INFLUÊNCIA DA PRODUÇÃO DE CITOCINAS, METABOLITOS DE ÓXIDO DE NITROGÊNIO E TROCA DE LÍPIDOS NA FORMAÇÃO DE ESTÁGIOS EXTERNOS DE ENDOMETRIOSE GENITAL NOS PACIENTES EM IDADE REPRODUTIVA**INFLUENCE OF CYTOKINES PRODUCTION, NITROGEN OXIDE METABOLITES AND LIPIDS EXCHANGE ON THE FORMATION OF EXTERNAL GENITAL ENDOMETRIOSIS STAGES IN PATIENTS OF REPRODUCTIVE AGE****ВЛИЯНИЕ ПРОДУКЦИИ ЦИТОКИНОВ, МЕТАБОЛИТОВ ОКСИДА АЗОТА И ОБМЕНА ЛИПИДОВ НА ФОРМИРОВАНИЕ СТАДИЙ НАРУЖНОГО ГЕНИТАЛЬНОГО ЭНДОМЕТРИОЗА У ПАЦИЕНТОК РЕПРОДУКТИВНОГО ВОЗРАСТА**

ERMOLOVA, Natalya Victorovna^{*1}; PETROV, Yuriy Alekseevich¹;
LEVKOVICH, Marina Arkadevna¹; KOLESNIKOVA, Ludmila Valerievna¹;
DRUKKER, Nina Aleksandrovna¹;

Rostov State Medical University, Russian Federation

** Correspondence author*
e-mail: rniiap.ermolova@gmail.com

Received 10 April 2020; received in revised form 26 May 2020; accepted 20 June 2020

RESUMO

A relevância do estudo se deve à prevalência e aumento da incidência de endometriose, principalmente em pacientes jovens, sua influência na função reprodutiva e a falta de diagnóstico não invasivo confiável da doença e seus estágios. Não existem opiniões unificadas sobre a etiologia e patogênese multifatorial desta doença. Este artigo tem como objetivo revelar os mecanismos de formação de estágios de endometriose com base no estudo do efeito da produção de citocinas, metabólitos de óxido nítrico e metabolismo lipídico. A principal abordagem para estudar esse problema é investigar os níveis sistêmico (soro sanguíneo) e local (líquido peritoneal), sua comparação. Isso permite uma consideração abrangente da patogênese da doença e a determinação do valor dos fatores biologicamente ativos mencionados, dependendo dos estágios da doença. O artigo apresenta novos dados sobre o conteúdo de lipoproteínas de alta e baixa densidade, colesterol, fator de crescimento transformador $\beta 1$ e fator de necrose tumoral α , conteúdo de metabólitos de óxido nítrico e sua interpretação clínica é dada de acordo com os estágios da doença. Os materiais do artigo são de valor prático para pesquisadores de endometriose e praticantes de ginecologistas e obstetras.

Palavras-chave: *endometriose genital externa, lipoproteínas, óxido nítrico, fator de crescimento transformador beta 1, fator de necrose tumoral alfa.*

ABSTRACT

The relevance of the study is due to the prevalence and increase in the incidence of endometriosis, especially in young patients, its influence on reproductive function, and the lack of reliable non-invasive diagnosis of the disease and its stages. There are no unified views on the etiology and multifactorial pathogenesis of this disease. This article is aimed at revealing the mechanisms of the formation of endometriosis stages basing on the study of the effect of cytokines production, nitric oxide metabolites and lipid metabolism. The main approach to study this problem is both to investigate systemic (blood serum) and local (peritoneal fluid) levels, their comparison. This allows comprehensive consideration of the pathogenesis of the disease and determination of the value of the mentioned biologically active factors depending on the stages of the disease. The article presents new data on the content of high- and low-density lipoproteins, cholesterol, transforming growth factor $\beta 1$ and tumor necrosis factor α , the content of nitric oxide metabolites

and their clinical interpretation is given according to the stages of the disease. The materials of the article are of practical value for researchers of endometriosis and practicing obstetrician-gynecologists..

Keywords: *external genital endometriosis, lipoproteins, nitric oxide, transforming growth factor beta 1, tumor necrosis factor alpha.*

АННОТАЦИЯ

Актуальность исследования обусловлена распространенностью и ростом заболеваемости эндометриозом, особенно у пациенток молодого возраста, влиянием его на репродуктивную функцию, отсутствием надежной неинвазивной диагностики заболевания и его стадий. Нет единых взглядов на этиологию и многофакторный патогенез этого заболевания. В связи с этим, данная статья направлена на раскрытие механизмов формирования стадий эндометриоза на основании изучения влияния продукции цитокинов, метаболитов оксида азота и обмена липидов. Ведущим подходом к исследованию данной проблемы является исследование как системного (сыворотка крови), так и местного (перитонеальная жидкость) уровней, их сопоставление. Это позволяет комплексно рассмотреть патогенез заболевания и определить значение обозначенных биологически активных факторов в зависимости от стадий заболевания. В статье представлены новые данные по содержанию липопротеинов высокой и низкой плотности, холестерину, трансформирующего фактора роста $\beta 1$ и фактора некроза опухолей α , содержанию метаболитов оксида азота и дана их клиническая интерпретация в соответствии со стадиями заболевания. Материалы статьи представляют практическую ценность для исследователей эндометриоза и практикующих врачей акушеров-гинекологов.

Ключевые слова: *наружный генитальный эндометриоз, липопротеины, оксид азота, трансформирующий фактор роста бета 1, фактор некроза опухоли альфа.*

1. INTRODUCTION

Endometriosis is a chronic, recurring, genetically determined, inflammatory disease. Its main clinical manifestations are persistent pain and infertility. According to morphological and functional properties It is a benign growth outside the uterine cavity tissue similar to the endometrium (Adamyan *et al.*, 2016).

