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## MARKERS OF INFLAMMATION AND ANTIOXIDANT PROTECTION IN THE ORAL FLUID OF CHILDREN WITH DIFFERENT BODY MASS INDEX BEFORE AND AFTER THE USE OF THE THERAPEUTIC AND PROPHYLACTIC COMPLEX

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It is known that the inflammatory reaction performs a protective function in the body. Applications of a mucosoadhesive gel containing lipopolysaccharide are proposed in order to enhance the inflammatory response and activate leukocytes migrated into the parodontium. Thus, the inflammatory process does not have the ability to become chronic. A method for the treatment of inflammatory parodontal diseases in children of 12 years old has been developed, substantiated and tested, which provides for the use of a complex of anti-inflammatory adaptogens, the effectiveness of which is confirmed by the normalization of the oral microbiocenosis (a decrease in the degree of dysbiosis by 2.1–4.2 times to normal values), a decrease in the activity of inflammatory processes (elastase by 2 times, malondialdehyde by 1.7 times), restoration of antimicrobial protection (decrease in urease activity by 1.4–2 times; increase in lysozyme levels by 1.6–1.7 times) in the oral cavity of children as with hypo- and with hypertrophy. The obtained results indicate the absence of inflammatory processes and demonstrate the high therapeutic and prophylactic efficacy of the proposed complex.

**Key words:** children, inflammation, body mass index, oral fluid, treatment-and-prophylactic complex, dental diseases.

## О.Е. Рейзвіх, Д.О. Сухомейло, С.А. Шнайдер, С.В. Кленовська, Ю.Г. Романова МАРКЕРИ ЗАПАЛЕННЯ ТА АНТИОКСИДАНТНОГО ЗАХИСТУ В РОТОВІЙ РІДИНІ ДІТЕЙ З РІЗНИМ ІНДЕКСОМ МАСИ ТІЛА ДО ТА ПІСЛЯ ЗАСТОСУВАННЯ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ

Відомо, що запальна реакція виконує захисну функцію в організмі. Аплікації мукозо-адгезивного гелю, що містить ліпополісахарид, запропоновані з метою посилення запальної реакції і активації лейкоцитів, що мігрували в пародонт. Таким чином, запальний процес не має можливості перейти в хронічну форму. Розроблено, обґрунтовано та апробовано спосіб лікування запальних захворювань пародонту у дітей 12 років, який передбачає використання комплексу протизапальних адаптогенів, ефективність якого підтверджується нормалізацією мікробіоценозу порожнини рота (зниження ступеня дисбіозу в 2,1–4,2 рази до показників норми), зниженням активності запальних процесів (еластази в 2 рази, малонового діальдегіду в 1,7 рази), відновленням антимікробного захисту (зниження активності уреазу в 1,4–2 рази; зростання рівня лізоциму в 1,6–1,7 рази) в порожнині рота дітей як з гіпо-, так і з гіпертрофією. Отримані результати свідчать про відсутність запальних процесів та демонструють високу лікувально-профілактичну ефективність запропонованого комплексу.

**Ключові слова:** діти, запалення, індекс маси тіла, ротова рідина, лікувально-профілактичний комплекс, стоматологічні захворювання.

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It is known that one of the most important inducers of inflammation is intestinal endotoxin-lipopolysaccharide (ET-LPS). Already in minimal doses (for children, 0.5–2.5 µg/day or 0.002–5.0 g per 100 g of the dosage form), it causes the development of an inflammatory reaction, which is manifested by an increase in the antimicrobial potential due to reactive oxygen forms [9].

In dental practice, LPS are used in cases of chronic infectious-inflammatory diseases of the maxillofacial area and in chronic recurrent aphthous stomatitis. At introduction of LPS pyrogenic effect is noted [15]. The effectiveness of pyrotherapy in some diseases is associated with an increase in vascular permeability and tissue barriers for not only immune cells and factors of humoral immunity, but also for antibiotics and other drugs. [14]. Dysbiotic and inflammatory reactions in the oral cavity are protective against the parodontium.

