Research Article

Immune-metabolic disorders inner endometriosis; pharmacological correction of disorders

A. Konoplya Aleksey[1], Ivanova Oksana Yurevna[2]*, I. Konoplya Aleksandr[3], V. Telegina Olga[2], V. Khorlyakov Kirill[3]

ABSTRACT

Aim: The complicacy of endometriosis pathogenesis makes it difficult to choose medical preparations for optimal effects on the main elements that determine the development and progression of this disease. The aim of the research: to study immune and metabolic disorders in female patients with internal endometriosis (adenomyosis) and to determine the effectiveness of immunomodulatory drugs and antioxidants inclusion into conventional pharmacotherapy of this disease. **Method:** A total of 62 female patients with verified adenomyosis Stage II confirmed by clinical and instrumental (ultrasound and hysteroscopic examination) methods were under observation. **Result and discussion:** Twenty female patients received only standard treatment, 21 examined ones additionally received the combinations of antioxidant and immunomodulatory drug: Ridostin and hypoxen or cycloferon and cytoflavin. All the preparations were administered according to the recommendations set forth in the Federal Manual on the Use of Drugs. In the peripheral blood, indicators of the cytokine system, complement and functional metabolic activity of neutrophils were detected. The intensity of lipid peroxidation (LPO), the state of the antioxidant system was assessed at the systemic (circulating blood plasma) and local (aspiration biopsy of the uterus cavity) levels. **Conclusion:** It was established that in patients with adenomyosis there is a combination of LPO activation with the development of oxidative stress with endothelial dysfunction and immune inflammation. The inclusion of drugs with immune modulating and antioxidant effects into standard pharmacotherapy corrects the development of immune inflammation, levels oxidative stress, restoring the activity of the antioxidant system enzymes and reduces the activation of LPO.

KEY WORDS: Adenomyosis, Antioxidants, Correction, Immune metabolic disorders, Immunomodulatory drugs

INTRODUCTION

Endometriosis is traditionally subdivided into genital and extragenital, and genital in its turn is divided into internal (endometriosis of the uterus body) and external (endometriosis of the cervix, vagina, perineum, retrocervical region, ovaries, fallopian tubes, peritoneum, rectum, and rectouterine pouch). In recent years, "internal endometriosis" is increasingly being considered as a very special disease and is designated with the term "adenomyosis."[1,2]

Prolonged and progressive course of endometriosis, the severity of clinical manifestations, persistent reproductive function impairment, reduced the quality of life and work decrement determine both medical and social significance of this pathology, the pathogenesis of which, despite numerous studies, statements and assumptions, still keeps many problems.[3-5]

Endometriosis is a multifactorial disease based on such interrelated, possibly genetically determined components of pathogenesis as excessive proliferation; non-physiological angiogenesis; inadequate local immune response; and aseptic inflammation. With the development of endometriosis, locus minoris resistentiae in endometriosis development is the organs of the reproductive system in which the processes of implantation, invasion, and growth of endometrioid heterotopias occur, which are realized in the clinical picture of dysmenorrhea, chronic pelvic pain syndrome, and infertility.[6-8] However,

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

[1]Department of Obstetrics and Gynecology, Faculty of Postgraduate Education, FSBEI HE Kursk State Medical University of the Ministry of Health of the Russian Federation, Kursk 305041, Russia, [2]Department of Obstetrics and Gynecology, FSBEI HE Kursk State Medical University of the Ministry of Health of the Russian Federation, Kursk 305041, Russia, [3]Department of Biological Chemistry, FSBEI HE Kursk State Medical University of the Ministry of Health of the Russian Federation, Kursk 305041, Russia

***Corresponding author:** Ivanova Oksana Yurevna, Department of Obstetrics and Gynecology, FSBEI HE Kursk State Medical University of the Ministry of Health of the Russian Federation, Kursk, Russia. E-mail: ivanovao1@mail.ru

Received on: 12-03-2019; Revised on: 18-05-2019; Accepted on: 25-06-2019

according to modern scientific researches, in presence of pathology not only local but also systemic changes occur, characterized by impaired lipid peroxidation (LPO), antioxidant protection factors, structural, and functional changes in the red blood cells, which results in the development of endothelial dysfunction, impaired microrheology, hypoxia, and dysmetabolism.[2,3,8,9]

In recent time, the thesis that immune inflammation is the basis of endometriosis pathogenesis is increasingly heard. The authors of this statement testify that the disease is accompanied by increased production of pro-inflammatory cytokines, prostaglandins, the complement components, hydrolytic enzymes, increased angiogenesis processes, abnormalities of the ectopic endometrium and impaired function of cell-mediated immunity, which in its turn causes the development of metabolic disorders at the systemic and local levels.[10,11]