About 2-10% of women of the reproductive age in the population suffer from this disease (Adamyan *et al.*, 2016; Falcone and Lebovic, 2011) and up to 50% of women screened for infertility (Vercellini *et al.*, 2011). Still there are no non-invasive markers of external genital endometriosis (EGE). Scientists discuss the problem of the systematic progression of this disease but no solution is found.

Processes occurring in the peritoneal cavity play an important role in the development of external genital endometriosis in patients of the reproductive age. The amount of peritoneal fluid (PF) is known to be increased in women with endometriosis comparing with healthy women without endometriosis (Sonova *et al.*, 2010). PF in these patients is characterized by a high content of activated macrophages (Berbic *et al.*, 2009) producing cytokines: TNF- α (Sonova *et al.*, 2011), TGF- $\beta 1$ (Ermolova, 2009) stimulating angiogenesis (Wang *et al.*, 2009) and also

contains a large amount of lipoproteins, especially of low density, which generate oxidized lipid components as part of a macrophage inflammatory medium (Anderson Sanches Melo *et al.*, 2010)

The TGF- $\beta 1$, found in high concentrations in the PF of patients with EGE (Charles *et al.*, 2010), has anti-inflammatory properties and is able to inhibit TNF- α , IL-1 β and other cytokines synthesis, inhibit endothelial cell proliferation.

Information on the TNF- α role in the cell is rather contradictory, and often it produces opposite effects on the same cell types (Laschke, 2011). TNF- α activates lipolysis and inhibits lipogenesis.

The development of so-called local aseptic inflammation and immunocompetent cell dysfunction is generally considered as a fact established both in an experimental model of endometriosis in mice and in the abdominal cavity of patients with endometriosis (Schmidt *et al.*, 2015). In endometriosis macrophages are not able to perform their all functions, and an estrogen-dependent chronic inflammation is triggered (Takebayashi *et al.*, 2014). Imbalance in the formation of free radicals makes the basis of the inflammatory process in endometriosis (Darling, 2013).

Nitric oxide plays the role of a universal regulator of many biological functions having both protective and damaging character. Thus, being an important regulator of vascular tone, providing immune responses, neuronal transmission and antioxidant protection, nitric oxide can under certain conditions also act as a free radical, which has a pronounced destructive effect (Babushkina, 2009; . Bryan *et al.*, 2009). Nitric oxide is formed in cells from L-arginine under the action of NO-synthase enzyme (Vanin, 2008). There is a second way of converting L-arginine, by means of arginase, it is hydrolyzed to ornithine and urea, followed by the formation of proline (Babushkina, 2009), which in turn is a source of tissue hardening, and, it is well known, moderate and severe degree of EGE in 100% of cases is accompanied by the development of the adhesive process (Luciano and Adhesion, 2008). In this case, the problem is not only in the clinical manifestations of the adhesive process (pelvic pain, infertility) (Chernukha, 2011), but also in the high recurrence rate after mechanical adhesiolysis during laparoscopy. Arginase and NO-synthase compete with each other for a common substrate - L-arginine. Arginase, an enzyme in the urea synthesis cycle, has a higher activity and exceeds that of NO-synthase (Vanin, 2008).

N. Santanam *et al.* (2002), found the presence of an acidic environment in the PF of women with endometriosis. PF lipoproteins in patients with endometriosis have a greater ability to oxidation compared with blood plasma lipoproteins.

Many scientists search for new non-invasive markers of endometriosis, and biochemical and immunohistochemical indicators already developed are unfortunately not specific (Vodolazkaia, 2012; Gajbhiye *et al.*, 2012). Additional studies are necessary to determine the effectiveness of the early diagnosis of dyslipidemia in order to prevent endometriosis in patients of reproductive age. Of particular interest is the participation of cytokines, metabolites of nitric oxide and lipoproteins in the formation of the stages of the disease, which will allow to personalize the treatment tactics of these patients.

The purpose of the study was to determine clinical significance of lipid and cytokine markers status, nitric oxide metabolites in the formation of disease stages in patients of reproductive age with external genital endometriosis.

2. MATERIALS AND METHODS

The study, approved by the Local Ethics Committee of the Research Institute of Obstetrics and Pediatrics at Rostov State Medical University, included 96 patients, 74 were patients with EGE and 22 women without endometriosis (control group) who underwent examination and treatment at the gynecology department of the Research Institute of Rostov State Medical University.

All patients were divided into 3 clinical groups: group I consisted of 28 patients with I-II stages of EGE according to r-AFS classification (1985), group II - 46 patients with stages III-IV of the disease. The III control group consisted of 22 patients without endometriosis.

To achieve the above purpose, the following methods were used: clinical (medical history, examination), laboratory, ultrasound, endoscopic (laparoscopy, hysteroscopy, colposcopy), histological examination of biopsy specimens obtained during laparoscopy.

The following criteria were used to include the patients into the study: Criteria for the inclusion of patients in the study: 1. The patient underwent endoscopic surgery (laparo- and hysteroscopy), confirming EGE with subsequent morphological verification of the diagnosis, in patients without EGE to exclude tubal-peritoneal infertility factor, sterilization. 2. Reproductive age of patients. 3. Complaints of infertility and/or pain. 4. Body mass index 18.5 - 25 kg/m².

The criteria for the exclusion from the study were the puberty and perimenopausal age of the patients, obesity or metabolic syndrome.

The patients were included into the study after obtaining informed consent and were recorded according to the standards of the Ethics Committee of the Russian Federation (protocol No. 46 dated 04/17/2014). Blood, peritoneal fluid of patients obtained by laparoscopy was used in the study.