Every year, the percentage of children and adolescents with metabolic syndrome and, in particular, overweight, is growing, which is caused by an unfavorable environmental situation, excessive stress loads, the spread of bad habits and unhealthy lifestyles [11], as well as a deterioration in the quality and structure of children's nutrition and adolescents, as well as a sedentary lifestyle. In the body of such children, in most cases, immunological and functional reactions, carbohydrate metabolism, and microbiocenosis of the oral cavity are impaired. These shifts can have a significant impact on the state of hard tissues of teeth, parodontal tissues, the level of oral hygiene in children, which requires the development and application of effective methods for the prevention and treatment of major dental diseases [12]. In medical practice, ET-

LPS is used primarily to stimulate immunity and nonspecific resistance of the organism. The most well-known drugs are pyrogenal, prodigiosan, benketom, pyrexal, salmozan, zymosan, and others. Currently, in dentistry are increasingly used drugs based on LPS in connection with the emergence of new purified bacterial lysates. The main indication for the appointment of bacterial polysaccharides is a chronic course of infectious diseases [13].

In developing the treatment-and-prophylactic complex (TPC), we took into account the need to use in children drugs that would have anti-inflammatory and wound-healing effects, do not inhibit the vital microflora; stimulated immunity at the cellular and systemic level, had an antioxidant effect.

The enzyme lysozyme, which is part of TPC, has antibacterial, immunomodulating, hepatoprotective effects. Also, lysozyme in the composition of the dental elixir has the ability to dissolve the cell membrane of bacteria and fungi, suppress the multiplication of viruses, stimulate immunity and enhance the antimicrobial effect of immunoglobulins.

Quercetin is a bioflavonoid (vitamin P). It has anti-oxidant, membrane-protective, angioprotective, anti-inflammatory action.

Calcium citrate is the most effective form of calcium, which is easily absorbed in the body and is involved in many enzymatic processes (blood clotting, osteogenesis, immune reactions, etc.).

Ovomucoid and gelatin (collagen-like protein) stabilize and protect lysozyme from the destructive action of microbial proteases, enhance the action of other antimicrobial agents, without inhibiting the vital activity of beneficial microflora. In addition, ovomucoid has anti-inflammatory and wound healing effects. All biologically active substances (bioflavonoids, B vitamins, vitamin E, carotenoids) in the preparation obtained from wheat seedlings are preserved to the maximum. Peppermint extract has analgesic, antiseptic and vasodilating effects [8].

Children 12 years old are a special group for observation due to active morphological changes in the body. Children of this age group are a particularly vulnerable contingent in the initiation of inflammatory processes in the parodontium due to a number of reasons: immaturity of bone tissue and parodontium; hormonal instability, rapid growth, insufficient motivation for oral hygiene, irregular and inappropriate nutrition, etc.

Unreasonably, little attention has been paid to the problem of prevention and complex treatment of parodontal diseases in adolescents with the use of LPS preparations. Further study of various aspects of the targeted action of drugs based on LPS in the treatment of parodontal disease is a promising area of modern dentistry.

**The purpose** of the work was to determine the effectiveness of a treatment-and-prophylactic complex containing lipopolysaccharide on the level of inflammatory response in the tissues of the oral cavity in children with different body mass index.

**Materials and methods.** The study involved 78 children of 12 years of age (32 girls and 46 boys). Using indicators of age, height and weight, comparing them with the data of centile tables, the physical development of children was assessed (correspondence of body weight to the height of the child). All children were divided into three groups depending on body mass index (BMI): normotrophy (BMI=20–25), hypertrophy (BMI>25), hypotrophy (BMI<20).

The TPC included: “Pyrogenal” preparation (Research institute of epidemiology and microbiology, Russian Federation), used in the composition of mucosa-adhesive gel with a concentration of LPS 2 mcg/ml; dietary supplement “Lizotsym-forte” (RPE “Biotekhnolohiya”, Ukraine); anti-inflammatory dental elixir “Lizomukoid” (RPE “Biotekhnolohiya”, Ukraine); phyto-gel “Biotrit” (RPE “Biotekhnolohiya”, Ukraine).

Children during 2 weeks, before bedtime, after brushing their teeth used the “Pyrogenal” gel with a concentration of LPS 2 mcg/ml in a dose of 0.5 ml per one application on the gums. After 2 weeks, TPC was continued with preparations of “Lizotsym-forte” 1 pill sublingually 2 times a day 30–40 minutes before meals in the morning and tooth elixir “Lizomukoid” 1 teaspoon of elixir per ¼ glass of water, rinsed the oral cavity after each meal. Children also applied 0.5 ml of “Biotrit” phytogel to the gums before going to bed, after brushing their teeth. Biochemical analysis was performed in the liquid portion of the mixed oral fluid of children, collected after month of conducting of comprehensive prevention. The collection of mixed unstimulated saliva was carried out in the morning, before meals, in centrifuge tubes for 10 minutes [6].