Since the main cellular substrate of inflammatory reactions is phagocytic cells and immune-competent T-cells, which separate the whole spectrum of cytokines, the definition of mechanisms that regulate their expression in endometriosis will significantly deepen our understanding of the etiology and pathogenesis of this disease. It should be born in mind that changes in the immune status and metabolic disorders are interrelated and interdependent processes in the presence of the inflammatory reaction, which is extremely important to consider when developing effective methods of treatment and rehabilitation.[6,10-12]

The complicacy of endometriosis pathogenesis makes it difficult to choose medical preparations for optimal effects on the main elements that determine the development and progression of this disease. The use of antioxidants and immunomodulatory drugs along with hormonal preparations is actively discussed in the literature, the inclusion of which is standard treatment (ST) corrects the modified relevant laboratory parameters and reduces the severity of clinical symptoms.[12-15] However, a small number of such studies and the ambiguity of the obtained results dictate the need for further researches in this direction.

From there, the aim of the study was to study immune and metabolic disorders in patients with internal endometriosis (adenomyosis) and to determine the effectiveness of immunomodulatory agents and antioxidants inclusion in the traditional pharmacotherapy of this disease.

MATERIALS AND METHODS

A total of 62 female patients with verified Stage II adenomyosis (Am),[1] confirmed by clinical and instrumental (ultrasound and hysteroscopic examination) methods were under observation. They were examined in SHI Lipetsk regional perinatal center in 2016–2019. All female patients were randomized by their age (34.7 ± 2.6 years), body weight index (no more than 26 kg/m^2). This examination did not include female patients with extragenital pathology in the acute stage, acute bacterial and viral infections, blood diseases, gastric and duodenal ulcers, necrotizing ulcerative colitis, cancer, taking anticoagulants, disaggregates, and nonsteroidal anti-inflammatory drugs.

All the patients underwent a complex clinical and instrumental examination according to generally accepted standards, herein in all the cases Am verification took place by means of ultrasonography. On the 3–7th days of the menstrual cycle, an ultrasound examination of the pelvic organs was carried out with followed by further hysteroscopic examination with simultaneous aspirate collection from the uterus cavity.

After the diagnosis verification, all the female patients received ST (clinical guidelines of the Ministry of Health of the Russian Federation, 2016[1]) consisting in the therapy with dienogest (Vizanna) at a dose of 2 mg/day orally within 3 months. Twenty patients received only ST (1st subgroup). Forty-two examined patients in addition to ST received various combinations of antioxidant + immunomodulatory agent and were divided into two equal subgroups, which, in addition to ST, respectively, received sodium ribonucleate (ridostin) 1.0 IMin 48 h No. 5 and hypoxen 1 tab. orally 3 times a day or inosine + nicotinamide + riboflavin + succinic acid (cytoflavin) intravenously by drop infusion 10 ml 2 times a day for 7 days and meglumine acridone acetate (cycloferon)-3 tablets orally in 24 h No. 10. All the drugs were administered in accordance with the recommendations set forth in the Federal Manual on the Use of Drugs (formulary system).[16]

The control group was formed over the same period of time from gynecologically healthy women contingent who applied for an outpatient visit to undergo an annual medical examination and accounted for 38 patients.

Laboratory methods of research were carried out on patients' admission to the in-patient hospital and the 15th day following the treatment onset. Immunological and metabolic laboratory parameters were evaluated at the systemic (circulating blood plasma) and local (aspiration biopsy of the uterine cavity) levels. The indicators obtained in the control group patients are taken as a formal norm.

LPO processes were assessed by the content of acyl hydroperoxides (AHP) and malondialdehyde (MDA)

using a set "TBK-Agat" ("Agat-Med" Russia), using the spectrophotometer Apel-330 (Japan) at a wavelength 535 nm and 570 nm. The status of the antioxidant system was assessed by direct/competitive solid-phase enzyme immunoassay with the detection of reaction products in the wavelength range 405–630 using ready-made commercial kits: Superoxide dismutase (SOD) activity "Bender Medsystems" and catalase activity "Cayman chemical" (USA). Total antioxidant activity (TAA) was detected by a method based on inhibition degree of ascorbate and ferro induced tween-80 oxidation to MDA. The level of stable nitrogen oxide metabolites (CM_{ON}) was detected using the kit of "R and D" company (England), neopterin "IBL" (Germany) by means of enzyme-linked immunosorbent assay. In addition, the content of α_1-antitrypsin (α_1-AT), α_2-macroglobulin, and ceruleoplasmin (CP) was detected by means of immunoturbidimetry using the kit "Sentinel" (Spain), C-reactive protein by "vector-best" (Russia) on BTS-350 semi-automatic analyzer (BioSystems, Spain).