Analysis of clinical data showed that in all patients menstrual cycle was preserved, its mean duration in group I (EGE stage I-II) was 28.04 ± 1.2 days, in group II (EGE stage III-IV) - 28.7 ± 1.4 days, in group III (control) - 26.1 ± 1.3 days. Early menarche (up to 11 years old) was more common in patients with EGE from group II (60.9%) and group I (52.7%), i.e. in patients with EGE, early menarche was found, unlike patients in the control group. The mean age menstruation beginning was in group I 10.2 ± 0.11 years, in group II - 11.8 ± 0.19 years, in group III - $13.4 \pm$

0.1 years. Algomenorrhea occurred in 50% of patients with EGE with menarche, in 85.1% of patients with I-II stage of EGE and only in 21.7% of patients with III-IV stage of EGE.

34.9% of patients with EGE complained of progressively increasing pelvic pains, before and during menstruation 54%, dyspareunia 22%, spotting blood excretions before or during menstruation 28%. It should be noted that with minimal forms of the disease, pelvic pain was found only in 4% patients, while in severe forms (stage III-IV of EGE) this symptom was present in more than half (55.2%) of patients which is significantly higher ($p < 0.05$) when conducting intergroup comparisons. Psychoemotional disorders were in 38% in patients with EGE. Progression of the disease leads to increased frequency of symptoms. The severity and intensity of complaints prevailed in the group of patients with III - IV stage of EGE.

40.8% with EGE had primary infertility. In patients with EGE I-II stages it was registered in 68% of cases, which was 1.2 times higher than in the group with EGE III-IV stages, 39.5%. Secondary infertility was in 21.2% with EGE: in the first group of patients it was in 28% of patients and in the second group – in 23.6% of patients.

79.7% of the patients with stage I-II EGE (group I) had in their anamnesis sexually transmitted infections (STIs), and inflammatory diseases of the pelvic organs (PID) in 57.4% of patients, while in group II patients with EGE of the III-IV stage of STIs were found in 78.3%, and PID - in 60.9% of women.

Analysis of the duration of the disease from the onset of clinical manifestations to the 1st hospitalization revealed that this period ranged from 3 months to 6 years.

In 53.9% of patients ultrasound examination showed endometrioid cysts from 4 to 10 cm in size with a fine suspension of both unilateral (4%) and bilateral (95%). Only in 18% of patients with EGE typical endometrioid infiltrates from 2.5 to 3 cm were identified in the area of the uterine isthmus.

Colposcopy revealed background diseases of the cervix in 60.1% of patients with EGE stage I-II, in 76.6% of patients with EGE stage III-IV, while cervical dysplasia was found in 16.9% of in group I patients and in 21.7% group II patients. In the control group of patients, background cervical pathology was found in 30%, and cervical dysplasia in 10% of women.

In all cases surgical treatment was performed by laparoscopic access in the organ-saving volume. The main tasks of laparoscopic treatment were: maximum removal of all endometrioid foci; elimination of the adhesive process; restoration of the normal anatomy of the internal genital organs. Endometriosis prevalence was determined by laparoscopy using the recommendations of the revised classification developed by the American Society for Fertility (r-AFS) (1985). In our study, laparoscopy revealed a high percentage of endometrioid ovarian cysts (53.97%), which may indicate the untimely diagnosis of EGE. Superficial foci of endometriosis in the ovaries were detected in 42.8% of cases. Sacro-uterine ligaments (77.7%) and the rectum-uterine space (69.8%) lesions were most common, while in the vesicoureteral space foci of endometriosis were detected only in 31.7% of cases. On the wide uterus ligament endometriotic lesions were detected less often - 4.7% of cases.

In patients of the first group with EGE stage I-II, adhesions of the I degree were found in 12.8% of patients, of the II degree – in 10.8%, of the III degree – in 2.7%, and of the IV degree – in 2%. In women of the second group, with EGE stage III - IV, adhesions were found in 12%, 16.3%, 17.4%, 16.3% of cases, respectively.

In stage I-II, ovarian lesions were registered in 31% of cases, endometrioid cysts in 4%. Only peritoneum lesion was in 66.2%, combined forms - (peritoneum and ovaries) – in 48.7%. In EGE stages III - IV ovarian lesions in the form of endometrioid cysts were revealed in 94.6% of cases. Damage to the peritoneum was in 40.2% of cases. Combined forms (peritoneum and ovaries) were in 78% of patients.

Hysteroscopy revealed endometrial hyperplastic process in the form of simple hyperplasia without atypical signs was detected in 27% of group I patients and in 35.3% of group II patients, while in the control group this pathology was found only in 5% of women.

EGE was histologically confirmed in all patients of clinical groups I and II. Endometriosis verification was carried out according the degree of the process prevalence and the morphofunctional characteristics of heterotopic foci. The morphological diagnosis was based on the microscopic detection of ectopic endometrioid epithelium in combination with elements of the endometrioid stroma. Macrophages containing hemosiderin were also found in it. Endometrioid heterotopy was microscopically presented by an

accumulation of cellular formations, tubular, branching or cystically dilated. Internally the glands were lined with a cylindrical epithelium, sometimes even with cilia. In heterotopic foci, there were two types their existence – progression and regression. Proliferation of glandular epithelium of varying severity, secretory changes, decidualization of the cytogenic stroma were most common and typical for the progressing EGE foci. Regressing OGE foci without signs of functional activity were characterized by the presence of cystic gland transformation, epithelial atrophy, fibroplastic restructuring and angiomatosis of the cytogenic stroma in 19.6% of all patients, in EGE I-II stages – in 13.5% of patients, III- IV stages - in 24.5% of women. Histological examination of the surgical material revealed endometrial glands in endometrioid heterotopia in 71.7% of cases, 49.6% of patients had it in the proliferation phase, 50.4% of patients – in the secretion phase, and 18.3% in the middle secretion phase – 18.3%, in late - 81.7%. Since laparoscopy was performed in the first phase of the menstrual cycle, the data obtained indicate that in half of cases there was no correlation of the state of endometrioid heterotopy to the uterine cycle.

