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THE ROLE OF ENDOCRINE FACTORS AND HEAT SHOCK PROTEINS (HSP60 AND GROEL) IN PREDICTING THE EFFECTIVENESS OF TREATMENT OF CLIMACTERIC SYNDROME

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The research was carried out during the examination and treatment of 158 patients with the climacteric syndrome. The patients were divided into two groups: 1 group included 80 patients receiving menopausal hormone therapy; group 2 included 78 women who were not treated. All patients from two groups had their levels determined: anti-Müllerian hormone, follicle-stimulating hormone, luteinising hormone, and thyroid-stimulating hormone. In addition, a study of the level of antibodies (IgG) to human heat shock protein 60 (HSP60) and its bacterial homolog (GroEl) was conducted. As a result of the multivariate analysis, a reduction (p=0.012) of the risk of not achieving the effect of treatment due to symptoms of estrogen-deficiency state was found for treated patients, OR=0.29 (95 % CI 0.11–0.76) in comparison with the untreated group female patients (when standardised by the level of anti-Müllerian hormone). A higher (p=0.017) risk of not achieving the effect of treatment due to symptoms of an estrogen-deficient state revealed at a higher level of anti-Müllerian hormone, OR=6.1 (95 % CI 1.4–27) for every 1 ng/ml. Indicators of HSP60 and GroEl do not affect the effectiveness of treatment of clinical manifestations of estrogen deficiency.

Key words: perimenopause, climacteric syndrome, menopausal hormone therapy, HSP60.

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РОЛЬ ЕНДОКРИННИХ ФАКТОРІВ ТА БІЛКІВ ТЕПЛОВОГО ШОКУ (HSP60 ТА GROEL) У ПРОГНОЗУВАННІ ЕФЕКТИВНОСТІ ЛІКУВАННЯ КЛІМАКТЕРИЧНОГО СИНДРОМУ

Науково-дослідна робота проводилася при обстеженні та лікуванні 158 пацієнток з клімактеричним синдромом. Пацієнтки були розподілені на 2 групи: до 1 групи увійшло 80 пацієнток, які отримували менопаузальну гормональну терапію; до 2 групи увійшло 78 жінок, яким не проводилось лікування. Всім пацієнткам з двох груп проведено визначення рівнів: антимюлерового гормону, фолікулостимулюючого гормону, лютеїнізуючого гормону, тиреотропного гормону. Додатково проводилось дослідження рівня антитіл (IgG) до людського білку теплового шоку 60 (HSP60) та його бактеріального гомолога (GroEl). В результаті проведеного багатофакторного аналізу виявлено зниження (p=0,012) ризику не досягнення ефекту лікування за симптомами естроген-дефіцитного стану для пролікованих пацієнток, ВШ=0,29 (95 % ДІ 0,11–0,76) у порівнянні із групою не пролікованих пацієнток (при стандартизації за рівнем антимюлерового гормону). Виявлено вищий (p=0,017) ризик не досягнення ефекту лікування за симптомами естроген-дефіцитного стану при більш високому рівні антимюлерового гормону, ВШ=6,1 (95 % ДІ 1,4–27) на кожний 1 нг/мл. Показники HSP60 та GroEl, не впливають на ефективність лікування клінічних проявів дефіциту естрогенів.

Ключові слова: перименопауза, клімактеричний синдром, менопаузальна гормональна терапія, HSP60.

The study is a fragment of the research project "Actual issues of homeostasis, reproductive potential, the relationship of reproductive and somatic health of women, improvement of methods for predicting, diagnosing and preventing obstetric complications and gynaecological diseases, studying the impact of maternal health on the fetus and newborn", state registration No. 0119U100601.

Perimenopause is the process of the physiological transition of the female body from the reproductive stage to postmenopause. The perimenopausal transition includes several physiological changes that cause discomfort and affect a woman's quality of life. Changes during perimenopause lead to endocrine dysregulation of the reproductive system: the cycles become shorter and more sporadic, and the ovaries synthesise less estrogen and progesterone, which leads to disruption in the "ovary-pituitary-

hypothalamus" axis. These changes in the hormonal background form the main repertoire of symptoms called menopausal syndrome (MS) [3]. Symptoms of MS include vasomotor, psychological, urogenital and sexual symptoms. All of the above manifestations of perimenopause complement and modulate each other, which leads to the disruption of the adaptive mechanisms of the female body. Hormonal therapy is a pathogenetically substantiated treatment method [5].