Cytokines (tumor necrosis factor α [TNFα], interleukin 1 beta [IL-1β], IL-6, IL-8, interferon-γ [IFNγ], IL-2, IL-18, Granulocyte-colony stimulating factor [G-CSF], IL-4, IL-10, IL receptor antagonist [IL-1RA]) were detected by enzyme-linked immunosorbent assay using the kits of ZAO "vector-best" (Russia), components of the complement system (C_3, C_{3a}, C_4, C_5, and C_{5a}) and factor H – by means of diagnostic kit of OOO "Tsitokin" (Russia). The activity of the C_1 inhibitor was determined using the chromogenic method by its ability to inhibit C_1 esterase. Registration of all the ELISA results was carried out using a microplate photometer "Sunrise," Tecan (Austria).

Phagocytic activity of blood polymorphonucleocytes after their isolation from the blood on the velocity sedimentation gradient ficoll-urografin (d = 1.077) was assessed by determining the phagocytic index (PI), phagocytic number (PH), and phagocytosis activity index (PAI).[17] The activity of neutrophil oxygen-dependent systems was assessed on PD 303 SApel spectrophotometer (Japan) by nitro-blue tetrazolium reduction reaction (NBT-test), spontaneous and zymosan-stimulated (NBT-sp., NBT-st.), and neutrophils functional reserve (NFR).[18]

Statistical processing of the research results was carried out according to the criteria of variation statistical analysis with the calculation of average values (M), arithmetic average errors (m) using computer software Microsoft Excel, 2010. The significance of differences was estimated by U-criterion. Differences with $P < 0.05$ were considered statistically significant.

The degree of laboratory parameters disorders was calculated by the following formula: [19]

$$\left[\frac{\text{Patients index}}{\text{Healthy donors index}} - 1\right] \times 100\%$$

Note: In the range from 1 to 33% the obtained value corresponds to the first degree of laboratory disorders, from 34 to 66% to the second one, more than 66% to the third one.

RESULTS

The main complaints presented by the patients with Am were indications to dysmenorrhea (78%), chronic pelvic pain (65%), dyspareunia (72%), and inability to get pregnant within a year of regular sex life without using contraceptive methods (48%).

The age of menarche among the patients of the main and control groups was comparable and amounted to 13 ± 1.4 years (p1-2 > 0.05). The volume of menstrual blood loss in women with Am was 134 ± 10.2 ml, which was almost 3 times higher than that in the control group (43.2 ± 2.3 ml) (p1-2 < 0.05). The level of ferritin among patients with Am was 10.6 ± 1.2 ng/ml, which was significantly lower in comparison with the control data (84.6 ± 4.9 ng/ml). A comparative analysis of contraceptive methods showed that the hormonal contraceptive method is used by 15% of patients included in the main groups, which was significantly less in comparison with the control group (64%). The overwhelming majority of patients in the control group (72%) had one or more pregnancies in history, and in 78% births passed through the natural parturient canal, and 84% of them did not have any intra-uterine manipulations. Among the patients of the main groups in every third case (31.2%) delivery was performed by cesarean section; 86% underwent instrumental emptying of the uterus due to abortion.

Ultrasound data from patients with Am in 15% of observations revealed heterogeneity and vagueness of endometrial contours in combination with multiple hypo-and hyperechoic inclusions into the myometrium extending to the depth of no more than 1/3 of the myometrium. In 85% of cases, pathognomonic ultrasound signs of diffuse adenomyosis involved more than 2/3 of the myometrium. The data of the clinical and ultrasound picture in 92% of observations were confirmed by the results of the hysteroscopic study.

Determination in plasma of the patients with Am established an increase in the concentration of pro-inflammatory (TNFα, IL-1β, IL-6, IL-8, and IL-18), a decrease in anti-inflammatory (IL-4, and IL-1RA) cytokines. The content of IFNγ, IL-2, and G-CSF growth factor turned out to be higher than the parameters of healthy donors, the level of anti-inflammatory cytokine IL-10 remained

unchanged. After ST, there is an approach to the indicators of healthy donors, but not to their level, the concentration of all investigated anti-inflammatory cytokines and IL-1RA, a compensatory increase in the IL-10 content, the level of other cytokines remained unchanged [Table 1].