Angiomatosis was more pronounced (24.5% of cases) in patients with EGE stage III-IV. Moreover, in the stroma surrounding the gland, a mild lymphoid and plasmocytic infiltration was in 11.7% of cases of a focal character. As a percentage, there were no differences in EGE stages. Thus, at stage I-II they made 13.4% and in EGE stage III-IV - 12.5%. Stromal sclerosis was found in 55.7% of EGE patients. Moreover, diffuse sclerosis, which develops at the end of inflammation, or chronic insufficiency of blood supply, was in 50% of cases, while focal sclerosis occurred in 25% of patients. In OGE stage I-II it was revealed in 43.9% of patients, in stage III-IV – in 65.2% of patients. Hyalinosis was found in 33.4% of patients, and it was more pronounced with OGE stage III-IV – 42.9% of patients, versus 21.6% of patients with stage I-II of the disease. Inflammation in the stroma of endometrioid heterotopia in was found in 34.9% of cases in the general group, in OGE stages I-II – in 43.2% of patients, in stages III-IV – in 28.3% of patients. It should be noted that inflammatory changes in endometrioid heterotopies are more pronounced in EGE stage I-II, while sclerosis and tissue hyalinosis as outcomes of this process prevail in patients with EGE stage III-IV.

Serum lipoproteins and EGE were determined using Randox kits (Germany) on

Sapphire-400 biochemistry analyzer (Japan). The endogenous level of nitric oxide in the form of a nitrite anion (NO-) was determined using Griss reagent. Nitroxide synthase (NOS) activity was assessed by the increased nitric oxide production from L-arginine in the presence of NADPH. Growth factors, cytokines, were determined by enzyme-linked immunosorbent assay using “R&D Systems” kits (USA). Statistical data processing was carried out using the Statistica licensed software package (version 5.1, made by Stat Soft). The sample size in the work completely corresponds to the range of obtaining a confidence interval of probability, 0.95, and accuracy of calculation of statistical parameters, 0.05. Results are presented as median and interquartile interval. The significance of differences between the compared parameters was determined by the Mann-Whitney criterion for nonparametric distributions. The results were considered to be statistically significant at $p < 0.05$. In correlation analysis of the data, the Pearson criterion was used in the case of a normal distribution of variables and otherwise the Spearman criterion, and the significance level was 0.05.

3. RESULTS AND DISCUSSION

The results obtained in patients with stages I-II of EGE are presented in Table 1. It shows that the content of TGF- β 1 in the blood serum in these was 1.4 times higher compared to the control group of women ($p < 0.004$).

The study of lipoproteins revealed significant changes in the content of HDL in the blood serum, characterized by their increase of 1.1 times ($p < 0.013$) (Table 1). Nitric oxide (NOx) metabolites content in the blood serum of patients with I-II stages of EGE was 6.9 times higher than in the control group ($p < 0.041$), while NO synthase activity was not change significantly and remained at physiological level.

At stage III-IV of EGE, the content of TNF- α in the blood serum of patients was 1.9 times higher compared with the data of women in the control group ($p < 0.014$).

Significant changes in the content of the studied lipid fractions concerned only HDL both in minimal and severe forms of EGE ($p < 0.013$ and $p < 0.06$, respectively). The content of NOx in the blood serum of patients with III-IV stages of EGE (Table 1) was 13.4 times higher than in the control group ($p < 0.001$).

The study of the lipid spectrum in the PF in patients with stages I-II of EGE showed the increase of free cholesterol 1.43 times content, HDL - 1.73 times and HDL - 1.6 times ($p < 0.008$, $p < 0.004$, $p < 0.003$, respectively) (Table 2).

The content of TNF- α in the PF in patients with stages III-IV of EGE was 1.5 times less compared with the control group of women ($p < 0.050$) (Table 2), while the level of TGF- β 1 in these patients was 1.7 times higher than the control ($p < 0.001$).

The content of NOx in the PF of patients with III-IV stages of EGE was 3.3 times higher than in patients in the control group ($p < 0.039$) (Table 2). The activity of NO synthase exceeded physiological parameters by almost 1.5 times ($p < 0.001$).

There is no doubt that for the most successful solution of the EGE problem it is necessary to use modern methodological approaches, in particular, a comprehensive study of the molecular basis of the disease development and the relationship of metabolic disorders at the local and systemic levels. Formation of the disease stages is of special interest since it makes the basis for the further management of patients. The proliferation factors of endometrioid heterotopia (cytokines and growth factors) and metabolic (lipid) parameters that affect the activity of enzymes involved in the processes of angiogenesis were studied.

Biologically active polypeptides - growth factors that stimulate or inhibit angiogenesis, and also regulate cellular mitogenic effects play an important role in the regulation of intracellular metabolism. This refers to TGF β 1 (Burlev, 2012; Ermolova, 2008; Ermolova, 2009). TGF β 1 inhibits proliferation and induces differentiation of most types of cells that have been studied already. As for TGF β 1 metabolic features, it inhibits NO synthase activity and activates arginase (Babushkina, 2009). Significance of the systemic level of regulation of metabolic processes that control cell growth (TGF- β 1) in the study indicates the presence of compensatory reactions in patients with I-II EGE stages.

