

Estrogens hypersensitivity syndrome in endometriosis

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Objectives. The aim of the study was to develop a diagnostic method for estrogen hypersensitivity syndrome in proliferative benign diseases of the female reproductive system. Using the example of endometriosis, changes in the activity of the chaperone protein Hsp90 α were determined as a diagnostic biomarker.

Methods. The study included 60 women aged 30 to 50 years, thirty of whom had a laparoscopically confirmed diagnosis of endometriosis and 30 were included in the control group. After blood sampling to determine the baseline levels of Hsp90 α , the patients were prescribed a single oral dose of estradiol valerate 2 mg tablet. The plasma Hsp90 α levels were remeasured 6 h later.

Results. No significant change ($p>0.05$) in baseline Hsp90 α concentrations was found between the group of patients with endometriosis and the control group. Six hours after estradiol valerate administration, a significant difference ($p<0.001$) in Hsp90 α levels was observed between the group of patients with endometriosis and the control group. The increase in Hsp90 α concentration following the test-load was also more pronounced in women with confirmed endometriosis compared to controls (27.29 ± 15.62 ng/ml and 3.16 ± 4.36 ng/ml, respectively) ($p<0.001$). ROC analysis indicated that the absolute increase in Hsp90 α level of more than 8.6 ng/ml in patients with endometriosis has a sensitivity of 0.93 and a specificity of 0.97 (compared to the control group). This change in relative concentration corresponded to an increase of over 28.7%.

Conclusion. The increase in Hsp90 α plasma level of more than 30% within 6 h after the test-load is a specific criterion for estrogen hypersensitivity syndrome in endometriosis. Further investigation including other nosological forms (myoma, adenomyosis, endometrial hyperplasia) is required.

Keywords: hypersensitivity, estrogen-dependent diseases, uterine fibroids, endometrial hyperplasia, endometriosis, heat shock protein, Hsp90, estrogen receptors

Among diseases of the female reproductive system, hormone-dependent nosological forms occupy an important place. These include endometriosis, endometrial hyperplasia, uterine fibroids (Artymuk et al. 2012; Altukhova et al. 2020) and several others. Disruption of steroid hormone reception is a risk factor for the development of these conditions and also promotes their progression, potentially leading

to malignancy (Babaeva et al. 2017; Serov 2018; Sviridova et al. 2024).

Estrogens are a group of “key” sex hormones of the female reproductive system, whose elevated levels lead to excessive proliferation of target tissues such as the glandular tissue of the mammary glands, myometrium, eutopic and ectopic endometrium (Babichev et al. 2009; Adamyan et al. 2016; Serov

2018). Hormone-dependent diseases of the female reproductive organs are caused by the influence of hyperestrogenism, which can be absolute – characterized by a significant increase in estrogen levels in the blood and tissues – or relative, involving an excessive receptor response to estrogens despite normal or even decreased levels (Serov 2018; Altukhova et al. 2020). An increased receptor response, as the basis of the estrogen hypersensitivity syndrome, may contribute to the development of recurrences in 15–20% of patients (Lo et al. 2018).

The estrogen hypersensitivity syndrome is a pathological condition in which target tissues exhibit increased sensitivity and an excessive response to estrogens (both normal and decreased levels), provoking excessive cellular proliferation and other physiological responses (Rivero et al. 2008).

Assessment of estrogen hypersensitivity syndrome remains an under-researched issue despite its significance, although several publications address this topic in the scientific literature. Itsekson and co-authors (2011) developed a method for evaluating estrogen sensitivity by injecting estriol or estrone into the patient's skin and then monitoring the skin's reaction to the hormone. The appearance of a reaction exceeding a certain threshold confirms the presence of estrogen hypersensitivity syndrome (Itsekson et al. 2011).

In another study, an increased likelihood of hypersensitivity to estrogens or estrogen-active substances in the presence of a deletion of 2,244 nucleotides in intron 6 of the human estrogen receptor alpha gene (ESR1) has been demonstrated (Rivero et al. 2008). The developed genetic diagnostic method is costly and requires a lengthy wait for results.

