



## SUBPOPULATION COMPOSITION OF LYMPHOCYTES IN WOMEN WITH GENITAL ENDOMETRIOSIS

<sup>1</sup>Musakhodjaeva Diloram Abdullayevna\*, <sup>1</sup>Aripova Tamara Uktamovna, <sup>2</sup>Magzumova Nargiza Makhkamovna, <sup>2</sup>Ismoilova Dildora Uktamovna, <sup>1</sup>Djumaeva Dilnoz Narmuratovna, <sup>1</sup>Azizova Zukhra Shukhratovna.

<sup>1</sup>Republican Scientific Center of Immunology, Tashkent, Uzbekistan.

<sup>2</sup>Tashkent Medical Academy, Uzbekistan.

**Corresponding Author: Musakhodjaeva Diloram Abdullayevna**

Republican Scientific Center of Immunology, Tashkent, Uzbekistan.

Article Received on 03/03/2018

Article Revised on 24/03/2018

Article Accepted on 14/04/2018

### ABSTRACT

**SUMMARY:** 58 patients with genital endometriosis were examined. Of these, 27 had internal endometriosis, and 31 had external genital endometriosis. 18 practically healthy women made up a control group. All examined women were examined the state of the immune system (cellular immunity, phagocytosis and cytokine status). It was found that in patients with endometriosis, the level of T-lymphocytes and its subpopulation composition were reduced, and the level of killer activity varied in different ways depending on the form of genital endometriosis. Functional activity of neutrophils changed only in the group of women with EGE.

**KEYWORDS:** external and internal genital endometriosis, immune status, phagocytosis, cytokines.

### INTRODUCTION

To clarify the fundamental mechanisms of the development of endometriotic foci, the study of local immune processes is of great interest. However, the study of the systemic immune response in endometriosis also provides important information about the pathogenetic factors of the development of the disease, since the functional state of circulating immunocompetent cells can largely reflect the direction of immune disorders occurring at the local level.

The aim of the research was to study the features of some parameters of congenital and adaptive immunity in peripheral blood of women with genital endometriosis.

### MATERIAL AND METHODS OF RESEARCH

We observed 58 women with internal (27 women) and external genital endometriosis (31 women), the diagnosis of which was verified at the stage of clinical and instrumental examination, including therapeutic and diagnostic laparoscopy. All patients were comparable in age, complaints, anamnesis, gynecological and extra genital pathology, extent of disease (grade II-III according to the classification of the American Fertility Society r-AFS).<sup>[4]</sup> The indicators of 18 practically healthy women with normal reproductive function, who were enrolled in planned surgical sterilization, were used as a control group for immunological studies.

Immunological studies were performed by studying the level of lymphocytes by identification on the surface of their clusters of differentiation of CD3, CD4, CD8,

CD16, CD20, CD25 and CD95 using monoclonal antibodies of the LT series (Sorbert LLP, Moscow, Russia). Functional activity of phagocytes was studied in the Nitro Blue-Tetrazolium Test (NBT). The level of pro- (IL-1 $\beta$ , IL-2, IL-6, IL-8, IFN $\gamma$ ) and anti-inflammatory (IL-4) cytokines in serum was studied by ELISA (test system «Cytokine», St. Petersburg, Russia).

### RESULTS AND ITS DISCUSSION.

Analysis of medical records showed that the age of women was between 22-38 years.

A study of the duration of the course of the disease showed that women with IGE had an average of 4.7 years, while in the case of the EGE, it was 2.8 years.

The study of somatic anamnesis showed that the transferred gynecological diseases most often met dysmenorrhea, acute and chronic adnexitis, uterinemyoma, wearing intrauterine spirals (IUD), colpitis, bacterial vaginosis and dysfunctional uterine bleeding (DUB).

Among the transferred obstetric and gynecological operations, such as ovarian surgery for various ovarian cysts, conservative myomectomy, reconstructive and plastic surgery for uterine malformations, cesarean section, suturing of perforation holes on the uterus, ectopic pregnancy, etc. were observed. Such surgical interventions as operations on the cervix (diathermosurgical and cryosurgical manipulations) were extremely rare (2 women).

Studying the complaints of patients from both study groups showed that all women had painful menstruation of one or another intensity. The pains had a cyclic periodicity that increased before the menstruation and weakened and disappeared after them.