The determination of the activity of urease in the oral fluid was carried out by a method based on the ability of urease to split urea to ammonia, which with Nessler's reagent gives a yellow color. The intensity of the color of the sample is directly proportional to the activity of urease, which was expressed in  $\mu\text{catal/l}$  of ammonia, formed in 1 second in 1 liter of oral fluid [6].

Determination of the level of lysozyme in the oral fluid of children was carried out by the bacteriolytic method, based on the ability of lysozyme to lyse the walls of bacteria. The degree of clarification is proportional to the activity of lysozyme, which was expressed in u/l of oral fluid [5].

The degree of dysbiosis was calculated from the ratio of the relative activities of urease and lysozyme according to Levitsky [7]. Normally, in healthy individuals, this indicator equals 1. In the presence of dysbiotic phenomena  $>1$ .

In saliva, the level of inflammation markers was determined: the content of malondialdehyde (MDH) and elastase activity [1]. MDH content – by reaction with 2-thiobarbituric acid. Normally, the concentration of MDH in the oral fluid is  $0.3 \pm 0.07$  mmol/l.

The degree of inflammation in the oral cavity was assessed by the level of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) (“Sigma”, USA) by the Visser method. Elastase activity is expressed in microcatal per 1 liter of oral fluid. Normally, elastase activity in the oral fluid is  $8.0 \pm 1.0$   $\mu$ kat/l. The results were processed by variational and statistical methods of analysis on an IBM PC in SPSS SigmaStat 3.0 and StatSoft Statistica 6.0 software using Student's t test [2].

**Results of the study and their discussion.** A series of biochemical studies was carried out to assess the dynamics of changes in the oral microbiocenosis and to determine the level of inflammation markers under the influence of TPC. Study conducted 1 month after taking TPC.

Among the many factors influencing the development of parodontitis, the bacterial flora of oral fluid occupies a special place. Obviously, there is a causal relationship between the development of a pathological process in the parodontal tissues and a violation of the bacterial balance in the oral cavity towards the prevalence of pathogenic forms of microorganisms, characterized by their own metabolic characteristics and, therefore, their specific set of enzymes.

Table 1 shows the results of determining in the oral fluid of children an indicator characterizing microbial contamination of the oral cavity – the activity of urease and another enzyme – lysozyme, which is key in the system of antimicrobial protection of mucous membranes, including the oral cavity. Microbiocenosis is a collection of representatives of different groups of microorganisms that inhabit the oral cavity and enter into biochemical, immunological and other interactions with the human body. Conditionally-pathogenic and pathogenic microflora has high urease activity.

A change in the activity of lysozyme in the oral fluid indicates an increase or decrease in antimicrobial protection and indicates the state of the adaptive response of the oral cavity. The activity of lysozyme in all examined children in the initial state was lower than the norm (by 34–44 %).

Table 1

**Effect of treatment-and-prophylactic complex with LPS on the activity of urease and lysozyme in the oral fluid of children with different BMI**

No.	Groups / number of children	Research stages			
		before	after	Before	after
		Urease activity, $\mu$ kat/l		Lysozyme activity, u/l	
1	BMI=20–25 n=26	0.093 $\pm$ 0.011	0.067 $\pm$ 0.008 $p_1 < 0.05$	86 $\pm$ 12	134 $\pm$ 176 $p_1 < 0.05$
2	BMI>25 n=29	0.108 $\pm$ 0.012 $p > 0.05$	0.053 $\pm$ 0.007 $p > 0.05$ $p_1 < 0.05$	92 $\pm$ 15 $p > 0.05$	157 $\pm$ 18 $p > 0.05$ $p_1 < 0.01$
3	BMI<20 n=23	0.126 $\pm$ 0.018 $p > 0.05$	0.061 $\pm$ 0.005 $p > 0.05$ $p_1 < 0.004$	78 $\pm$ 9 $p > 0.05$	129 $\pm$ 15 $p > 0.05$ $p_1 < 0.009$

Note: significance of differences calculated: p – compared to gr. 1;  $p_1$  – in comparison with the indicator before applications.