The introduction of rydostin and hypoxen into ST additionally normalized the concentration of TNFα and IL-1RA, corrected the levels of IL-1β, IL-6, IL-8, IFNγ, and IL-2 to a greater degree, increased the content of IL-10 even more, the level of other cytokines remained unchanged. Compared to the previous combination of drugs, the inclusion of cycloferon and cytoflavin in ST additionally normalizes the concentration of IL-18 and IL-6, higher than the control values, it increases compensatory the content of the studied anti-inflammatory cytokines and corrects to a greater degree the level of IL-1β, IL-8, G-CSF, and IL-2 [Table 1].

Before the treatment in plasma of the patients with Am, activation of the complement system (an increase in the content of all its components studied) and a multidirectional change in its inhibitors (an increase in C_1-inhibitor and a decrease in factor H) were found. ST approximates to the control indicators the concentration of complement components (with the exception of C_5), increases the level of inhibitors above the donor values [Table 2].

The addition of ridostin and hypoxen to ST has normalized the content of C_{3a}, C_4, and C_{5a}-components of the complement and to a greater degree brought the concentration of C_3 and C_5-components closer to the control values. Compared to the previous group of patients, the use of cycloferon and cytoflavin in ST additionally normalizes the concentration of C_5-component and increases the inhibitors level of the complement system significantly higher than that of the control [Table 2].

Table 1: Cytokine level in blood plasma of patients with adenomyosis before and after treatment

Indicators	Unit of measure	Healthy	1	2	3	4
			Patients (adenomyosis)			
			Before treatment	Standard treatment	ST+ridostin +hypoxen	ST+cycloferon +cytoflavin
TNFα	pkg/ml	3,3±0,2	8,4±0,18*[1]	5,2±0,21*[1,2]	3,9±0,22*[2,3]	3,1±0,2*[2,3]
IL-1β	pkg/ml	1,9±0,21	8,3±0,24*[1]	6,3±0,5*[1,2]	4,9±0,32*[1-3]	3,4±0,4*[1-4]
IL-6	pkg/ml	5,8±0,21	22,9±1,87*[1]	14,6±2,17*[1,2]	10,1±1,6*[1-3]	5,7±0,23*[2-4]
IL-8	pkg/ml	5,2±0,12	37,7±2,1*[1]	24,8±1,8*[1,2]	14,3±1,3*[1-3]	8,1±0,87*[1-4]
IL-18	pkg/ml	291,4±22,5	409,9±23,6*[1]	367,9±21,4*[1,2]	371,5±19,1*[1,2]	309,9±18,5*[2-4]
IL-4	pkg/ml	10,0±0,45	7,5±0,6*[1]	7,5±1,1*[1]	7,7±0,45*[1]	15,45±0,47*[1-4]
IL-10	pkg/ml	3,7±0,21	4,1±0,2	6,6±0,34*[1,2]	8,4±0,42*[1-3]	10,5±0,75*[1-4]
IL-1RA	pkg/ml	370,0±17,9	43,9±9,2*[1]	277,6±15,0*[1,2]	349,5±33,1*[2,3]	463,67±14,9*[1-W4]
IFNγ	pkg/ml	2,4±0,1	15,3±1,4*[1]	13,3±2,2*[1]	6,1±0,42*[1-3]	5,9±0,44*[1-3]
G-CSF	pkg/ml	73,6±1,8	112,5±12,[1]*[1]	123,2±5,9*[1]	119,5±6,8*[1]	90,33±3,11*[1-4]
IL-2	pkg/ml	0,6±0,56	6,2±0,21*[1]	5,9±0,8*[1]	4,1±0,8*[1,2]	2,6±0,42*[1-4]

On this and the following tables, an asterisk indicates significant differences in arithmetic means ($P<0.05$); the numbers next to the asterisk-in relation to the indicators of which group these differences are given. TNFα: Tumor necrosis factor α, IFNγ: Interferon-γ, G-CSF: Granulocyte-colony stimulating factor, IL-1RA: Interleukin-1 receptor antagonist, ST: Standard treatment

Table 2: Compliment system and functional metabolic activity of blood neutrophils in patients with adenomyosis before and after treatment