Xia and Huang (2004) have proposed to detect estrogen receptor (ER) levels via immunohistochemistry using high-affinity monoclonal antibodies in order to assess estrogen hypersensitivity. This method is applicable only to biopsy material.

Lo et al. (2018) have demonstrated that measuring the absolute concentration of the chaperone protein Hsp90 α in blood plasma samples provides valuable information for evaluating the effectiveness of therapy in breast cancer, bladder cancer, and prostate cancer.

Estrogen receptors (ERs) (primarily ER α and ER β) are ligand-dependent transcription factors whose activation and stability depend on chaperone complexes. In their inactive state, ERs are associated with chaperone proteins such as heat shock proteins (Hsp), specifically Hsp90 α with a molecular weight of 90 kDa. Hsp90 α ensures proper folding of the

ligand-binding domain of the ER, maintains the receptor in a conformation competent for estradiol binding, and prevents its proteasomal degradation. Estrogens, passing through the plasma membrane of target tissue cells, bind to specific inactive receptors, inducing conformational changes and releasing chaperone proteins, which facilitates receptor dimerization. After ligand binding and conformational rearrangement, the ER dissociates from the Hsp90 complex and translocates into the nucleus to regulate transcription. The activated complex interacts with DNA regions, regulating gene transcription and executing hormonal signaling (Han et al. 2015; Yasar et al. 2016; Fuentes and Silveyra 2019; Marquardt et al. 2019; Backe et al. 2022; Yu et al. 2022).

Normoestrogenism in severe forms of endometriosis, recurrent endometrial hyperplasia, and other estrogen-dependent proliferative diseases necessitates the identification of modulation in the receptor apparatus to endogenous and/or exogenous estrogens, which pathogenetically contributes to the development of estrogen hypersensitivity syndrome (MacLean and Hayashi 2022; Sviridova et al. 2024).

The objective of the current study was to develop a diagnostic method for estrogen hypersensitivity syndrome in proliferative benign diseases of the female reproductive system, using endometriosis as an example, by assessing changes in the activity of the chaperone protein Hsp90 α .

Materials and Methods

Subjects. The study was conducted in the Department of Gynecology of Russian State Pediatric Medical University, St. Petersburg from May 2024 to July 2025. The design of the study was approved by local ethic committee of the University #34/3/2 5/20/2023.

The study included 60 women aged 30 to 48 years, 30 of whom had a laparoscopically confirmed diagnosis of endometriosis within 1 year (stage II or higher according to the ASRM, i.e., more than 16 points) and 30 women formed the control group. Inclusion criteria for the control group were: painless, regular menstruation, absence of ultrasound data indicating adenomyosis, uterine fibroids, endometrial hyperplasia, ovarian pathology, and a history of at least one spontaneous pregnancy. Women who had previously undergone surgery for uterine fibroids, endometrial hyperplasia or endometriosis were excluded from the control group.

All patients had not taken any hormonal medications for at least 14 days prior to inclusion in

the study. They were advised to abstain from intense physical and psycho-emotional activities, alcohol consumption, sexual activity, and smoking for 24 h before blood sampling and the test-load.

Hsp90α concentration measurement. After blood collection to determine the baseline Hsp90α level, patients orally received a single dose of estradiol valerate 2 mg tablet (test-load). The subsequent measurement of Hsp90α levels in plasma was performed after 6 h (the time of maximum concentration of the 17β-estradiol hydrolysate). The concentration of Hsp90α in plasma was measured using the ELISA method by a Kit for Heat Shock Protein 90 (HSP90α) Human (Cat. SEA533Hu) produced by Cloud-Clone Corp (China).