A simultaneous decrease in lysozyme activity and an increase in urease activity (33–80 %) in the oral fluid indicates a high microbial contamination in the children's oral cavity and the need to introduce antibacterial, anti-inflammatory and microbiocenosis-regulating drugs into the treatment-and-prophylactic complex.

Urease activity in children with BMI=20–25 after the use of TPC decreased by 1.4 times, in children with hypertrophy and malnutrition – by 2 times. Accordingly, the level of lysozyme in the oral fluid of 12-year-old children increases. The greatest changes in the activity of lysozyme were found in children with a BMI>25 one month after the start of the use of the treatment-and-prophylactic complex. The level of this indicator was 1.7 times higher than the initial level and exceeded the indicator of the norm. With normotrophy – by 1.6 times, with malnutrition – by 1.7 times, but did not reach 140 u/l.

In this regard, it can be assumed that the proposed TPC contributes to an increase in the effectiveness of nonspecific antimicrobial protection in the oral cavity and, as a consequence, to a decrease in the number of conditionally-pathogenic microflora representatives in children with different BMI.

The state of the system “antimicrobial protection and conditionally-pathogenic microbiota” in the oral cavity clearly reflects the indicator of the degree of dysbiosis. Figure 1 shows the results of determining the degree of oral dysbiosis by the method of A.P. Levitsky.

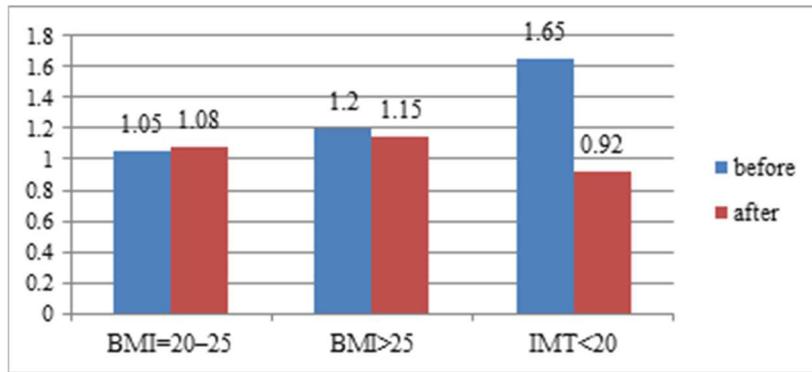


Fig. 1. Influence of treatment-and-prophylactic complex on the degree of oral dysbiosis in children with different BMI.

Before treatment in the oral cavity of children with deviations in body weight in both the direction of hypotrophy and hypertrophy, the degree of dysbiosis exceeded that indicator in children with normal body weight. After using TPC in the oral cavity of children with BMI<20, the dysbiosis index decreased 1.8 times ( $p<0.005$ ). The degree of oral dysbiosis in children with normotrophy corresponded to the norm.

The results obtained indicate that the use of the proposed treatment-and-prophylactic complex leads to an effective increase in the level of nonspecific antimicrobial protection, the degree of contamination of pathogenic microflora decreases, as a result of which the microbiocenosis in the oral cavity was normalized.

As noted in table 2, before the use of TPC, in the oral fluid of children of all observed groups, inflammation markers (elastase activity (norm 0.20  $\mu\text{kat/l}$ ) and MDH level (norm 0.17 mmol/l)) were increased on the background of an increase in urease (norm 0.070  $\mu\text{kat/l}$ ) and a decrease in lysozyme activity (norm 140 u/l).

□bl□2

**Effect of treatment-and-prophylactic complex with LPS on the degree of oral dysbiosis in children with different BMI**

No.	Groups / number of children	Research stages			
		before	before	Before	before
		Elastase activity, $\mu\text{kat/l}$		MDH content, mmol/l	
1	BMI=20-25 n=26	0.372±0.048	0.194±0.025 $p_1<0.005$	0.26±0.03	0.15±0.02 $p_1<0.01$
2	BMI>25 n=29	0.307±0.039 $p>0.05$	0.148±0.018 $p>0.05$ $p_1<0.001$	0.31±0.04 $p>0.05$	0.19±0.02 $p>0.05$ $p_1<0.05$
3	BMI<20 n=23	0.394±0.051 $p>0.05$	0.183±0.019 $p>0.05$ $p_1<0.001$	0.28±0.03 $p>0.05$	0.28±0.05 $p<0.05$ $p_1<0.05$

Note: significance of differences calculated: p – compared to gr. 1;  $p_1$  – in comparison with the indicator before applications.