Indicators	Unit of measure	Healthy	1	2	3	4
			Patients (adenomyosis)			
			Before treatment	Standard treatment	ST+ridostin +hypoxen	ST+cycloferon +cytoflavin
C_3	mg/dl	88,4±2,3	138,4±4,8*[1]	106,6±5,1*[1,2]	96,1±4,1*[1-3]	94,3±3,1*[1-3]
C_{3a}	mg/dl	66,0±7,9	190,4±14,1*[1]	105,0±4,58*[1,2]	57,4±4,3*[2,3]	68,3±7,3*[2,3]
C_4	mg/dl	9,6±0,8	27,9±1,1*[1]	20,4±2,0*[1,2]	8,7±0,93*[2,3]	9,9±1,6*[2,3]
C_5	ng/ml	39,6±3,1	62,7±3,3*[1]	60,3±2,4*[1]	45,7±2,1*[1-3]	40,6±2,8*[2,3]
C_{5a}	ng/ml	16,4±1,9	45,9±3,5*[1]	34,6±1,39*[1,2]	19,8±1,6*[2,3]	17,4±2,5*[2,3]
C_1-inh.	ng/ml	29,9±2,7	71,4±3,9*[1]	85,6±4,9*[1,2]	85,9±4,1*[1,2]	99,6±6,5*[1-4]
Factor H	ng/ml	223,8±16,9	148,8±27,2*[1]	283,0±12,3*[1,2]	259,2±11,3*[1,2]	354,4±10,8*[1-4]
PI	abs.	74,2±5,4	60,1±4,8*[1]	69,5±3,2*[2]	70,8±4,2*[2]	77,6±4,7*[2]
PN	%	6,0±0,33	4,3±0,41*[1]	4,7±0,78*[1]	5,8±0,7*[2,3]	6,1±0,5*[2,3]
PAI	-	4,5±0,23	2,6±0,14*[1]	3,3±0,27*[1,2]	4,1±0,2*[2,3]	4,7±0,3*[2-4]
NBT-sp.	%	8,0±0,7	12,9±0,54*[1]	11,9±0,6*[1]	9,4±0,3*[1-3]	8,9±0,31*[2,3]
NBT-st.	%	23,3±3,4	34,8±2,8*[1]	29,2±3,6*[1]	26,1±2,3*[2]	27,8±3,1*[2]
NFR	%	15,3±1,1	21,9±2,4*[1]	17,9±2,1*[2]	16,7±2,0*[2]	18,9±2,1*[2]

PI: Phagocytic index, PAI: Phagocytosis activity index, NFR: Neutrophils functional reserve, ST: Standard treatment

On patients with Am admission to the clinic due to the study of functional metabolic activity (FMA) of neutrophils in the peripheral blood, a decrease in the activity and intensity of phagocytosis (PI, PN, and PAI) and an increase in the activity parameters of their oxygen-dependent systems (NBT-sp., NBT-st., NFR) were determined. ST normalized two indicators of FMA (PI and NFR) and corrected PAI; the other parameters of neutrophils remained unchanged. Ridostin and hypoxen additionally normalized the parameters of phagocytic (PN and PAI) and oxygen-dependent (HBT-st.) activity and corrected HBT-sp. of polymorphonuclear circulating leukocytes. The use of cycloferon and cytoflavin additionally normalized NBT-sp. and increased PAI [Table 2].

Before treating the patients with Am at the systemic level (circulating blood plasma), there was an increase in LPO products (MDA by 2.6 times, AHP by 4.7 times), a decrease in antioxidant defense factors (TAA by 1.3 times, CP level by 1.5 times, the activity of SOD and catalase 4.8 and 1.2 times, respectively). Besides, the concentration of neopterin increased by 2.3 times, α2-MG by 1.5 times, CRB by 2.3 times, CM_{ON} by 2.6 times, and the level of α1-AT reduced by 2.1 times in the blood plasma [Table 3].

Conducted ST revealed an approach to the indicators of the control group, but not to their level, the content of LPO products, the activity of antioxidant enzymes, the concentration of neopterin, α2-MG, CRB, and CM_{ON}. The inclusion of rydostin and hypoxen to the ST additionally, in comparison with ST normalized the level of α2-MG, corrected to a greater degree TAA, and the content of AHP and neopterin. Compared to the previous combination of drugs, the use of cycloferon and cytoflavin with ST additionally normalizes the concentration of LPO products, CP and brings the level of α1-AT and CRB closer to the parameters of healthy donors [Table 3].