Statistical analysis. Data analysis using the Shapiro-Wilk test indicated that the distributions in the groups were not normal: the p-values before and after the test-load were 0.048 and 0.0029, respectively, in the control group; and 0.0005 and 0.0035, respectively, in the endometriosis group. Therefore, for further comparisons, we employed non-parametric tests: the Wilcoxon signed-rank test for paired comparisons within groups and the Mann-Whitney U test for independent samples between groups. To determine a diagnostic cutoff value that could be used to verify the presence of estrogen hypersensitivity syndrome, ROC analysis was performed along with the Youden index ($J = \text{Sensitivity} + \text{Specificity} - 1$). The F-statistic (or F-criterion, F-ratio) is the ratio of two variances (variability measures) used to assess how strongly groups differ from each other relative to the variability within groups. It is calculated as $F = (S_{\text{within groups}})^2 / (S_{\text{between groups}})$ (Altukhova et al. 2020). We employed this value to compare intergroup dispersion as an overall indicator of the statistical significance of the entire model.

Results

The mean baseline Hsp90α concentrations in the group of patients with endometriosis did not differ from the values in the control group (32.78 ± 20.49 ng/ml and 27.34 ± 13.33 ng/ml, respectively) ($p > 0.05$). However, six hours following estradiol valerate administration, a significant difference in Hsp90α levels was observed between the group of patients with endometriosis and the control group (60.07 ± 28.88 ng/ml and 30.50 ± 15.31 ng/ml, respectively) ($p < 0.001$). The change in concentration (Δ) after the test-load was more pronounced in the group of patients with verified endometriosis (27.29 ± 15.62 ng/ml) compared to the control group

Table 1

Plasma concentrations of Hsp90α (ng/ml) before and after a single test-load with estradiol valerate 2 mg tablet in the control group and in the patients with endometriosis

Control group		Patients with endometriosis	
Before	After	Before	After
9.0	11.0	21.0	77.0
11.3	14.0	17.0	36.0
8.0	10.0	11.4	20.0
26.0	29.0	24.0	29.0
12.0	15.0	14.0	35.0
19.0	21.0	16.0	34.0
34.0	37.0	44.0	68.0
44.0	44.0	34.0	40.0
26.0	29.0	55.0	76.0
19.0	21.0	23.0	44.0
45.0	46.0	43.0	65.0
22.0	25.0	55.0	110.0
30.0	33.0	38.0	99.0
22.0	23.0	22.0	80.0
19.0	22.0	12.0	45.0
17.0	19.0	87.0	112.0
24.0	28.0	94.0	132.0
25.0	27.0	45.0	88.0
67.0	69.0	37.0	67.0
53.0	77.0	41.0	68.0
15.0	18.0	14.0	30.0
19.0	22.0	15.0	29.0
24.0	29.0	34.0	80.0
27.0	31.0	22.0	31.0
30.0	32.0	24.0	44.0
35.0	34.0	28.0	56.0
25.0	22.0	51.0	83.0
35.0	37.0	13.0	45.0
44.0	52.0	19.0	34.0
34.0	38.0	30.0	45.0

(3.16 ± 4.36 ng/ml). The statistical significance of these changes assessed with non-parametric tests was high and significant in the group of patients with endometriosis: the Wilcoxon test for Δ before and after the test-load yielded $p = 1.9 \times 10^{-9}$, indicating a very significant increase after the test-load. Although slightly lower, the change was also statistically significant in the healthy group with the Wilcoxon test $p = 1.7 \times 10^{-5}$. The highly significant difference in

Hsp90 α levels between the groups – Mann-Whitney test $p=1.5\times10^{-10}$ – indicates that the change in Hsp90 α concentration in patients with endometriosis is markedly greater than in healthy individuals. In all cases, the differences are statistically significant ($p<0.001$).

The use of ROC analysis evaluates the diagnostic accuracy of the change in the Hsp90 α concentration (Δ) for distinguishing the control group from the group of patients with verified endometriosis. The AUC=0.980 indicates very high predictive accuracy of the group of patients (healthy/endometriosis) based on the change in the Hsp90 α concentration (Figure 1). This means that the magnitude of the change in Hsp90 α concentration after the test-load can almost precisely predict which group a patient belongs to, thus allowing the prognosis of estrogen hypersensitivity syndrome based on the endometriosis model. Therefore, in both groups, the test-load causes a significant increase in Hsp90 α concentration with a notably more pronounced response in women with endometriosis.