Elastase is one of the most powerful destructive enzymes produced by leukocytes and microorganisms. The use of TPC and the application of LPS gel on the mucous membrane of the gums helped to reduce the activity of elastase by 2 times in the oral fluid of children, as evidenced by the results obtained 1 month after the start of TPC use. This indicates the extinction of the acute inflammatory process in the parodontium, and in children of all observation groups with different BMI indicators.

The greatest amount of elastase is found in neutrophils. Since this enzyme is of neutrophilic origin, it can be assumed that there is a delayed immunodeficiency state in adolescents with deviations in body weight, both in the direction of hypotrophy and in the direction of hypertrophy. Neutrophil elastase reduces the inflammatory response to invading microorganisms.

An interesting fact is the maximum increase in the level of elastase in schoolchildren of all groups of observation of BMI before the use of TPC (by 1.5–2 times). Elastase is characterized by a powerful destructive effect on tissue proteins. The activity of this enzyme increases significantly in the oral fluid during inflammatory processes in the oral cavity.

The results obtained indicate an increase in anti-inflammatory efficacy with the use of lipopolysaccharide-based TPC.

Table 2 also presents the results of a study of another marker of inflammation, the level of MDH, which characterizes the intensity of lipid peroxidation (LPO) in the oral cavity of children in the observation groups. Analysis of the oral fluid before treatment revealed an increase by 1.5–1.8 times in the content of this indicator in all groups of children with BMI deviations. After the application of TPC, a decrease in the level of MDH was noted; the maximum increase was recorded in children with malnutrition (by 2.2 times).

For the most part, LPS have been studied from the point of view of the participation of endotoxin in the pathogenesis of certain diseases (cardiovascular, gynecological, diseases of the upper respiratory tract and lungs, neurological diseases, diabetes mellitus, etc.), including dental diseases, as well as the

development of dysbiosis, which in turn can be both a consequence of various diseases and lead to an exacerbation of the course of caries, gingivitis, parodontitis, stomatitis and other dental diseases, can complicate their diagnosis and treatment [9]. Thus, the microflora of the oral cavity is a highly sensitive indicator system that reacts with qualitative and quantitative shifts to changes in the state of various organs and systems of the body, especially in childhood, and requires further study. However, unreasonably little attention has been paid to the problem of prevention and treatment of parodontal diseases in adolescents with the use of LPS preparations. Previously, we assumed that an insufficient level of inflammatory response might be the cause of the formation of chronic parodontitis. [10]. LPS has a pro-inflammatory effect, however, in small doses, LPS stimulates the body's defense systems, transferring them from a state of rest to the stage of active action. These properties of small doses of LPS served as the basis for its clinical use.

The mucosa-adhesive gel used by us is a highly active non-specific immunomodulator of a wide spectrum of action: causes activation of the reticuloendothelial, hypothalamo-pituitary and fibrinolytic systems. Pyrogenal activates macrophages, enhances phagocytosis, stimulates the production of interleukin-1, which causes the proliferation of a number of body cells (fibroblasts, endothelial cells of hematopoietic cells, etc.); interleukin-2, which is necessary to maintain the growth of lymphocytes (primarily T cells), induction of endogenous interferon. An increase in the functional activity of phagocytes leads to an increase in the antimicrobial resistance of the organism, acceleration of the formation of antibodies. As a result of the activation of cells of the macrophage series and the secretion of cytokines by them, the functional activity of both the cellular and humoral immune response increases [4].

Our studies confirmed the presence of inflammatory reactions, intensification of lipid peroxidation and reproduction of conditionally-pathogenic and pathogenic microbiota on the background of a decrease in nonspecific antimicrobial and antioxidant protection in the oral cavity in children with different BMI.

The fact of shifts in the oral microbiota in children with hypotrophy and hypertrophy requires the appointment of more correct differentiated schemes of prevention and therapy for not only restoring the microscopic landscape of the oral cavity, but also to reduce the inflammatory process of the mucous tissues in it. This situation requires more frequent and detailed dental examinations in children with deviations in the BMI indicator, which in this case it is advisable to carry out timely and effective pharmacotherapy and especially preventive measures in this contingent of children in order to prevent major dental diseases.