The study of aspirate obtained from the uterine cavity of patients with Am established an increase in the concentration of MDA and AHP by 1.8 and 2.4 times, respectively, CMON by 4.9 times, a decrease in TAA, SOD, and catalase activity by 1.2, 1.5, and 1.3 times, respectively. After ST normalization of TAA, the activity of SOD and catalase was detected, and an approach to the control values, but not to their level, the content of MDA and CM_{ON}. Inclusion of rydostin and hypoxen in the ST normalizes the content of MDA and increases SOD and catalase activity above the control level. The use of cycloferon and cytoflavin in comparison with the previous combination of drugs additionally normalizes the content of AHP and CM_{ON} and to a greater degree increases the activity of antioxidant enzymes [Table 4].

A quantitative comparison of disturbed immunometabolic parameters number revealed that in Am before treatment 97.6% of the studied parameters on the system (blood plasma) and local (aspirate of the uterus) level were changed from the normal values, it was also revealed that disorders of Stage I totaled 15%, and Stages II and III 35% and 50%, respectively. It should be noted that disorders of Stages II–III that required compulsory specific correction[19] totaled 85%. After ST, 87.8% of the parameters studied were changed, and the disorders of Stages I, II, and III totaled 27.5%, 20%, and 35%, respectively. After administration of rydostin and hypoxen in ST, 75.6% of the indices remained changed from the values of the control group (disorders of Stages I, II, and III totaled 22.5%, 17.5%, and 27.5%, respectively). The combination of cycloferon and cytoflavin turned out to be the most effective pharmacotherapy regimen, since 56.1% of the indicators turned out to be changed, while the disorders of Stages I, II, and III totaled 17.5%, 12.5%, and 20% of the parameters, respectively [Table 5].

Table 3: Pharmacological correction of metabolic disorders in patients with adenomyosis at the systemic level

Indicators	Unit of measure	Healthy	1	2	3	4
			Patients (adenomyosis)			
			Before treatment	Standard treatment	ST+ridostin +hypoxen	ST+cycloferon +cytoflavin
MDA	mcmol/l	2,47±0,21	6,31±0,23*[1]	3,35±0,24*[1,2]	3,4±0,25*[1,2]	2,37±0,09*[2,4]
AHP	Standard unit	0,25±0,03	1,17±0,05*[1]	0,81±0,04*[1,2]	0,42±0,03*[1,3]	0,27±0,02*[2,4]
TAA	%	57,2±0,92	45,2±0,93*[1]	47,9±0,78*[1]	51,5±1,1*[1,3]	50,4±1,08*[1,3]
SOD	St. u./ml	59,0±4,6	12,35±0,32*[1]	17,28±0,39*[1,2]	16,2±0,53*[1,2]	16,1±0,31*[1,2]
Catalase	cat/l	12,5±0,42	10,7±0,88*[1]	17,6±0,94*[1,2]	19,9±0,47*[1,2]	18,6±1,84*[1,2]
CP	mg/dl	60,3±3,1	39,4±2,8*[1]	42,6±3,3*[1]	50,3±4,8*[1,2]	62,2±5,0*[2,4]
Neopterin	pg/dl	7,12±0,15	16,2±1,3*[1]	12,4±1,2*[1,2]	9,02±0,71*[1,3]	8,1±0,45*[1,3]
α_1-AT	g/l	90,4±3,93	43,7±3,34*[1]	40,3±2,22*[1]	42,4±2,36*[1]	79,4±5,3*[1,4]
α_2-MG	g/l	4,85±0,32	7,4±0,4*[1]	6,3±0,2*[1,2]	4,3±0,31*[2,3]	4,47±0,33*[2,3]
CRB	mg/dl	3,3±0,32	7,55±0,5*[1]	5,2±0,3*[1,2]	5,7±0,6*[1,2]	4,47±0,33*[1,4]
CM_{ON}	mcmol/l	1,87±0,2	4,82±0,33*[1]	2,84±0,15*[1,2]	2,67±0,12*[1,2]	2,56±0,11*[1,2]

MDA: malondialdehyde, SOD: Superoxide dismutase, TAA: Total antioxidant activity, α_2-MG: α_2-macroglobulin, CP: Ceruleoplasmin

Table 4: Pharmacological correction of metabolic disorders in patients with adenomyosis at the local level