Depending on the form of the disease, various additional symptoms of endometriosis are fixed.

Menstrual dysfunction in the form of DUB prevailed in women with IGE and amounted to 76.5% of women, which was accompanied by an irregular cycle, smearing intermenstrual secretions (51%), plentiful monthly (19%) and bleeding (6.5%).

During menstruation, nausea (30%), vomiting (11%), fever (7%) were often observed in women with both IGE and EGE, and leukocytosis (19%), high ESR (17%) and low hemoglobin (27%).

The general condition of women is greatly worsened by the fact that there is an effect of endometriosis on the work of all internal organs. They feel weakness (45%), work capacity decreases (27%), irritability arises due to persistent pain, and due to heavy blood loss, dizziness (25%), tachycardia and dyspnea (15%).

One of the serious complications of endometriosis is the absence of pregnancy for more than 1 year (primary infertility) and secondary infertility.

In endometriosis due to hormonal disorders, ovulation is often absent, or there is a failure of the second phase of the cycle. Thus, in the group of women with IGE, the insufficiency of the second phase of the cycle prevailed, which was 58.8%, while in the EGE group anovulation was more common and amounted to 32.3%.

It was found that for women with ovarian endometriosis 42% of cases were characterized by an increase in the level of FSH and LH, which indicates damage to the follicular apparatus of the ovaries and a decrease in the ovarian reserve. In addition, 8% of patients experienced an increase in estrogen, prolactin (10%), and a decreased level of progesterone up to 70% of cases.

The study of the nature of the pain syndrome in women with various forms of endometriosis showed that, depending on the location of the sites of hemorrhage, additional symptoms of this disease appear. Thus, for 44.7% of patients with adenomyosis was characterized by increased pain, radiating into the anus during intercourse (dyspareunia).

In 15 women with EGE (71.4%), the focus of endometriosis was in the posterior Douglas space. In such cases, women often complained of pain during the act of defecation.

With the location of the focus of endometriosis on the wall of the bladder, complaints were on pain during urination.

The results of immunological studies showed that the number of CD3 + and CD4 + lymphocytes significantly decreased in women with EGE in the peripheral blood and the content of CD16 + cells sharply increased in comparison with similar data of healthy women ( $P < 0,01$  in all cases).

Changes in the phenotypic profile of peripheral lymphocytes with internal endometriosis had a different orientation (Table 1). In this group of women, we observed a significant decrease in the CD8 + cell count and an increase in the number of CD16 + lymphocyte lymphocytes, compared with the control group ( $P < 0,05$ ).

**Table 1: Characteristics of the population composition of peripheral blood lymphocytes in women with endometriosis, (M±m).**

Indicators, %	Control group, n=18	IGE, n=27	EGE, n=31
CD3+	55,8±1,9	50,7 ±1,4*	48,4 ±1,7*
CD4+	35,6± 1,4	31,3± 1,1	28,6± 1,2*
CD8+	21,8±1,0	20,8±0,8	17,3±0,6*
CD16+	13,6 ± 1,1	8,7 ±0,5*	21,9 ± 1,3*
CD20+	15,7±0,9	16,3±0,7	13,7±0,8
CD45RA+	54,3 ±1,4	48,8 ±1,2*	38,3 ±1,5*
CD25+	18,3 ±1,4	10,3 ±1,4*	15,3 ±1,4
CD95+	26,8±1,6	19,8±1,6*	23,8±1,6

Note: \* The value is reliable compared to the control group ( $P < 0,05$  to 0.001)

The level of functional activity of peripheral neutrophils was also evaluated by us in terms of spontaneous and zymosan-stimulated NBT-test with calculation of phagocytic reserve index (Table 2).

