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Plasma cortisol and insulin levels as a biomarkers of stress in girls with oligomenorrhea in the frontline city Kharkiv, Ukraine

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Резюме. Menstrual dysfunction remains a pressing problem of modern medicine. Along with many adverse factors affecting the health of girls who should be considered as future mothers, the impact of traumatic events occupies one of the leading places. Stress is a complex adaptive reaction that requires additional energy expenditure. Under its influence, the functioning of both the hypothalamic-pituitary-adrenal axis (HPAA) and insulin (In) secretion changes. **The aim** of the study was to determine the characteristics of the stress response in girls with oligomenorrhea (OM) in a frontline city. **Material and methods.** To assess the adaptive capabilities, the serum levels of cortisol (C) and In and their ratio were studied in 116 girls with OM. All patients were divided into groups depending on the duration of OM. The 1-st group included 20 girls who sought medical help in the first year after menarche. The 2-nd group included 28 adolescents with the disease for up to 2 years, the 3-rd group consisted of 39 patients with OM for up to 3 years, and the 4-th group included 39 patients with OM for more than 3 years. **Results.** The function of the adrenal glands and the pancreas in terms of C and In synthesis was preserved in half of the girls with OM, as evidenced by the C/In ratio, which fluctuated within the normal range. A decrease in this index was noted in more than thirds, and from 3 to 10% of girls had an increase in the stress index. It was established that when OM occurs in the first year of menstruation, high numbers of the stress index (C/In) are not noted, which indicate a decrease in the reserves of the body's compensatory capabilities and a disruption in the energy supply of adaptive processes. With the extension of the OM duration, signs of exhaustion already appear, that is an inadequate response of the body to the chronicity of the process. **Conclusions.** As a result of the conducted research and analysis of the identified violations, it was found that 39.7% of patients with OM have adaptation disorders. These deviations emphasize the need to identify adaptive resources to address the issue of their correction in order to increase the body's ability to tolerate stress and strengthen adaptive capabilities.

Keywords: adolescent girls, cortisol, insulin, adaptive-compensatory capabilities, oligomenorrhea, voltage coefficient – C/In ratio.

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Life in modern society, especially Ukrainian, has been full of unpredictable events for 10 years. More than a decade ago, UNICEF attributed wars (along with hunger and AIDS) to the factors that deprive more than half of children on the globe of their childhood, and every year this threat is becoming more and more serious. Ukraine, unfortunately, has also faced all the manifestations of full-scale war. The stay of a person in an emergency zone, migration, as scientists' studies indicate, affect somatic, mental, and psychological health, which can be considered as the consequences of psycho-emotional tension and stress [1-3].

Stress is a complex adaptive response that requires additional energy expenditure. An increase of glucose in the blood of patients – hyperglycemia – is an important component of stress [4]. The HPAA is the main mediator of stress, and C is the main adaptive signaling regulator of stress. It increases the availability of glucose by increasing its production by the liver through transcriptional and posttranscriptional activation of gluconeogenic enzymes. In an acute response to a stressful event, the secretion of C, as a hormone that responds to danger, promotes rapid metabolic adaptation, while the prolonged action of C gradually leads to In resistance and, ultimately, to cardiometabolic disorders. It is believed that C is a powerful activator of In resistance and an inhibitor of In secretion [5, 6]. C and In play a leading role in the implementation of nonspecific reactions that ensure the development of the body's resistance to stress [7]. Recognizing differences between different levels of C concentration in the body is crucial, since it is the main glucocorticoid biomarker in the homeostatic response of the body to stress. The vago-insular system also plays an important role in regulating balance in the body. The close functional relationship between C and In, aimed at enhancing physiological effects, allows us to consider these systems as leading links of single regulatory mechanisms [8].

The aim of the study was to clarify the features of the stress response in girls with OM in a frontline city.

Material and methods

The investigation was carried out at the State Institution «Institute for Children and Adolescents Health Care of the National Academy of Medical Sciences of Ukraine». Under surveillance, there were 116 girls aged 12-17 years with impaired menstrual function of the OM type. All patients were divided into groups depending on the duration of OM. The 1-st group included 20 girls who sought medical attention in the first year after menarche. The 2-nd group included 28 adolescents with the disease for up to 2 years, the 3-rd group consisted of 39 patients with OM, that was observed for up to 3 years, and the 4-th group included 39 patients with OM, that existed for more than 3 years.

