Modifying effect of obesity on the content of sex hormones and their receptors in endometrial adenocarcinoma and its surrounding tissue

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Abstract

Aims. To study the effect of comorbid pathology: obesity of degree 2-3 on the level of sex steroid hormones and their receptors in the tumor and its surrounding tissue in patients with endometrial cancer (EC).

Materials and methods. In 30 patients with endometrioid adenocarcinoma T1-3N0-1M0 (the main group, 15 females with obesity grade 2-3 (BMI ≥35); the reference group 15 females with normal BMI) in samples of the tumor and its perifocal zone taken after surgical treatment, the levels of estradiol (E2), estrone (E1), testosterone (T), progesterone (P4), androgen receptors (AR), progesterone receptors (RP4), estrogen receptors (ERα and ERβ) were determined by ELISA method. Statistical analysis was performed with STATISTICA 10.0.

Results. Obese EC patients showed longer healing of postoperative wounds, slow recovery, and more frequent tumor metastasizing to regional lymph nodes. In the tumor samples in all patients, compared with the intact endometrium, the levels of estrogens, testosterone and their receptors were higher. Obesity accompanying the malignant process led to a local increase in the levels of estrogens, testosterone, progesterone and AR, ERα and ERβ in the tumor. In the tumor samples, there were no significant differences from the presence of obesity in the levels of RP4. In the perifocal zone of the tumor in patients with comorbid pathology, compared with the parameters in the reference group, the level of E2, P4 and T was also higher, but the content of all steroid receptors was lower.

Conclusion. Obesity aggravates hyperestrogenism and progesterone deficiency in adenocarcinoma and increases its enrichment with the androgen and estrogen receptors with the prevalence of ERα over ERβ that may cause the autocrine-paracrine regulation of the growth and metastasizing of the malignant process in patients with endometrial cancer.

Keywords
Endometrial cancer, Obesity, Estrogens, Progesterone, Testosterone, Steroid hormone receptors

Imprint

Introduction
Endometrial cancer (EC) is a widespread heterogeneous disease, the progression of which is due to some genetic factors, comorbidities and environmental factors [1,2,3].

According to epidemiological studies, obesity may play an important role in the development of gynecological diseases, especially in uterine corpus cancer (UCC) [4,5]. Thus, with a rise in the body mass index (BMI) per each 5 kg/m², an increase in the risk of endometrial malignant tumors by 60% is noted [6]. Females with BMI ≥30 kg/m² have a 3-fold increased risk of UCC compared with the non-obese females (BMI <25), reaching an 8-fold risk in females with BMI ≥40. Obesity is most often associated with endometrioid carcinomas and may also increase the risk of non-endometrioid tumors [7].

Adipose tissue is now fully recognized as a metabolically active endocrine organ that secretes sex steroids, including estrogens, as well as adiponectin, visfatin, resistin, leptin, and tumor necrosis factor-α (TNFα) [5,8,9]. It is assumed that obesity affects the tissues of the uterus through its hormonal activity, pro-inflammatory effect and hyperinsulinemia [10]. However, the relationship between obesity and cancer of the reproductive organs remains largely a controversial issue due to the complexity of epidemiological studies to identify the actual causal relations [1].
Adipose tissue expresses aromatase, an enzyme, which catalyzes the endogenous conversion of androgen to estrogen. Thus, with the growth of the adipose tissue, the estrogen levels increase. Similarly, the amount of the sex-hormone binding globulin decreases hence the level of the bioactive estrogen in the circulating bloodstream elevates even more [9]. In addition, obesity is associated with diabetes, metabolic syndrome, and a pro-inflammatory state, which may contribute to endometrial carcinogenesis through an increased exposure to growth factors and other non-estrogenic mechanisms [11]. Taking into account the pandemic of metabolic and endocrine disorders in the modern world that indicates that a human being lives in a chronic stressful environment, characterized by the consumption of high-energy food combined with low physical activity [12], the study of the impact of obesity as comorbid pathology on the malignant process is topical.

The aim of our research work was to study the effect of the presence of comorbid pathology: obesity of degree 2-3 on the level of sex steroid hormones and their receptors in the tumor and its surrounding tissue in patients with endometrial cancer.

Materials and methods
Examined were 30 patients with endometrioid adenocarcinoma T1-3N0-1M0, mean age 64±3.2 years, including the main group with 15 females with obesity grade 2-3 (BMI≥35) and the reference group covering 15 females with the normal BMI values. Conditionally intact endometrium was obtained after surgical treatment of the patients with uterine myoma, who had the normal BMI values. All patients gave their written informed consent to conduct our scientific research. In the samples of the intact endometrium, the tumor and the perifocal zone of the latter, obtained after surgical treatment of the patients, the levels of estradiol (E2), estrone (E1), testosterone (T), progesterone (P4) (Cusabio, China), androgen receptors (AR), progesterone receptors (RP4), estrogen receptors (ERα and ERβ) (Cloud-Clone Corp. China) were detected by ELISA method. Our statistical analysis was performed with STATISTICA 10.0. The normality of distribution was assessed by the Shapiro-Wilk test, and the significance of differences between the groups was identified by the Kruskal-Wallis method.