Methods for diagnosing MS consist of a complex of instrumental and laboratory studies. In diagnosing MS, an important marker is anti-Mullerian hormone (AMH), which reflects the pool of non-growing follicles or those at rest [9, 11]. The decrease in AMH begins five years before menopause, which corresponds to the age of onset of perimenopause [8]. Another important diagnostic marker is a follicle-stimulating hormone (FSH). During perimenopause, FSH levels rise sporadically, which is explained by the fact that the ovarian follicular pool requires more intense stimulation to form a preovulatory follicle. A decrease in ovarian reserve subsequently leads to an increase in the intermenstrual interval. However, prolonging the menstrual cycle does not occur suddenly since ovulatory cycles alternate with anovulatory ones and lead to hormonal fluctuations, directly affecting the woman's well-being. Determination of FSH levels allows monitoring of dynamic changes in the ovarian reserve [1]. The determination of FSH and AMH makes it possible to assess the dynamics of exhaustion and the magnitude of the ovarian reserve, which helps to optimise treatment tactics and achieve maximum efficiency in managing perimenopausal symptoms [4].

However, one cannot categorically state the monopoly influence of the endocrine system on the course of perimenopause since immune regulation also plays an indirect role in the formation of clinical performance. A violation of immune regulation, namely its shift to autoimmune aggression, causes intracellular stress. In this context, the search for immune markers of intracellular stress, which will be an addition to diagnostic measures, looks promising. One such marker is the human heat shock protein HSP60 [10]. A significant advantage of choosing this marker is its availability for detection. In a healthy body, heat shock proteins are found intracellularly and involved in protein refolding and maintaining intracellular homeostasis. However, the HSPs are expressed on the cell surface during inflammatory processes. HSP60 has a bacterial homologue GroEl expressed on the surface of microorganisms such as *Chlamydia trachomatis, Chlamydia pneumonia, Mycobacterium tuberculosis, Helicobacter pillory* and other pathogens. As a result of infection, the body's immune system produces antibodies to GroEl, which can cross-react with HSP60 and potentiate an autoimmune response [2, 10]. Existing research evidence suggests a link between intracellular stress and estrogen influence. Menopausal hormone therapy reduces antibody titer to HSP60 [12]. The possible influence of perimenopausal insufficiency in the production of sex hormones and replacement therapy on the state of the response of immunocompetent cells. Therefore, studying changes in the levels of antibodies to HSP60 and Groel can become a reliable marker of the course and compensation of menopausal symptoms in perimenopause.

The purpose of the study was to determine the role of the levels of Anti-Mullerian, follicle-stimulating, luteinising, thyroid-stimulating hormones and antibodies (IgG) against human heat shock protein 60 (Hsp60) and its bacterial homologue (Groel) in the diagnosis and treatment of perimenopause symptoms.

Materials and methods. A prospective study of 158 women with the climacteric syndrome in perimenopause, who were examined and treated according to the National Consensus on the Management of Patients in Menopause, was conducted in the period October 2019 – March 2022 at the Bogomolets national medical university based on the public non-profit enterprise "Kyiv City Maternity Hospital No. 5".

All patients were divided into two groups to determine the treatment factors for menopausal syndrome in perimenopause. The distribution of patients into groups was performed randomly: by the method of even and odd visits.

Main group (group 1) included 80 patients (mean age 50 years) who received treatment according to the standard scheme (estrogens (17β-estradiol) + gestagens (dydrogesterone)); group 2 (comparison group) included 78 women (mean age 50.5 years) who were not treated.

Inclusion criteria for the study: women up to two years after the last menstruation and aged 45 to 53 years, in whom the first symptoms of an estrogen-deficient state appeared, namely urogenital and sexual manifestations (dryness and itching of the vagina, frequent urination, pain during sexual intercourse, bleeding after intercourse, decreased libido, difficulty achieving orgasm). Patients assessed urogenital and sexual manifestations according to the questionnaire (NAMS Menopause Health Questionnaire) [14].

Exclusion criteria from the study: disease of the cardiovascular system, blood clotting disorders, viral hepatitis, neurological disorders, and oncological diseases.

All patients from the two groups underwent a set of clinical and laboratory measures: the examination of patients was carried out by the National Consensus on the Management of Patients in Menopause [12], which included an examination of the hormonal profile, namely Anti-Mullerian hormone (AMH), follicle-stimulating hormone (FSH), luteinising hormone (LH), thyroid-stimulating hormone (TSH). Additionally, the level of antibodies (IgG) to the human heat shock protein 60 (HSP60) and its bacterial homologue (GroEl) was studied using an indirect enzyme-linked immunosorbent assay.

The study was carried out by the principles of the Declaration of Helsinki. The Local ethics committee adopted the study protocol. Informed consent was obtained from the women for the study.