The C concentration in blood serum was determined using an additional enzyme-linked immunosorbent assay on the «Rayto RT 2100C» analyzer (Germany) and kits from the company «Best-Diagnostic» (Ukraine). Reference values were considered as Lower quartile 318.0 nmol/L, Upper quartile 673.0 nmol/L.

Tests for immunoreactive insulin (IRI) were carried out using commercial reagent kits («DRG Instruments GmbH», Germany), and glucose using commercial kits («Genesis»). The normative IRI fluctuations were considered Lower quartile 8.10 μU/mL, Upper quartile 15.95 μU/mL.

The In resistance index (HOMA) is based on the formula:

$$\text{HOMA} = \frac{G_0 \times Ins_0}{22.5}$$

de G_0 – fasting blood glucose, mmol/L;
Ins_0 – fasting In, μU/mL.

The presence of In resistance in patients was diagnosed at the level of HOMA, which was 3.5 years CU. The severity of stress was determined not by the absolute ratio of C and In, but by the magnitude of the coefficient of stress, which was distributed between C and In (C/In). The equal group consisted of 35 girls of the same age with a normal menstrual cycle, placed in the early foliculin phase until the beginning of war. The value of the C/In ratio fluctuated in a wide range and on average was 64.53±38.94 units (Lower quartile 32.29 units, Upper quartile 85.51 units). In 27.3% of girls, deviations in the stress coefficient were noted, mainly due to a decrease in adaptive capabilities, and only 4.5% had an exhaustion of the body's protective mechanisms.

Statistical analysis was carried out using the additional software package Statustica 5.5. To compare the indicators, Student's t-test, Wilcoxon-Mann-Whitney, χ^2 were used. All p values less

than 0.05 were considered statistically significant. The results were expressed as average ± standard deviation (SD) and a hundred percent difference.

The ethical principles set forth in the Declaration of Helsinki of the World Medical Association were adhered to throughout the research study. The study was approved by the Bioethics and Deontology Committee at the State Institution «Institute of Children's Health and Children's Health of the National Academy of Medical Sciences of Ukraine».

Results

Analysis of the serum C content revealed that in the absolute majority of girls with OM it was within physiological values (**Table 1**). Its reduced and increased levels did not differ significantly. In acute stressful conditions, the adrenocortical system is activated, which leads to increased C secretion. However, exposure to chronic stress reduces C secretion, which is the final hormonal effect of the HPAA, and the so-called «adrenal fatigue» occurs. That is, under conditions of prolonged stress, the effect of glucocorticoids becomes maladaptive [9-12].

Table 1. Percentage of patients with OM and different levels of C and IRI

Indicator	Percentage, %
Cortisol, nmol/L	
normal	76,1
< 10 percentile	13,8
>75 percentile	10,1
IRI, μU/mL	
normal	56,4
>75<90 percentile	24,8
>90 percentile	18,8

IRI fluctuations in the normal zone were recorded significantly less often than C (P<0.0000), and elevated levels were significantly more frequent (p<0.0000-0.005; Table 1).

Correlation analysis revealed that there are negative relationships between C, IRI and the NOMA index in OM, which does not exceed 2 years. Thus, C has a negative relationship with IRI (r=-0.61; p<0.05) and the HOMA ratio (r=-0.66; p<0.03) in the first year of OM existence, as well as in the second year of the disease – C and IRI (r=-0.51; p<0.01), C and HOMA (r=-0.51; p<0.01). With the extension of the term of OM existence, these relationships disappear.

Reference values of IRI were recorded in more than half of girls with OM regardless of the C level. An increase in IRI concentration was most often observed at low C values (p<0.02) (**Table 2**).

It is known that C can affect In secretion and, at high levels, provoke the development of In resistance [6]. At all C levels, an increase in IRI above 90 percent was accompanied by In resistance.

