relation to alpha receptors in a specific cell response to steroids. It is possible that the change in mutual proportions of both estrogen receptor expression levels with the advantage of beta receptors is a step towards loss of maturity and specificity of cells leading to tumor formation. In the assessed material, most of the tumors diagnosed in higher clinical stages were the cases with lower histological grades. It could also be the reason for the stated differences in receptor expression levels. In cases of diagnosed sarcomas it was revealed that strong beta receptors were significantly often related to lower clinical stages of disease with $p = 0.03$. The low number of cases in the sampled group does not allow unambiguous conclusions.

The differences in tissue distribution of both estrogen receptors could indicate their different biological roles. The presence of high ER-β expression levels is often

accompanied by low ER-α expression levels and such arrangements allow the overt biological function of a dominant receptor. Due to the presence of the second type of receptor, even with low activity, the biological action of a dominant receptor could be modulated. The presented relations between estrogen receptors were based on a statistically significant limit and require further research with a high quantity of test groups. High ER-β expression level accompanied by low ER-α expression level or the lack of ER-α expression should probably be considered as an unfavourable prognostic factor in endometrial cancers, indicating a low grade of cancer and tendency to faster progression.

## Conclusions

1. High ER-β expression levels are often significantly connected with low ER-α expression levels. It could indicate the different biological functions of these two types of receptor.

2. High ER-β expression levels statistically often are connected with low grade and high clinical stage of tumour and could be considered as an unfavourable prognostic factor of disease. On the other hand, the presence of ER-β creates theoretical grounds for the introduction of hormonal therapy. From this aspect its presence could be regarded as a positive prognostic factor for therapy.

3. ER-β is the dominant form of estrogen receptor in cases of mixed mesodermal tumour, its presence indicates hormonal dependence of this tumour.

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