The results were processed according to generally accepted methods when analysing the results, the statistical package "EZR v. 1.54" (graphic interface R statistical software v. 4.0.3, R Foundation for Statistical Computing, Vienna, Austria). The critical significance level was taken equal to 0.05.

Results of the study and their discussion. The average age of the examined patients was 48.8±4 years in the main group and 49.6±3.8 years in the control group. A total of 158 patients underwent a hormonal profile examination (FSH, LH, AMH, TSH) and a study of the level of antibodies (IgG) to human heat shock protein (HSP60) and its bacterial homologue (GroEl). According to the results of the study, there were no differences between the groups in terms of TSH (in the first group – 3.1 mIU/l (2.25–3.89 mIU/l), in the second group – 3.05 mIU/l (2.3–3.2 mIU/l) p=0.167), HSP60 (in the first group – 0.068 units of optical density (0.039–0.086 units of optical density), in the second group - 0.065 units of optical density (0.043–0.084 units of optical density) p=0.709), and GroEl (in the first group – 0.488±0.21 units of optical density, in the second group – 0.52±0.19 units of optical density, p=0.537), except for AMH levels (in the first group – 0.12 ng/ml (0.04–0.4 ng/ml), in the second group – 0.4 ng/ml (0.06–0.6 ng/ml) p=0.013), LH (in the first group – 25 mIU/ml (23.1–34.05 mIU/ml), in the second group – 21.1 mIU/ml (13.8–23.4 mIU/ml) p<0.001), and FSH (in the first group – 76.5 mIU/ml (63–88.5 mIU/ml), in the second group – 52.1 mIU/ml (45.3–66.1 mIU/ml), p<0.001). For further analysis, these differences were taken into account.

The achievement of the effect was assessed according to the questionnaire (NAMS Menopause Health Questionnaire) after the treatment. With a total score of "0" points for the symptoms of an estrogen-deficient state after treatment, the treatment was effective in 82 patients, of which 55 patients (68.7 %) from the main group and 27 patients (34.65 %) in the comparison group, otherwise the treatment effect was considered as not achieved in 42 patients, of which 25 patients (31.3 %) from the main group and 51 patients (65.35 %) from the comparison group, respectively.

Constructing and analysing logistic regression models were used to analyse the relationship between the risk of not achieving the treatment effect for the symptoms of an estrogen-deficient state with factor signs. As factor signs, seven indicators were analysed: treatment, follicle-stimulating hormone (FSH), luteinising hormone (LH), anti-Mullerian hormone (AMH), thyroid-stimulating hormone (TSH), HSP60, GroEl. Table 1 shows the results of the one-way analysis.

Table 1

Analysis of univariate models of logistic regression for predicting the risk of not achieving the effect of treatment in terms of urogenital parameters

Factor sign		Model coefficient, b±m	Difference between OR (odds ratio) and 1, p	Model odds ratio, SR (95 % CI (confidence intervals))
Treatment	No	Reference value		
	Yes	-1.42±0.48	**0.003**	0.24 (0.09–0.61)
FSH, per 100 mIU/ml		1.13±0.95	0.35	-
LH, per 10 mIU/ml		-0.24±0.24	0.323	-
AMH, per 1 ng/ml		2.06±0.73	**0.005**	7.8 (1.9–33)
TSH, per 1 mIU/ml		0.01±0.20	0.961	-
HSP60		-0.76±3.9	0.836	-
GroEl		1.43±1.81	0.431	-

As a result of the univariate analysis, it is possible to conclude that there is no correlation (p>0.05) between the risk of not achieving the effect of treatment on the symptoms of an estrogen-deficient state

from FSH, TSH, LH, HSP60, GroEl. There was a decrease (p=0.007) in the risk of not achieving a high treatment effect on the symptoms of an estrogen-deficient state for treated patients, OR=0.24 (95 % CI 0.09–0.61) compared with the group of untreated patients. A higher (p=0.005) risk of not achieving the effect of treatment on the symptoms of an estrogen-deficient state was revealed with a higher level of AMH, OR=7.8 (95 % CI 1.9–33) per 1 ng/ml.

To identify a set of signs associated with the risk of not achieving the effect of treatment for the symptoms of an estrogen-deficient state using multivariate logistic regression models, a selection of indicators significantly associated with the resulting sign was carried out. For the selection, step-by-step switching on/off of factor signs was used (with the switching threshold p<0.1 and the switching off threshold p>0.2). During the analysis, out of 7 risk factors, two independent signs were identified: treatment and the level of AMH.