It was shown in the experiments that incubation of recruited macrophages with cholesterol causes a significant increase in TGF- β 1 (Schwartz, 2009). Cholesterol can directly induce the synthesis and secretion of TGF- β 1 in monocytes - macrophages.

Variations in the lipid content of cell membranes can also significantly change the

activity of proteins that are their components (Keelet, 2012). In the presence of inflammatory processes in the body, cholesterol and phospholipids content changes. Cell membranes, mainly the middle phospholipid layer, with intensively occurring processes, serve as a screen to reflect the aggressive effects of the cascade of toxic products. As a result of the changes caused, the structure, membrane functions, balance between radical oxidative processes and the functional antioxidant defense system are disturbed. A large number of low-density lipoproteins (LDL) generate very low-density lipoproteins (VLDL) (oxidized components), which enhance the growth of heterotopia by activating endometrial cell growth factors (Verit, 2013; Melo, 2010).

HDL increase causes paraoxonase-1 (PON-1) increase in the content, which is a part of lipoprotein complex, and, consequently, its activity. Study of the paraoxonase gene polymorphism (Kolesnikova *et al.*, 2012) confirms this proposition, which showed the absence of its differences compared with the parameters in patients of the control group. HDL modification in women with EGE I-II stages at the systemic level is compensatory in nature, aimed at preventing the formation of more "significant" endometrioid heterotopia in the peritoneal cavity.

Increase in nitric oxide production is due to the activation of the nitrite reductase pathway for its synthesis. NO metabolites hyperproduction contributes to the progression of endometrioid heterotopia due to vasodilation of the existing vessels and the formation of new ones by stimulating migration and proliferation of endothelial cells (Sonova *et al.*, 2010). Nitric oxide is also known as a powerful anti-apoptotic compound, high generation of which plays an important role in the synthesis and deposition of collagen, which contributes to the formation of adhesions (Adamyan *et al.*, 2016; Guidice, 2010).

An almost 2-fold increased TNF- α level enhances the induction of phospholipase A2 (FLA2), which is involved in the triggering of the arachidonic acid cascade, resulting in the synthesis of prostaglandins that cause pain syndrome. According to our study, 68.9% of patients with EGE suffered periodic pains before or during menstruation, 8.1% had dyspareunia. 66.0% of patients had dysmenorrhea with menarche, which was significantly higher than in the control group (13.6%). TNF- α acts on proliferating cells by enhancing apoptosis. These patients had an inflammatory process, since TNF- α synthesis is observed in stimulated cells,

suggesting that its high level is the result of infection, which was found in 78.3% (pelvic inflammatory diseases) and in 14.4% (sexually transmitted infections) of patients.

LDL in patients with stages III-IV of the disease at the system level corresponded to control values. This is provided by an increased HDL content, which includes the enzyme paraoxonase, which protects against LDL oxidation. This enzyme promotes the breakdown and elimination of oxidized cholesterol. High levels of HDL cholesterol (hLDPV) in patients with external genital endometriosis at all stages of the disease suppresses inflammation. This situation follows from the previously identified inflammatory process in the pancreas, confirmed by the increased content of proinflammatory cytokines (TNF- α and IL-1 β) in it (Ermolova, 2008). However, it was found that in the inflammatory process, HDLPs change their function to pro-inflammatory due to a decrease in the concentration of the main HDL apolipoprotein - ApoA. It provides reverse transport of cholesterol to the liver and increases the activity of paraoxonase. If the detected high level of HDL in the pancreas increases the anticoagulant properties of red blood cells - on the one hand, and on the other hand, it manifests pro-inflammatory properties on the background of the inflammatory process, which enhances inflammation, then a complex metabolic situation is revealed when it is difficult to determine which HDL function predominates.

In the pancreas of women with I-II stages of EGE, the increased LDL level ($p < 0.003$) was found comparing to the control data, which may be the result of compensatory reactions aimed at increasing the activity of PLA2, LDL structural unit.

A correlation analysis of the actual material was carried out in the experiment. Significant negative correlation between the content of free cholesterol and metabolites of nitric oxide ($r = -0.8$) in the blood serum and between free cholesterol and the activity of NO synthase ($r = -0.85$) is of special interest. The revealed regularity shows that at the systemic level there is a dependence of nitric oxide production and NO-synthase activity on the lipid spectrum, and in particular, on the level of free cholesterol in women with EGE I-II stages.

Changes in TNF- α production suggest that there is a reduced function of this cytokine as an angiogenesis factor (Guidice, 2010) and cell proliferation in the pancreas. TNF- α regulatory

capabilities in the pancreas are determined by its ability to induce apoptosis and produce antibacterial protective effect. In this case they are sharply reduced.

Change of metabolic processes at the systemic level can play a significant role in their disruption at the local level. First, TGF- β 1 high level as a factor that reduces proliferation is controlled by the central cerebral structures. However, modification of the molecular relationships of lipid metabolism (HDL) with angiogenic compounds in endothelial cells increases NO generation, causing an increase in heterotopies blood supply. It should be pointed out that a high level of TGF- β 1 inhibits NO-synthase, reducing one of the reactions of NO formation, i.e. the development of compensatory processes occurs at the systemic level.

Thus, the metabolic features of the formation of I-II stages of EGE in patients at the systemic level (blood serum) are significantly increased levels of TGF- β 1, HDL and nitric oxide metabolites. Besides, significant changes in the content of lipid metabolism products were found in the pancreas (local level). They were characterized by a significant increase in cholesterol, HDL and LDL, which contribute to the formation of oxidative stress in the abdominal cavity.