Results
In the patients with endometrial cancer in the main group, where the malignant process developed against the background of obesity, more frequent tumor metastasizing to regional lymph nodes, slow healing of postoperative wounds and long recovery were found, compared with the patients in the reference group with the normal BMI values.

It was revealed that in the tumor samples collected from the patients both in the main and the reference group, compared with the parameters in the intact endometrium (Table 1), the following levels were higher: the level of E1 was 3.3 times higher and 10.7 times higher, respectively; E2 greater by a factor of 1.5 and 3.6; T greater by a factor of 1.4 and 3.2, respectively; RA higher by a factor of 2.3 and 4.7, respectively; ERα greater by a factor of 3.2 and 6.1 respectively; ERβ greater by a factor of 1.3 and 3.2, respectively. At the same time, we found that only in the tumors of the patients of the main group the level of P4 was 1.6 times higher than that in the intact endometrium. Significant differences in the content of RP4 in the tumor samples in the patients of the main and reference groups were not identified.

Our comparative analysis of the perifocal zones showed that in the patients of the main group, the level of E2 was 1.4 times higher, P4 5.7 times higher, T 4.3 times greater, but the content of all receptors was lower: RP4 1.9 times lower, AR 1.7 times smaller, ERα 1.9 times and ERβ 1.9 times lower, respectively.

In the tumor tissue of the patients with EC, the levels of E1 and E2 exceeded those in the samples of the perifocal zones: in the main group by a factor of 15 and 3.3, and in the reference group by a factor of 6.2 and 2, respectively. The content of testosterone in the main group was 1.5 times higher in the perifocal zone compared with the tumor region, while in the reference group, on the contrary, it was 1.3 times higher in the tumor than it was detected in the corresponding perifocal zone.

It is noteworthy that in the patients of the main group in the tumor and the perifocal zone, the level of progesterone did not have significant differences, exceeding the levels found in the intact endometrium, while in the reference group, on the contrary, the level of P4 in the perifocal zone was 3.9 times lower than that recorded in the tumor and, at the same time, did not exceed the values in the intact endometrium in any sample. In addition, the levels of AR, ERα and ERβ in the main group were higher in the tumor by a
factor of 2.5, 1.9 and 1.4, respectively, compared with its perifocal zone, while in the reference group, on the contrary, the content of AR, ERα and ERβ in the perifocal zone exceeded those in the tumor by a factor of 1.4, 1.9 and 3.2, respectively.

An analysis of the ratios in the patients of the main and the reference groups (see Table 2 herein) showed that hyperestrogenism was revealed in the tumor samples, compared with the intact endometrium, due to the prevalence not of estradiol, but estrone, and, so E1/T was 3.3 times and 2.4 times higher, respectively, while E2/T had no significant differences.

In addition, a progesterone deficiency was detected in the tumor samples, compared with the intact endometrium: E1/P4 and E2/P4 in the main group were 6.5 times higher and 2.2 times higher, respectively, and the same was applicable to the reference group: E1/P4 and E2/P4 were greater by a factor of 3.2 and by a factor of 1.4, correspondingly. It should be noted that against the background of the prevalence of estrone in the tumor samples, both in the reference and main group, the ratio of the estrogen receptors changed, with the prevalence of the alpha form: REα / REβ were 2.4 times and 1.9 times higher, respectively, compared with the intact endometrium.

In the perifocal zone of the tumor in the patients only of the reference group, an imbalance between estrogen and progesterone towards the prevalence of estrogens was revealed, compared with the intact endometrium: E1 / P4 and E2 / P4 were 2.4 times and 1.9 times higher, while in the perifocal zone of adenocarcinoma in the patients of the main group, E1/P4 and E2/P4 were 2.1 and 1.4 times lower than those in the intact endometrium, respectively. The balance between est-