Table 2. Comparison of percentage of girls with OM and different levels of IRI at different C indicators

Cortisol, nmol/ L	IRI		
	normal	>75<90 percentile	>90 percentile
normal	56,2	25,7	18,1
>75 percentile	50	38,5	11,5
< 10 percentile	52,6	26,3	21,1

An important role in the formation of the body's response to stress is played by hormones such as C and In. They play a leading role in the implementation of nonspecific reactions that ensure the development of the body's resistance to internal and external loads. It is assumed that the possible effects of hormones are determined by the balance between interdependent hormones [11, 13]. Taking into account that the level of hormones varies in different ways, it is not very informative to analyze the levels of C and In separately, the C/In ratio was determined to quantitatively assess the severity of the stress state. This ratio is one of the objective criteria for the severity of the stress effect and the activity of compensatory and adaptive processes developing in the body.

The average values of the C/In index in all groups are given in **Table 3**.

Table 3. Average values of the C/In index in patients with OM depending on the duration of the disease, M±SD (n)

Groups	C/In, unit
All	34,82±26,51 (116) *
I	37,37±22,23 (20) *
II	31,05±25,33 (28) **
III	37,78±36,77 (29) *
IV	34,11±20,492 (39) *
Comparison	64,53±38,94 (35)

Note. * – p<0.001 and ** – p<0.01 in comparison with group «Comparison»

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In girls with OM, regardless of the disease duration, the stress coefficient was significantly lower than in the comparison group, which may indicate the instability of protective mechanisms. Individual analysis revealed that in 48-68% of adolescents, the C/In ratio was within the normative values (**Table 4**). Finding this ratio in the intermediate zone may indicate a compensated state. A decrease in this coefficient was recorded in 30–40% of patients. That is, more than a third of patients have instability of protective mechanisms, or even exhaustion. No significant difference was found depending on the group.

Table 4. Percentage of different values of the C/IRI ratio in girls with OM taking into account the duration of the disease

C/IRI, unit	Groups			
	I	II	III	IV
normal	68,4	57,2	48,3	66,7
>75 percentile	0	7,4	10,3	2,6
< 10 percentile	31,6	35,7	41,4	30,7

Increase in C/In ratio was registered in a small percentage of patients. Moreover, in girls of group 1, increase in C/In ratio was not observed at all. Its high values appear as the duration of OM increases.

Discussion

The impact of traumatic events can lead to mental, behavioral, emotional problems, which cannot but affect the general state of health.

This problem is especially acute for girls during puberty, the most vulnerable, even in terms of its physiological course. The lack of menstrual function is also a psychological trauma that occurs as a result of an imbalance between the requirements (to have menstruation like everyone else) and the body's resources (coordinated work of all links in the regulation of the menstrual cycle).

During stress, the HPAA is activated, causing an increase in plasma cortisol. This is a physiological reaction, on a global scale, glucocorticoids are presented as «run and fight» hormones, and in this sense catabolic hormones are necessary for the mobilization of reserves to provide the body with energy. However, hyperactivity of the HPAA can lead to significant deviations in mental health (depression, anxiety) [14, 15]. Stress also affects In secretion. The concentration of the blood In levels, in addition to glucose, directly depends on the C content and is inhibited at high levels [6].

Assessing disorders of menstrual function as a response to stress, one of the biological responses of which is the activation of the HPAA and the insular apparatus, one can draw conclusions about the nature of the endocrine reaction of the body and the tension state of compensatory systems based on the C/In ratio. Determining of the C and In effects on the adaptive capabilities of adolescent girls with OM revealed that their ratio is the most informative as a marker of a nonspecific stress response. The function of the adrenal glands and the pancreas in terms of the C and In synthesis in half of the girls with OM was preserved, as evidenced by C/In ratio, which fluctuated within the normal range. That is, these girls have an adequate hormonal response to changes in the parameters of the internal and external environment. A decrease in this index was observed in more than a thirds (30.4%) mainly due to an increase in In at normal C values. There are some reports where adaptation disorders may be associated with the formation of hyperinsulinemia [16-18]. A decrease in this ratio may indicate a possible depletion of the adaptive capabilities of a girl with menstrual disorders.

From 3 to 10% of girls had an increase in the stress index. An increase in the C/In coefficient indicates a low level of reserves, or their depletion and disruption of the energy supply of adaptive processes. The higher the stress coefficient, the smaller the reserve of the body's compensatory capabilities and the more threatening the prognosis for compensation of the function in a state of stress becomes. It has been established that when OM occurs in the first year of menstruation, high numbers of the stress index (C/In), which indicate a decrease in the reserves of the body's compensatory capabilities, are not observed. With the extension of the OM term, signs of exhaustion already appear, that is, an inadequate reaction of the body to the chronicity of the process.