In EGE stages III – IV a high level of TGF- β 1 is a factor in the development of fibrosis, which underlies the adhesive process. According to the data obtained by of N.V. Ermolova (2008), a high level of this growth factor is due to the inflammatory process both at the systemic and local levels.

Nonsteroidal products of the mevalonate lipid exchange pathway perform prenylation of small G-proteins (Rho, Rac), which ensure their integration into membranes and manifestation of functional activity. Modification of molecular bonds of lipid metabolism in endothelial cells increases NO generation, causing an increase in blood supply to endometrioid formations.

In this study, in women with EGE stages III-IV, different lipid fractions were differentiated in connection with the existing metabolic relationship of the mevalonate lipid exchange pathway and the generation of the main vasodilator, nitric oxide. In EGE stages III-IV, an increase in HDL content at the systemic level obviously plays a significant role in the state of lipid metabolism at the local level.

The increased blood supply to endometrioid heterotopia is due to the high content of nitric oxide metabolites in the pancreas of patients with EGE stages III-IV stages. This compound is a powerful vascular growth factor, vasodilator, and apoptosis inhibitor (Guidice, 2010). NOx excess is accompanied by oxidative stress and acts as a mutagenic, carcinogenic, and antiapoptotic factor (Sonova *et al.*, 2010; Adamyan *et al.*, 2016). High NOx generation in the pancreas, is due to the NO-synthase pathway, as evidenced by the high activity of this enzyme. There are few reports on the increased NO-synthase expression in endometrioid heterotopies, which can be explained by the presence of a functionally defective enzyme form or the secondary nature of this process (Kinugasa, 2011; Cayci, 2011; Rogers *et al.*, 2013).

The revealed very high NO generation in the peritoneal fluid can play an important role in the development of hypoxia. Recently, it has been proved that in the case of excessive NO production, its interaction with superoxidion (O_2^-) is observed with the formation of peroxynitrite ($ONOO^-$), a powerful oxidizing agent that modifies hemoglobin and changes its affinity for oxygen (Zinchuk, 2006). The latter leads to a decrease in the oxygen-binding properties of blood, and, consequently, to hypoxia, which leads to cellular metabolism disorders during endometriosis foci formation of (Taylor *et al.* 2002; Lee *et al.*, 2009).

Correlation analysis of the studied parameters in the blood serum of patients with EGE stages III-IV showed a close positive relationship between the HDL level and TNF- α content of ($r = 0.60$). In this case, a negative correlation between NO-synthase activity and the TGF- $\beta 1$ level ($r = -0.33$) was found. A significant negative relationship was in the peritoneal fluid between HDL and NO ($r = -0.75$).

There is no doubt that the accompanying inflammatory process, immunological dysregulation, apoptosis inhibition, angiogenesis activation, and oxidative stress in the abdominal cavity are pathogenetic factors contributing to the survival and growth of endometrioid heterotopies (Adamyan, 2016).

Patients from our clinical sample mainly complained of infertility and / or pain, so we tried to find an explanation of their origin within the limits of our studies. We found prevalence of primary infertility regardless of the EGE stage. However, in percentage terms, both primary and

secondary infertility prevailed in patients with EGE stage I-II stage (57.1% and 32.1%, respectively). Apparently, both ovarian factors and the adhesion process in the abdominal cavity, characterized in our conditions by the involvement of the peritoneum of the sub-ovarian fossa and cul-de-sac, which may interfere with fertilization. The decrease in the ovarian reserve was due to the existing formation of endometrioid ovarian cysts from 3 to 10 cm, marked high TGF- $\beta 1$ content, influencing arginase and, subsequently, enhancing the synthesis of proline, the main substrate of the connective tissue. On the other hand, it is not possible ignore in this connection (with TGF $\beta 1$) the importance of high nitric oxide generation due to nitrite reductase synthesis in the absence of NO-synthase activity changes, which also contributed to the formation of adhesions. In this metabolic situation, significantly high HDL levels both in blood serum and peritoneal fluid should be taken into consideration, since they contribute to high generation of nitric oxide metabolites and the formation of oxidative stress.

The pain syndrome in our patients was probably due to the significant production of prostaglandins, which, on the one hand, were stimulated by high TNF- α content, which activates phospholipase A2 (a structural unit of LDL). On the other hand, the high generation of nitric oxide metabolites, changing the metabolism of arachidonic acid, acts on cyclooxygenase and thereby stimulates the formation of prostaglandins.

The revealed changes in the relationship of the lipid spectrum, growth factors, and angiogenic compounds are the theoretical basis for additional therapeutic measures aimed at normalizing metabolic processes in OGE.

4. CONCLUSION

In patients with EGE stages I-II a high content of TGF- $\beta 1$, HDL and nitric oxide metabolites was found in the blood serum (systemic level). Modification of metabolic processes at the systemic level plays a significant role in disrupting them locally (peritoneal cavity). A change in the molecular interactions of lipid metabolism (HDL) and angiogenic compounds in endothelial cells increases nitric oxide generation, which enhances blood supply to heterotopies.

Compensatory processes develop at the system level, in the form of a nitrite reductase pathway for the synthesis of nitric oxide, since a

high level of TGF- β 1 inhibits NO-synthase. Adaptive reactions in the peritoneal cavity, contributing to a decrease in proliferation and inflammation, are caused by an increase in free cholesterol, LDL and HDL.

In patients with EGE stages III-IV there is relationship between HDL and TNF- α at the systemic level, which has anti-inflammatory properties. In the pancreas of patients of this group the parameters of TGF- β 1, nitric oxide metabolites and NO-synthase are significantly increased, which indicates NO-synthase pathway for the synthesis of nitric oxide.