Indicators	Unit of measure	Healthy	1	2	3	4
			Patients (adenomyosis)			
			Before treatment	Standard treatment	ST+ridostin +hypoxen	ST+cycloferon +cytoflavin
Aspirate from the uterus						
MDA	mcmol/l	0,3±0,04	0,54±0,02*[1]	0,48±0,02*[1,2]	0,31±0,03*[2,3]	0,29±0,04*[2,3]
AHP	St. U.	0,05±0,01	0,12±0,05*[1]	0,15±0,04*[1]	0,11±0,03*[1]	0,04±0,01*[2,4]
TAA	%	21,8±0,85	18,3±0,46*[1]	20,6±1,2*[2]	22,3±0,87*[2]	20,4±0,13*[2]
SOD	St. U./ml	10,8±0,37	7,31±0,33*[1]	10,52±0,26*[2]	15,5±0,39*[1,3]	18,4±0,4*[2,4]
Catalase	cat/l	10,4±0,78	8,3±0,58*[1]	9,9±1,03*[2]	17,8±0,5*[1,3]	19,6±1,4*[1,4]
CM_{ON}	mcmol/l	0,42±0,06	2,05±0,09*[1]	0,76±0,05*[1,2]	0,69±0,06*[1,2]	0,44±0,02*[2,3]

AHP: Acyl hydroperoxides, MDA: Malondialdehyde, SOD: Superoxide dismutase, TAA: Total antioxidant activity

Table 5: Comparative efficacy of metabolic disorders correction by various combinations of immunomodulatory agents and antioxidants in adenomyosis

Conducted treatment	Changed parameters from those ones of healthy donors		Changed parameters by the stage of disorder					
			I		II		III	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Before treatment	40	97,6	6	15	14	35	20	50
Standard treatment	36	87,8	11	27,5	8	20	14	35
Standard treatment, ridostin, hypoxen	31	75,6	9	22,5	7	17,5	11	27,5
Standard treatment, cycloferon, cytoflavin	23	56,1	7	17,5	5	12,5	8	20

DISCUSSION

The study shows that female patients with Am have oxidative stress, activation of LPO processes, which is evidenced by an increased concentration of LPO products (MDA, and AHP), CM_{ON} and CRB, a significant decrease in antioxidant defense indicators (TAA, CP, SOD activity, and catalase) in the systemic and local levels. These processes are combined with endothelial dysfunction and immune inflammation, which is evidenced by an increase in vasodilating (CM_{ON}) factor, an increased level of proinflammatory cytokines (TNFα, IL-1, IL-6, and IL-18), neopterin, and CRB.[20-24]

Conducted ST does not normalize 87.8% of the altered indicators of metabolic status that resulted in the administration of drugs with immunomodulatory and antioxidant effects in ST.[14,25]

Inclusion of drugs with the indicated action into the standard pharmacotherapy showed their high efficacy in the correction of the modified parameters of the immune-metabolic status in Am. Rydostin and cycloferon probably correct the development of immune inflammation, reducing the level of pro-inflammatory cytokines, and hypoxen and cytoflavin level oxidative stress, restore the activity of the antioxidant system enzymes, reduce LPO activation, thereby stabilizing the membranes not only of endometrium target cells but also immunocytes as well.

Most studies on the involvement of immune mechanisms in the pathogenesis of endometriosis are devoted to the external genital form. There is a decrease in the activity of natural killer cells, an increase in pro-inflammatory cytokines in peripheral blood and peritoneal fluid, a decrease in the ability of blood leukocytes to produce interferons, an increase in the number of peritoneal macrophages, as well as pro-inflammatory cytokines and chemokines secreted by them in both peritoneal fluid and endometrioid implants. It should be noted that some data from different authors are contradictory.[10,26-28]

In patients with Am, we found an increase in the systemic circulation of chemokine and pro-inflammatory cytokine levels (TNFα, IL-8, IL-1β, IL-6, and IL-18) with a decrease in the concentration of anti-inflammatory cytokines (IL-1RA, and IL-18 4), which reflects the response of resident and recruited innate immunity cells to molecular patterns associated with the damage.[29,30]

A high level of IFNγ (a cellular immune system response of the inflammatory type) and long-term maintenance of an increased IFNγ/IL-4 ratio in the blood, which does not decrease after ST, indicates the activation of IFNγ-producing NK-cells, which are lymphoid cells of type 1 innate immunity (ILC-1) that provides polarization of T-cell differentiation toward T-helper type 1, activating macrophages, expressing enzymes responsible for the development of reactive oxygen forms, and activation of NO synthase with the formation of NO. A significant increase in the content of the colony-stimulating factor G-CSF and IL-2, which does not also decrease after conducted

ST. It can activate mature neutrophils and support the growth of both mixed granulocyte-monocytic colonies and individual colonies of granulocytes and monocytes/ macrophages, and IL-2 with its pronounced ability to induce the activity of practically all clones of cytotoxic cells, activates monocytes and macrophages, thereby increasing the secretion and synthesis of chemokines, pro-inflammatory cytokines, and colony-stimulating factors.[30,31] The revealed changes in cytokine status, activation of the complement system, increased the oxygen-dependent activity of peripheral blood neutrophils (increased production of reactive oxygen forms as a result of a respiratory blast) indicates the presence of immune inflammation at the systemic level. Structural and functional changes of erythrocytes in Am known in the literature[32,33] can cause disruption of micro-rheological and hemostatic statuses, dysmetabolism, and lead to hypoxia, which is one of the causes of inflammatory reaction that is metabolic in nature. The identified changes contribute to the systemic subsequences of endometriosis.