The above data confirm the results obtained in patients with other pathologies [3] and give strong grounds to recommend TPC for the prevention of parodontal disease in children 12 years old with the introduction of a gel containing LPS.

Taking into account the results of our research, we recommend that pediatric dentists take into account the body mass index, which is a screening criterion and the most convenient for assessing underweight or overweight in diagnostic and treatment-and-prophylactic measures, as well as take into account the principles of dental prophylaxis based on the need for a unified approach to dental and somatic health of adolescents.

## Conclusions

1. It was found that in the development of inflammatory parodontal diseases, a weakened inflammatory response plays a significant negative role. In order to activate the inflammatory process, it is proposed to use LPS in the form of a mucosa-adhesive gel.
2. Components of a treatment-and-prophylactic complex complement each other, providing antibacterial, antiviral, immunomodulatory, antidiabetic, antioxidant and membrane-protective effects.
3. Biochemical analysis of the oral fluid, carried out a month after treatment and prophylactic measures, established the normalization of all studied markers. The obtained results indicate the absence of inflammatory processes, the restoration of antimicrobial protection and normalization, demonstrate a high therapeutic and prophylactic effectiveness of the proposed complex.

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### MORPHOLOGICAL FEATURES OF ESTROGEN AND PROGESTERONE EXPRESSION LEVELS IN ECTOPIC ENDOMETRIUM IN WOMEN WITH ENDOMETRIAL CYSTS

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Endometrial cysts occupy one of the leading positions among endometrial neoplasms (up to 30 % of women of reproductive age have a history of this disease). The purpose of the study was to evaluate the immunomorphological features of ovarian endometrial tissue in women with infertility to assess their prognostic and diagnostic value. Our study has shown lower levels of estrogen and progesterone receptors in eutopic endometrium in women with ovarian endometrial cysts than in women without genital pathology. We associate this with the expression variants of the ER and PR receptor systems. In the main group, this ratio was 1 (11 out of 50 women had a characteristic histochemical picture for this pathology); in the comparison group, this figure was 14 cases out of 50, and in the control group – in 18 patients out of 50 studied. This allows us to consider the problem of sex hormones dysregulation as a predictor and, at the same time, a potential point of influence on the pathogenesis of cysts of endometrial origin.

**Key words:** endometrial cyst, infertility, estrogen receptor, progesterone receptor.

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### МОРФОЛОГІЧНІ ОСОБЛИВОСТІ РІВНЮ ЕКСПРЕСІЇ ЕСТРОГЕНУ ТА ПРОГЕСТЕРОНУ В ЕКТОПІЧНОМУ ЕНДОМЕТРІЇ У ЖІНОК З ЕНДОМЕТРІОЇДНИМИ КІСТАМИ

Ендометріюїдні кісти займають одну з головуючих позицій серед новоутворень ендометрію (до 30 % жінок репродуктивного віку мають дане захворювання в анамнезі), тому на меті дослідження стояло вивчення імуноморфологічних особливостей тканини ендометрію яєчників у жінок із безпліддям для оцінки їх прогностичного і діагностичного значення. У ході наукової роботи було виявлено нижчий рівень експресії естрогенових та прогестеронових рецепторів еутопічного ендометрію серед жінок з ендометріюїдними кістами яєчників, ніж у жінок без генітальної патології. Ми пов'язуємо це з варіантами експресії рецепторних систем ER і PR, які розподілились наступним чином: у основній групі це співвідношення прямувало до 1 (11 з 50 жінок мали характерну для даної патології гістохімічну картину), у групі порівняння цей показник склав 14 випадків з 50, а у групі контролю – 18 пацієнтів з 50 досліджуваних. Це дозволяє говорити про проблему порушення регуляції статевих гормонів як про предиктор і водночас потенційну точку впливу на патогенез кіст ендометріюїдного походження.

**Ключові слова:** ендометріюїдна кіста, безпліддя, естрогеновий рецептор, прогестероновий рецептор.

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Endometriosis is a benign chronic disease in which the endometrium is formed outside the uterine mucosa. This can lead to the formation of superficial and deep endometriotic foci, in particular ovarian endometrial cysts [13]. This problem's genetic, cytological, immunological, endocrine, and inflammatory aspects have been intensively studied in recent years. Particular attention is drawn to the hormonal