The change in the relationship of the lipid spectrum, nitric oxide metabolites and cytokines is the theoretical basis for additional therapeutic measures aimed at normalizing metabolic processes in EGE.

5. REFERENCES

1. Adamyan, L. V.; Kulakov, V. I.; Andreeva, E.N. Endometriosis Clinical recommendations. Moscow, 2016.
2. Babushkina, A. V. L-arginine from the point of view of evidence-based medicine *Ukr. Honey. Chasis*. **2009**, 74 (XI / XII).
3. Burlev, V.A.; Dubinskaya, E. D.; Ilyasova, N.A.; Burlev, A.V.; Gasparov, A.S. Pro- and anti-inflammatory activity in patients with peritoneal form of endometriosis and adhesions of the pelvic peritoneum. *Problems of reproduction*. **2012**, 4, 6-11.
4. Vanin, A.F.; Adamyan, L.V. Dinitrosol complexes of iron with thiolate ligands as the basis for the development of drugs with different effects. *Problems of reproduction. Spec. Issue, 21st Century Technologies in Gynecology*. **2008**, 86-89.
5. Ermolova, N.V. The pathogenetic role of endothelial factors and sex hormones in the formation of external genital endometriosis. *Russian Bulletin of the Obstetrician-Gynecologist*. **2007**, 8(3), 29-32.
6. Ermolova, N.V.; Pathogenetic mechanisms of the formation of external genital endometriosis and its stages in patients of reproductive age. Ph.D. thesis, Rostov-on-Don, 2009.
7. Zinchuk, V.V. NO as a ligand that determines the oxygen-binding properties of blood. Mater. Int. Symptosis "Active forms of oxygen, nitrogen and chlorine in the regulation of cellular functions in normal and pathological conditions." Grodno 2006, 89-93.
8. Keelet, E.P.; Korneeva, I.E.; Shurshalina, A.V. Infertility of unclear origin: the focus of modern scientific research. *Probl. Reprod*. **2010**, 16(1), 32-35.
9. Kolesnikova, L.V.; Ermolova, N.V.; Linde, V.A.; Shiring, A.V.; Slesareva, K.V.; Tomay, L.R.; Skachkov, N.N. The absence of the negative effect of paraoxanase gene polymorphism in the exchange of lipoproteins with external genital endometriosis. *Journal of Obstetrics and Women's Diseases. Specialist. issue*, **2012**, LXI, 28-29.
10. Sonova, M.M.; Arslanyank, N.; Loginova O.N.; Maksimova Yu.V.; Muralova Z.Kh.; Shulyak I.Yu. Optimization of the treatment of external genital endometriosis using antioxidant agents. New technologies in the diagnosis and treatment of gynecological diseases. A. S. XXIII International Congress with a course of endoscopy. Moscow. 2010, 163-164.
11. Sonova; M.M.; Didenko, L.F.; Antonova, S.A.; Tikhonova, E.S.; Osipova, A.A.; Arslanyan, K.N. The participation of macrophages in the pathogenesis of genital endometriosis. *Reproduction problems. V International Congress on Reproductive Medicine. Specialist. issue*, **2011**, 152-153.
12. Chernukha, G. E. Endometriosis and chronic pelvic pain: causes and consequences. *Problems of reproduction*. **2011**, 5, 83-89.
13. Schwartz, Ya.Sh.; Khoshchenko, O.M.; Dushkin, M.I.; Feofanova, N.A. The effect of cholesterol and agonists of hormonal nuclear receptors on the production of transforming growth factor β in macrophages. *Bulletin of experimental biology and medicine*. **2009**, 148, 9, 294-297.
14. Anderson Sanches Melo; Julio Cesar Rosa-e-Silva; Ana Carolina Japur de Sa Rosa-e-Silva; Omero Benedicto Poli-Neto; Rui Alberto Ferriani; Carolina Sales Vieira. Unfavorable lipid profile in women with endometriosis. *Fertility and Sterility*. **2010**; 93, 7, 2433-2436.
15. Berbic, M.; Shuike, L.; Markham, R.;