The insufficient clinical and laboratory efficacy of ST in the correction of metabolic, immune and erythrocyte changes justified the use of drugs with immunomodulatory and antioxidant properties in pharmacologic therapy of Am, which was successfully used in the treatment of other diseases with similar changes.[14,25,32-36]

Pharmacological preparations hypoxen and cytoflavin used in the work, which is antioxidant energy correctors, level the manifestations of oxidative stress that occurs during endometriosis,[3,13] prevent the development of free radical oxidation and activation of LPO and stabilize phospholipid membrane layer damaged cells, including red blood cells and immunocytes. Ridostin and cycloferon are immunomodulatory agents whose function is an immune correction (modulation), i.e. correction of the immune system functioning, manifested in strengthening of the weakened and inhibition of stimulated immunity. Perhaps their normalizing effect on the complement system, cytokine and chemokine-producing cells, phagocytes function, which are important suppliers of non-oxidized radicals, determines their corrective influence on endometrial target cell membranes. It is logical to assume that the identification and correction of immune and metabolic disorders can affect the severity of Am. A more pronounced efficacy of cycloferon with cytoflavin combination is probably due to the presence of succinic acid, inosine and Vitamins PP and B_2 in the composition of cytoflavin. These components, carefully selected and balanced, have mutually potentiating metabolic and energy-correcting effects.[34] Cycloferon is a low molecular weight inducer of interferon with a broad spectrum of biological activity (antiviral, immune-modulating, anti-inflammatory, etc.). The main cells producing interferon after administration of cycloferon are macrophages, T-cells, and B-lymphocytes. The drug induces high titers of interferon in the organs and tissues containing lymphoid elements (spleen, liver, and lungs), activates bone marrow stem cells, stimulating the formation of granulocytes. It activates T-lymphocytes and natural killer cells, normalizes the balance between T-helper and T-suppressor subpopulations. At the same time, the direct positive effect of drugs on angiogenesis in the foci of endometriosis, controlled at the local level by angiogenic growth factors and cytokines, secreted by peritoneal macrophages, endometrial heterotopy cells, and endothelium, is not excluded.

CONCLUSION

The study of aspirate obtained from the uterine cavity of patients with Am established an increase in the concentration of MDA and AHP by 1.8 and 2.4 times, respectively, CMON by 4.9 times, a decrease in TAA, SOD, and catalase activity by 1.2, 1.5, and 1.3 times, respectively. After ST normalization of TAA, the activity of SOD and catalase was detected, and an approach to the control values, but not to their level, the content of MDA and CMON. Inclusion of rydostin and hypoxen in the ST normalizes the content of MDA and increases SOD and catalase activity above the control level. The use of cycloferon and cytoflavin in comparison with the previous combination of drugs additionally normalizes the content of AHP and CMON and to a greater degree increases the activity of antioxidant enzymes.

A quantitative comparison of disturbed immunometabolic parameters number revealed that in Am before treatment 97.6% of the studied parameters on the system (blood plasma) and local (aspirate of the uterus) level were changed from the normal values, it was also revealed that disorders of Stage I totaled 15%, and Stages II and III 35% and 50%, respectively. It should be noted that disorders of Stages II–III that required compulsory specific correction[19] totaled 85%. After ST, 87.8% of the parameters studied were changed, and the disorders of Stages I, II, and III totaled 27.5%, 20%, and 35%, respectively. After administration of rydostin and hypoxen in ST, 75.6% of the indices remained changed from the values of the control group (disorders of Stages I, II, and III totaled 22.5%, 17.5%, and 27.5%, respectively). The combination of cycloferon and cytoflavin turned out to be the most effective pharmacotherapy regimen, since 56.1% of the indicators turned out to be changed, while the disorders of Stages I, II, and III totaled 17.5%, 12.5%, and 20% of the parameters, respectively

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Source of support: Nil; Conflict of interest: None Declared