Table 2

Analysis of a two-factor logistic regression model for predicting the risk of not achieving the effect of treatment on the symptoms of an estrogen-deficient state

Factor sign		Model coefficient, b±m	Difference between OR (odds ratio) and 1, p	Model odds ratio, SR (95 % CI (confidence intervals))
Treatment	No	Reference value		
	Yes	-1.25±0.49	**0.012**	0.29 (0.11–0.76)
AMH, per 1 ng/ml		1.81±0.76	**0.017**	6.1 (1.4–27)

The two-factor model built on the selected signs is adequate, the area under the curve of operational characteristics (Fig. 1) AUC=0.73 (95 % CI 0.64–0.81), which indicates the presence of an average strength of the association of the risk of not achieving the effect of treatment according to symptoms of an estrogen-deficient state, according to signs (treatment regimens and AMH levels).

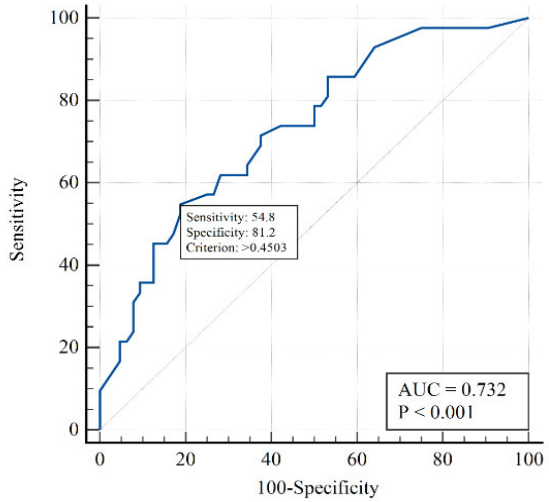

Fig.1. Operating performance curve of a two-factor risk prediction model of not achieving a treatment effect on estrogen deficiency symptoms

As a result of the multivariate analysis (Table 2), a decrease (p=0.012) in the risk of not achieving the effect of treatment on the symptoms of an estrogen-deficient state for treated patients was revealed, OR=0.29 (95 % CI 0.11–0.76) compared with a group of untreated patients (with standardisation by AMH level). A higher (p=0.017) risk of not achieving the effect of treatment on the symptoms of an estrogen-deficient state was revealed with a higher level of AMH, OR=6.1 (95 % CI 1.4–27) for each 1 ng/ml (with standardisation by treatment method).

Physiological changes during the menopausal period are associated mainly with decreased ovarian function. Evidence suggests that the menopausal transition spans an average of four years, with a median onset at age 47, progression to late menopause at median age 49, and transition to last menses at age 51-52 [7], suggesting comparable with the obtained results. Age-related changes in the ovaries lead to a compensatory increase in FSH, which is used as a marker of ovarian ageing. However, using this marker has limitations given its indirect correlation with decreased ovarian function. Thus, our results indicate no effect of FSH levels on the treatment outcome of the estrogen-deficient symptoms during perimenopause. Although the definition of perimenopausal status depends on the patient's age and menstrual history, further evaluation at the transition to menopause should also include other markers, mainly LH and AMH. The decrease in AMH level during the reproductive period reflects the follicular reserve of the ovaries [13]. As women approach menopause, AMH declines, and it has been found that two consecutive AMH measurements are used to determine the rate of change in ovulatory reserve, allowing for the age of menopause to be anticipated within 4 years [6]. However, a lower level of AMH at the time of initiation of treatment for symptoms of the estrogen-deficient state of perimenopause contributes to better treatment results, which can be associated with the effect on feedback links in the “ovary-pituitary-hypothalamus” axis. The transition from perimenopause to menopause is usually a stressful event for a woman's body. However, the stress in this situation should be understood more broadly. Heat shock proteins, which serve as a marker of cellular stress, are directly

involved in such processes. Agnieszka Rajtar-Ciosek et al. determined the positive effect of menopausal hormone therapy on the state of endothelial dysfunction [14, 2]. However, in our study, there was no statistically significant effect of HSP60 antibodies on the effectiveness of treating symptoms of the estrogen-deficient perimenopausal state.

Conclusion

1. Analysis of the hormonal profile during perimenopause is an essential component of the management algorithm for women with clinical manifestations of estrogen deficiency. Assessment of the state of the endocrine system before prescribing hormonal menopausal therapy makes it possible to predict the effectiveness of the prescribed treatment. The data obtained indicate that the most informative indicator for predicting the effectiveness of treatment is AMH.

2. Determination of human heat shock proteins HSP60 and its bacterial homologue GroEl as cellular stress markers may help assess immunoinflammatory processes during perimenopause. However, according to the data obtained, HSP60 and GroEl do not affect the effectiveness of treating clinical manifestations of estrogen deficiency.

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