- Tokushige, N.; Zhou, J. Macrophage expression in endometrium of women with and without endometriosis. *Hum Reprod* **2009**; 94: 623-31.
16. Bryan, N.S.; Bian, K.; Murad, F. Discovery of the nitric oxide signaling pathway and targets for drug development. *Frontiers in Bioscience*, **2009**, 14: 1-18.
 17. Cayci, T.; Akgul, E.O.; Kurt, Y.G.; Ceyhan, T.S.; Aydin, I.; Onguru, O.; Yaman, H. The levels of nitric oxide and asymmetric dimethylarginine in the rat endometriosis model. *ObstetGynaecol Res.* **2011**, 37(8), 1041-7.
 18. Charles, O.A. Omwandho; Lutz Konrad; Gulden Halis; Frank Oehmke; Hans-Rudolf Tinneberg. Role of TGF- β s in normal human endometrium and endometriosis. *Human Reproduction*, **2010**, 25, 101-109.
 19. Darling, A.M.; Chavarro, J.E.; Malspeis, S.; Harris, H.R.; Missmer S.A. A prospective cohort study of Vitamins B, C, E, and multivitamin intake and endometriosis. *J Endometr.* **2013**, 5(1), 17-26.
 20. Falcone, T., Lebovic, D.I. Clinical management of endometriosis. *Obstet Gynecol.* **2011**, 118(3), 691-705.
 21. Gajbhiye, R.; Sonawani, A.; Khan, S.; Suryawanshi, A.; Kadam, S.; Warty, N.; Raut, V.; Khole, V. Identification and evaluation of new serum markers for early diagnosis of endometriosis. *Human Reproduction.* **2012**, 2 (26), 35-45.
 22. Guidice, L.C. Endometriosis, *N Engl J Med*, **2010**, 362, 2389-2398.
 23. Gupta, S.; Agarwal, A.; Krajcir, N.; Alvarez, J.G. Role of oxidative stress in endometriosis. *Reprod Bio Med Online.* - **2006**, 13(1), 126-134.
 24. Kinugasa, S.; Shinohara, K.; Wakatsuki, A. Increased asymmetric dimethylarginine and enhanced inflammation are associated with impaired vascular reactivity in women with endometriosis. *Atherosclerosis.* **2011**, 219(2), 784-8.
 25. Laschke, M.W.; Giebels, C.; Menger, M.D. Vasculogenesis: a new piece of the endometriosis puzzle. *Hum Reprod Update.* **2011**, 17(5), 628-36.
 26. Lee, B.; Du, H.; Taylor, H.S. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. *BiolReprod* **2009**, 80, 79-85.
 27. Luciano, D.; Roy, G.; Luciano, A.A. Adhesion reformation after laparoscopic adhesiolysis: where, what type, and in whom they are most likely to recur. *J Minim Inv Gynecol.* **2008**, 15(1), 44-48.
 28. Melo, A.S.; Rosa-e-Silva, J.C.; Poli-neto, O.B.; Ferriani, R.A.; Vieira C.S. Unfavorable lipid profile in women with endometriosis. *Fertil Steril.* **2010**, 93(7), 2433-2436.
 29. Rogers, P.A.; D'Hooghe, T.M.; Fazleabas, A. Defining Future Directions for Endometriosis Research: Workshop Report From the 2011 World Congress of Endometriosis in Montpellier, France. *Reprod Sci.* **2013**, 20(5), 483-499.
 30. Santanam, N.; Murphy, A.A.; Parthasarathy, S. Macrophages, oxidation, and endometriosis. *Ann NY AcadSci* **2002**, 955, 461-467.
 31. Schmidt, N.; Laux-Biehlmann, A.; Boyken, J.; Koch, M.; Dahllof, H.; Zollner, T.M.; J. Nagel. Inflammation and pain in mouse model of retrograde menstruation. *Journal of Endometriosis and Pelvic Pain Disorders* **2015**, 7, 93-94.
 32. Takebayashi, A.; Kimura, F.; Kishi, Y.; Ishida, M.; Takahashi, A. The Association between Endometriosis and Chronic Endometritis. *PLOS ONE.* **2014**, DOI: 10.1371/journal.pone.0088354.
 33. Taylor R.N.; Lebovic, D.I., Mueller, M.D. Angiogenic factors in endometriosis. *Ann. N. Y. Acad. Sci.*, **2002**, 955, 89-100.
 34. Vercellini, P., Grossignani, P.G., Someglia, E. «Waiting for Go dot»: a commonsens approach to the medical treatment of endometriosis. *Hum Reprod* **2011**, 26, 3-13.
 35. Verit, F. F.; Yucek, O. Endometriosis, leiomyoma and adenomeiosis: the risk of gynecologic malignancy. *Asian Pac J Cancer Prev.* **2013**, 14(10), 5589-97.
 36. Vodolazkaia, A.; El-Alamat, Y.; Popovic, D. Panel of plasma biomarkers can predict endometriosis with high sensitivity in patients with ultrasound negative endometriosis. *Hum Reprod.* **2012**, 27(9), 2698-711.

37. Wang, G.; Tokushige, N.; Markham, R.; Fraser, I.S. Rich innervations of deep infiltrating endometriosis. *Hum Reprod* **2009**; 24:827-34.

Table 1. Parameters Indicators of cell metabolism regulators in patients with EGE (blood serum).

Parameter	EGE stage	Control	P
I-II EGE stage			
TGF- β 1 (pg/ml)	12936.0 [7650.0-28050.0]	9332.0 [4736.0-21100.0]	0.004
HDL (mmol/l)	1.25 [0.77-2.20]	1.10 [0.56-1.80]	0.013
NOx (mmol/l)	16.50 [0.80-61.50]	2.39 [0.10-62.50]	0.041
III-IV EGE stage			
TNF- α (pg/ml)	15.56 [4.70-64.00]	8.04 [0.40-23.60]	0.014
TGF (mmol/l)	1.25 [0.91-2.30]	1.10 [0.56-1.80]	0.006
NOx (mmol/l)	32.00 [0.48-56.00]	2.39 [0.10-62.50]	0.001

Note: Results are presented as median and interquartile interval.

p - significance of differences compared with the control group.

Table 2. Parameters of cell metabolism regulators in patients with EGE (peritoneal fluid)

Parameter	EGE stage	Control	P
I-II EGE stage			
Cholesterol (mmol/l)	2.0 [1.07 –3.09]	1.39 [1.21 – 1.81]	0.008
HDL (mmol/l)	0.78 [0.34-1.17]	0.45 [0.33-0.59]	0.004
LDL (mmol/l)	0.77 [0.0-1.21]	0.48 [0.01-0.70]	0.003
III-IV EGE stage			
TNF- α (pg/ml)	8.30 [0.02-29.86]	12.59 [4.0-31.20]	0.050
TGF-1 β (pg/ml)	2480.0 [1513.60-5720.0]	1425.40 [1011.0-2200.40]	0.001
NOx (mcmol/l)	24,00 [7.52-65.00]	7.25 [0.14-64.50]	0.039
NO-synthase (mcmol/l)	35.00 [28.60-67.40]	23.00 [0.79-57.60]	0.001

Note: results are presented as median and interquartile interval.

p - significance of differences compared with the control group.