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Normal endometrium</th>
<th>Reference group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tumor</td>
<td>Perifocal zone</td>
<td>Tumor</td>
</tr>
<tr>
<td>Estrone ng/g tissue</td>
<td>96.6±8.64</td>
<td>320.1±14.10³</td>
<td>1,029±50.64³</td>
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<td>P₁=0.000440</td>
<td>P₁=0.000923</td>
<td>P₁=0.000407</td>
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<tr>
<td>Estradiol ng/g tissue</td>
<td>563.44±27.3</td>
<td>847.75±32.89⁴</td>
<td>424.7±13.87⁴</td>
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<td>P₁=0.001918</td>
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<td>Progesterone ng/g tissue</td>
<td>0.86±0.05</td>
<td>0.9±0.087³</td>
<td>0.23±0.017³</td>
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<td>P₁=0.000410</td>
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</tr>
<tr>
<td>Testosterone ng/g tissue</td>
<td>1.0±0.058</td>
<td>1.4±0.068³</td>
<td>1.1±0.071³</td>
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<tr>
<td></td>
<td>P₁=0.000923</td>
<td>P₁=0.000410</td>
<td>P₁=0.000984</td>
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<tr>
<td>RP4 ng/g tissue</td>
<td>8.66±0.17</td>
<td>8.54±0.39³</td>
<td>34.52±1.23³</td>
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<td>P₁=0.000403</td>
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<td>P₁=0.000923</td>
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<tr>
<td>AR ng/g tissue</td>
<td>1.05±0.06</td>
<td>2.4±0.19³</td>
<td>3.37±0.16³</td>
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<tr>
<td>ERα ng/g tissue</td>
<td>0.65±0.022</td>
<td>2.1±0.22³</td>
<td>3.93±0.13³</td>
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<tr>
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<td>P₁=0.000125⁴</td>
<td>P₁=0.000412</td>
<td>P₁=0.000404</td>
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<tr>
<td>ERβ ng/g tissue</td>
<td>1.04±0.059</td>
<td>1.4±0.1³</td>
<td>4.4±0.26³</td>
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<td>P₁=0.000923</td>
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<td>P₁=0.000923</td>
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Notes: significant differences compared with: 1 – intact endometrium; 2 – indicators in similar samples of the reference group; 3 – indicators in the perifocal zone.
Table 2
Ratios of sex steroid hormones and their receptors in patients depending on the presence of obesity in tumor samples and perifocal zone

<table>
<thead>
<tr>
<th>Indicators (c.u.)</th>
<th>Normal endometrium</th>
<th>Reference group</th>
<th>Main group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tumor</td>
<td>p/zone</td>
<td>tumor</td>
</tr>
<tr>
<td>E1/T</td>
<td>96.6±4.2</td>
<td>228.6±12.3</td>
<td>46.8±3.1</td>
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<td>P1=0.000910</td>
<td>P1=0.000412</td>
</tr>
<tr>
<td>E2/T</td>
<td>563.4±23.4</td>
<td>605.6±36.2</td>
<td>386.1±22.7</td>
</tr>
<tr>
<td></td>
<td>P1=0.000412</td>
<td>P1=0.000912</td>
<td>P1=0.000912</td>
</tr>
<tr>
<td>E1/P4</td>
<td>112.3±5.4</td>
<td>355.6±21.1</td>
<td>223.6±15.4</td>
</tr>
<tr>
<td></td>
<td>P1=0.000410</td>
<td>P1=0.000912</td>
<td>P1=0.000380</td>
</tr>
<tr>
<td>E2/P4</td>
<td>655±32.7</td>
<td>942±35.0</td>
<td>1846.5±120.3</td>
</tr>
<tr>
<td></td>
<td>P1=0.000380</td>
<td>P1=0.000412</td>
<td>P1=0.000412</td>
</tr>
<tr>
<td>REα/ REβ</td>
<td>0.63±0.04</td>
<td>1.5±0.09</td>
<td>1.11±0.08</td>
</tr>
<tr>
<td></td>
<td>P1=0.000410</td>
<td>P1=0.000970</td>
<td>P1=0.000980</td>
</tr>
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</table>

Notes: significant differences compared with: 1 – intact endometrium; 2 – indicators in similar samples of the reference group; 3 – indicators in the perifocal zone.

trogens and androgens in the perifocal zones of the tumor in the patients with EC was shifted towards androgens: E1/T and E2/T were 6.7 and 4.4 times lower than those in the intact endometrium in the main group and 2.1 and 1.5 times smaller in the reference one. Similar to the case with the tumor tissue, the alpha form of the estrogen receptors prevailed in the perifocal zone compared with the intact endometrium: REα/REβ was 3 times higher in the main group and 1.8 times higher in the reference group.

Discussion

To properly explain the carcinogenesis of endometrial cancer, the “indisputable estrogen hypothesis” has conventionally been used, according to which a progestin deficiency against the background of an excessive amount of estrogens stimulates proliferation and suppresses endometrial apoptosis [13]. The endometrial cells, in response to sex steroid hormones, primarily estrogens and progesterone, multiply, differentiate and regress. These hormonal-induced physiological changes require complex paracrine interactions between certain endometrial cell types. When the hormonal balance between estrogens, progestins, and androgens is disrupted, the endometrium may become neoplastic that may lead to cancer [14].