Having established that the change in concentration (Δ) can serve as a reliable diagnostic criterion, it is also important to determine specific diagnostic thresholds. ROC analysis revealed that an absolute increase in Hsp90 α concentration of more than 8.6 ng/ml in patients with endometriosis has a sensitivity of 0.93 and a specificity (distinguishing

from the control group) of 0.97. Such a change in relative concentration corresponds to an increase of more than 28.7%. The F-statistic value was 12.78 at a significance level of $p<0.00001$, indicating the overall statistical significance of the entire model. Thus, the variables included in the regression model play a significant role in regulating the protein level and can be used for predicting its changes in future studies.

Discussion

Hsp90 α (HSPCAL4, 14q32.31, MIM# 140571) is a vital molecular chaperone abundantly present in cells, functioning in an ATP-dependent manner. Its expression is induced by heat shock and other forms of cellular stress. It plays a key role in maintaining protein homeostasis (proteostasis) by facilitating the remodeling and activation of client proteins, such as signaling proteins, transcription factors, and regulatory kinases (Obermann 2018).

The first known Hsp90 inhibitors belonged to the family of benzoquinone antibiotics. These compounds, including tanespimycin, inhibited the chaperone activity of the protein by competitively binding to the N-terminal ATP-binding site, inhibiting the ATPase activity of Hsp90. However, during clinical trials, tanespimycin and its analogs demonstrated toxicity and a lack of specificity toward individual Hsp90 α isoforms and client proteins (Obermann 2018; Wickramaratne and Wickner 2023).

Inhibitors targeting the C-terminal domain of Hsp90, including novobiocin and its analogs, have also been studied but demonstrated low efficacy and significant side effects in clinical trials. Therefore, there is a need to explore alternative approaches for inhibiting Hsp90 α , such as developing compounds that bind to other regions of the molecule (Wickramaratne and Wickner 2023).

The limited clinical success of these classical inhibitors can be attributed to two main factors. First, the conservative nature of the N-terminal ATP-binding pocket across all Hsp90 family members makes it difficult to achieve isoform-specific inhibition leading to off-target toxicity. Second, and perhaps more critically, the potent and complete inhibition of ATPase activity at the N-terminus triggers a compensatory cellular stress response. This involves the heat shock factor-1 (HSF-1)-mediated upregulation of other heat shock proteins, which can paradoxically promote cell survival and counteract the desired therapeutic effect. This phenomenon underscores the need for more nuanced strategies that modulate, rather than completely abolish, Hsp90 function.

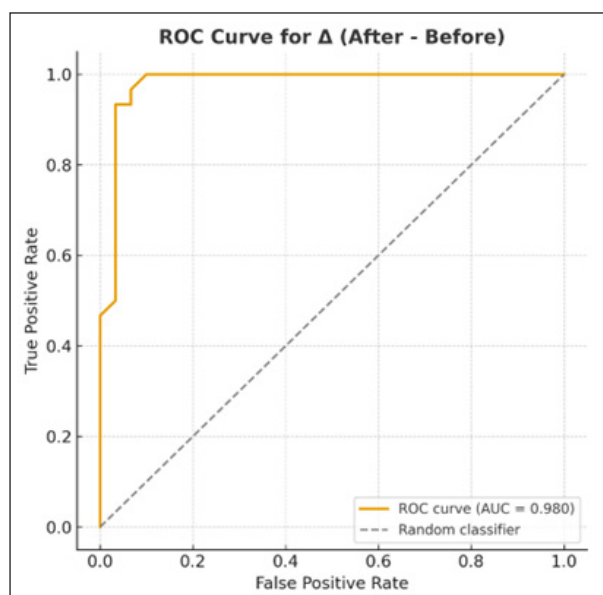


Figure 1. The receiver operating characteristic (ROC) analysis of changes in plasma Hsp90 α concentration of women with endometriosis and the control group after estradiol valerate test-load.