Thus, as a result of the study and analysis of the identified disorders, adaptation disorders in patients with OM have been identified. The identified changes in hormonal interaction deepen the understanding of the pathogenetic mechanisms of OM formation, make it possible to assess the state of adaptive-compensatory capabilities in girls with OM depending on the duration of its existence and outline a plan for therapeutic intervention. In addi-

tion to the main treatment of menstrual disorders, medical and psychological support, both through pharmacological drugs and psychotherapy should be included in programs of such intervention.

Conclusions

1. It was found that the stress coefficient varied in a wide range, which indicates different types of adaptive reactions in patients with OM. Three options for responding to stressful events were identified. The stress coefficient within physiological values indicates that stress hormones are in relative balance. The other two (increased and decreased stress coefficient) indicate maladaptive disorders.
2. All options indicate mechanisms by which the body reaches a state of resistance. In patients with an increased stress coefficient, the type of response indicates that the body is functioning at the limit of its adaptive capabilities and can quickly enter the exhaustion phase. A decrease in the C/In ratio indicates the tension of compensatory mechanisms, these girls cannot master the stress level and this can end with a transition to the exhaustion phase or a state of chronic stress.

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Abbreviations

C – cortisol
HOMA – insulin resistance index
HPAA – hypothalamic-pituitary-adrenal axis
In – insulin
IRI –immunoreactive insulin
OM – oligomenorrhea

Плазмові рівні кортизолу та інсуліну як біомаркеру напруги в дівчат з олігоменореєю в прифронтовому місті Харків, Україна

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Резюме. Порушення менструальної функції залишаються актуальною проблемою сучасної медицини. Поряд із багатьма несприятливими факторами, що впливають на здоров'я дівчат, яких слід розглядати як майбутніх матерів, вплив травматичних подій займає одне з провідних місць. Стрес є комплексною адаптивною реакцією, що потребує додаткових енергозатрат. Під його впливом змінюється функціонування як гіпоталамо-гіпофізарно-адреналової осі, так і секреція інсуліну. **Метою** нашого дослі-

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дження стало з'ясування особливостей реакції напруги в дівчат з олігоменореєю (ОМ) в прифронтовому місті. **Матеріал і методи.** Для оцінки адаптаційних можливостей було вивчено вміст у сироватці крові кортизолу (К) і інсуліну (Ін) та їх співвідношення в 116 хворих дівчат з олігоменореєю. Усі хворі були розподілені на групи в залежності від терміну існування ОМ. У першу групу увійшли 20 дівчинок, які звернулися по медичну допомогу в перший рік після менархе. До другої групи було включено 28 підлітків з існуванням захворювання до 2 років, третю групу становили 39 пацієнток, у яких ОМ спостерігалася до 3 років і в четверту групу було віднесено 39 хворих, у яких ОМ існувала понад 3 роки. **Результати.** Функція наднирників і підшлункової залози щодо синтезу К і Ін у половини дівчат з ОМ була збережена, про що свідчить співвідношення К/Ін, яке коливалось у межах нормативних значень. Зниження цього індексу відмічалося більш ніж у третини і від 3 до 10% дівчат мали підвищення індексу напруги. Встановлено, що при виникненні ОМ в перший рік існування менструацій не відмічається високих цифр індексу напруги (К/Ін), які свідчать про зниження резервів компенсаторних можливостей організму та порушення енергозабезпечення адаптаційних процесів. З подовженням терміну існування ОМ вже з'являються ознаки виснаження, тобто неадекватної реакції організму на хронізацію процесу. **Висновки.** У результаті проведеного дослідження і аналізу виявлених порушень встановлено, що в 39,7% хворих з ОМ мають місце розлади адаптації. Дані відхилення підкреслюють необхідність визначення адаптаційних ресурсів для вирішення питання щодо їх корекції з метою підвищення можливостей організму переносити стрес та посилення адаптаційних спроможностей.

Ключові слова: дівчата-підлітки, кортизол, інсулін, адаптаційно-компенсаторні можливості, олігоменорея, коефіцієнт напруги – співвідношення К/Ін.

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