In recent years, there has been an increase in the number of studies providing evidence of changes in tissue-specific concentrations of steroids and their receptors, which does not necessarily coincide with those in blood. At the same time, steroid metabolism in peripheral tissues is considered to be the key way in which, in particular, the endometrium can respond to local physiological demands and “fine tune” either activation or inhibition of processes dependent on the steroid hormone receptors [15]. The ability of malignant tumors to independently synthesize and metabolize hormones, against the background of the presence of their receptors, allows a neoplasm to autonomously regulate its growth [16, 17, 18].

Our study showed that, indeed, the tumor samples contained an increased amount of estrogens, with estrone prevailing over estradiol. The pathology accompanying the malignant growth in the form of obesity elevated the level of estrogens in the tumor without changing the dominant role of E1 over E2. This can be explained by the fact that adipose tissue is an endocrine organ capable of producing a large amount of steroid hormones, including estrogen [19]. In addition, in our study, it was found that against the background of obesity in the patients of the main group, the content of progesterone increased both in the tu-
There is evidence that obesity contributes to an increase in androgen synthesis, a phenomenon often observed in polycystic ovarian disease that is another risk factor for endometrial cancer [22]. Therefore, it can be assumed that an excess of androgens can also have a transforming effect on the endometrial cells [14].

At the same time, the controversial role of androgens in the endometrium is reported, which can demonstrate both their pro- and anti-proliferative effects [23]. Thus, in individuals with a sex change from a woman to a man, long-term use of testosterone contributes to uterine atrophy and thinning of the endometrium, which indicates the anti-proliferative and apoptotic effects [24]. However, in mice subjected to ovariectomy, data have been obtained indicating that in the absence of ovarian hormones (estrogen and progesterone), the use of dihydrotestosterone promotes the proliferation of endometrial cells [25].

In our study, the patients with EC of the main group showed a significant increase in their testosterone levels, both in the tumor and in the perifocal zone, compared with those in patients without comorbid pathology. The calculation of the ratio of estrogen to androgens showed that, despite the increased level of testosterone, in the tumor samples, the balance was shifted towards the prevalence of estrone. In addition, one should take into account the fact that androgens can act as a prohormone that increases the effects of estrogen, especially in postmenopausal and obese women, while the endometrium contains a sufficient amount of enzymes necessary for the synthesis of estrogens [19, 26].

The expression of steroid hormone receptors, as well as their etiological and prognostic role in endometrial cancer, has been the subject of extensive research in the current and past decades. The use of hormonal therapy in the treatment of endometrial cancer, targeting both RP4 and ER, demonstrates a low efficiency of such treatment [26].

In our study, it was found that both the tumor and its perifocal zone are characterized by a high level of estrogen and androgen receptors, compared with the intact endometrium, however, an increase in the content of progesterone receptors has been found only in the perifocal zone of adenocarcinoma. The presence of comorbid pathology, obesity, has made its own adjustments to the receptor status of the studied tissues, in the tumors in the patients of the main group, the level of AR and ER has been recorded to be higher than that in the perifocal zone, while in the reference group, it is the perifocal zone considered as the tissue more enriched with receptors. In a 2016 study by Kamal A.M. et al., it has been reported that in EC metastasizing, an increase in the AR expression is detected [27]. In addition, it is believed that the steroid hormone receptors are capable of co-regulation. Thus, REs bind to specific DNA sequences called estrogen response elements on the RP4 promoter and thus are able to increase their expression, while P4, on the other hand, can suppress the expression both of REs and its own ones. At the same time, testosterone can suppress the expression of the RP4 gene, and the maximum expression of AR in the endometrium can be detected during the follicular phase [28].

Another interesting feature is the change in the ratio of estrogen receptors towards the prevalence of ERα. In the human endometrium, ERα and ERβ exhibit cell-specific expression patterns during the menstrual cycle, ERα is present in the epithelial cells lining the glands and the lumen during the proliferative phase, at a time when the levels of circulating estrogens are rapidly elevated due to the growth of antral follicles containing cells aromatase-expressing granulose, but
decreased during the secretory phase, whereas ERβ does not reflect a dynamic change in expression in the stromal or epithelial cells and is present in endothelial cells and multiple immune cell populations that are ERα-negative [15]. The prevalence of the α-form of estrogen receptors may demonstrate the proliferative potential of a malignant tumor.

**Conclusion**

Obesity as a disease against the background of which endometrial cancer develops, has an essential effect on the hormonal receptor background in the tumor and its surrounding area, aggravating hyperestrogenism and progesterone deficiency in adenocarcinoma and increasing its enrichment with androgen and estrogen receptors, with a predominance of ERα over ERβ, which can cause the autocrine-paracrine regulation of growth and metastasizing of the malignant process.

**Statement on ethical issues**

Research involving people and/or animals is in full compliance with current national and international ethical standards.

**Conflict of interest**

None declared.

**Author contributions**

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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