Classical N-terminal Hsp90 inhibitors (such as geldanamycin derivatives) are limited by induced “heat shock response” and toxicity; this drives the development of non-classical strategies targeting other regions of Hsp90 or its co-chaperones. The discovery of the natural product diptoindonesin G (Dip-G) and its analog deoxy-Dip-G, which bind to the middle domain of Hsp90 and compete with ATP but are not traditional N-terminal inhibitors, opens a pathway for allosteric modulation of Hsp90 without inducing a pronounced “stress response.” By binding to the middle domain, such allosteric inhibitors are thought to induce a conformational change that disrupts the association of client proteins or co-chaperones without completely shutting down the chaperone cycle. This selective disruption may allow for the degradation of specific client proteins, like steroid hormone receptors, while leaving the general housekeeping functions of Hsp90 relatively intact.

For ER-dependent conditions (including endometriosis), such selectivity theoretically has the potential to weaken chaperone support of ER and reduce pathological activity without systemic side effects. Hsp90 is a central “hub” maintaining ER functionality and is involved in key pathogenic features of endometriosis, such as proliferation, inflammation, and resistance to apoptosis. ERs, particularly ER β , and their activity are key mediators of endometriosis pathogenesis. The chaperone protein Hsp90 α maintains the stability and activity of ERs; therefore, selective inhibition of Hsp90 may weaken estrogen-dependent pathological processes (such as inflammation, proliferation, and angiogenesis) without the side effects associated with classical therapies (Adamyant et al. 2016; Simmen and Kelley 2016; Chantalat et al. 2020; Eldafira et al. 2021; Guimaraes Ferreira et al. 2022; Bulun et al. 2023; Wickramaratne and Wickner 2023; Martire et al. 2025). Furthermore, the inflammatory microenvironment characteristics of endometriosis lesions is itself a potent inducer of Hsp90 expression, potentially creating a feed-forward loop that stabilizes pro-survival and pro-inflammatory client proteins. Intervening with an allosteric Hsp90 inhibitor could therefore break this cycle, reducing not only estrogen signaling but also the inflammatory cascades that contribute to pain and disease progression.

Hsp90 is actively being studied as a potential target for anticancer therapy because it facilitates the proper folding and activation of client proteins such as ER, which are often hyper-expressed in certain tumors. Furthermore, in cancer cells, Hsp90 α activity is higher than in normal tissues, leading to a phenomenon known as “oncogenic dependence,”

where mutant and overexpressed oncogenic proteins are protected from misfolding and degradation. Modulating Hsp90 α activity represents a promising approach for developing treatments not only for cancer but also for endometriosis, uterine fibroids, endometrial hyperplasia, and endometrial cancer (Raffone et al. 2019).

Conclusion

The study indicates that the chaperone protein Hsp90 α plays an important role in determining the strength of the biological response in women with endometriosis to exogenous estradiol. The intensity of the receptor response in endometriosis significantly differed from that in the control group of women without hyperplastic diseases. This formed the basis for a diagnostic method for estrogen hypersensitivity syndrome, which involves assessing changes in Hsp90 α concentration in plasma during the test-load.

Normoestrogenism is not an absolute marker of well-being. Typically, conditions such as uterine fibroids, endometrial hyperplasia, and endometriosis do not show deviations in estradiol levels. Moreover, reports of endometriosis cases in menopause further confirm that it is not the absolute plasma concentration of estradiol that serves as a marker or a pathogenetic factor in disease progression and the development of severe forms. An excessive receptor response to normal levels of endogenous or exogenous estradiol underpins the formation of estrogen hypersensitivity syndrome.

Our statistical analysis enabled the formulation of specific criteria for changes in Hsp90 α in endometriosis: an increase of more than 30% within 6 hours after the test-load. Increasing the sample size and including women with proliferative uterine fibroids and recurrent hyperplasia of the endometrium in future studies will allow the development of new diagnostic and prognostic criteria.

Further research into the characteristics of the receptor response of Hsp90 α in infiltrative endometriosis and severe forms of adenomyosis is promising. Additionally, exploring other pathogenetic components of steroid receptor signaling – such as HSP70, p23, HOP, FKBP51/52, Aha1, and others – may provide deeper insights into the mechanisms underlying these conditions and aid in the development of targeted diagnostic and therapeutic approaches.

Conflicts of interest: *The authors declare no conflicts of interest.*

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