**Table 2: Characterization of functional activity of peripheral blood neutrophils in women with various forms of endometriosis.**

Indicators, %	Control group, n=18	IGE, n=27	EGE, n=31
NBTs, %	16,8 ± 1,52	22,5 ± 1,7*	26, 8 ± 1,63*
HCTs, i.r.a.	0,24 ± 0,05	0,31 ± 0,03*	0,38 ± 0,02*
NBTh, %	38,3 ± 2,4	37,4 ± 2,0	37,9 ± 2,0
HCTh, i.r.a.	0,86 ± 0,06	0,83 ± 0,04	0,81 ± 0,03
IPR	1,46 ± 0,3	0,89 ± 0,2	0,64 ± 0,1*

Note: \* The value is reliable compared to the control group (P<0,05-0.001)

The analysis of the obtained results showed that in the peripheral blood of patients with EGE NBT activity of neutrophils in spontaneous NBT -test was significantly increased, and the index of dye reduction activity was increased in comparison with similar parameters in peripheral blood of healthy women (P<0,01, P<0,05, respectively). In addition, in this group there was a slight decrease in the normative values of reserve-functional neutrophil activity, estimated by the level of the index of phagocytic reserve (IPR), (P<0,05).

Changes in the indices of zymosan-stimulated NBT-test in peripheral blood of women with EGE were not observed in comparison with similar parameters in peripheral blood of healthy women (P<0,05). When comparing the indices of functional activity of peripheral blood neutrophils in patients with IGE and healthy women, we also did not have significant differences. The number of NBT-positive peripheral neutrophils in spontaneous and stimulated NBT -test in women with

internal genital endometriosis corresponded to normative values.

Thus, the results obtained by us indicate that the functional activity of neutrophils changed only in the group of women with EGE. Perhaps this is due to the AMGF- $\alpha$ 2 microglobulin fertility, or glycodepone, whose serum level in patients with endometriosis is significantly increased (Posiseeva L.V. with co-authors, 1998).

We also studied the synthesis of cytokines in serum in women with external and internal endometriosis. The obtained results indicate that in the group of women with internal endometriosis, the level of IL-1 $\beta$  was 3.2 times higher than the control values (P<0,01). At the same time the level of IL-2 had only a tendency to decrease. And the level of anti-inflammatory cytokine - IL-4 was 2.2 times lower than the values of the control group (P<0,01).

**Table 3: Features of synthesis of cytokines in serum of peripheral blood in women with endometriosis, (M ± m).**

Cytokines	Control group, n=18	IGE, n=27	EGE, n=31
IL-1 $\beta$	21,5 ± 2,2	69,7 ± 2,4*	73,4 ± 2,7*
IL-2	8,95 ± 1,7	7,3 ± 1,8	5,6 ± 1,5*
IL-4	20,9 ± 2,4	9,4 ± 1,3*	15,8 ± 1,6*
IL-6	27,9 ± 2,5	17,4 ± 1,0*	19,8 ± 1,4*
IL-8	16,3 ± 1,9	25,3 ± 2,0*	29,7 ± 2,3*
IFN $\gamma$	18,7 ± 2,7	15,6 ± 1,0	9,7 ± 0,9*

Note: \* The value is reliable compared to the control group (P<0,05-0.001)

Levels of IL-6 and IFN $\gamma$  were also reduced compared to the control group (P<0,05 for both cases).

Analysis of the women with external endometriosis showed that the levels of proinflammatory cytokines IL-1 $\beta$  and IL-8 were sharply increased (P<0,05), and the levels of IL-2, IL-4, IL-6 and IFN $\gamma$  were lower than in the control group (P<0,05).

Summarizing the data obtained by us on the phenotypic, cytokine profile and phagocytes of peripheral blood, it should be noted that the revealed changes in the parameters studied were common for various forms of endometriosis. Apparently, the systemic immune disorders in endometriosis are more associated with

concomitant clinical symptoms that are different for a certain form of endometriosis, and to a lesser extent reflect the general mechanisms of the development of the ectopic endometrium.

The obtained results convincingly prove the necessity of inclusion in the complex therapy of endometriosis immunocorrectors depending on the localization of endometriosis, clinical course and parameters of the immune system.

**REFERENCE**

1. Solodovnikova N.G., Niauri D.A. Role of immune system factors in the pathogenesis of external genital endometriosis // Zh. Bulletin of the federal center of the heart, blood and endocrinology. V.A. Almazova.-№.6.- 2011.- Izd: Fund of High Medical Technologies ISSN: 2311-4495.- P.23-28
2. Shishkov D.N. The role of immune disorders in the development of infertility in the case of small forms of external genital endometriosis // author's abstract ... .kand. diss. 2007, Ivanovo.
3. Selkov S.A., Yarmolinskaya M.I., Pavlov O.V. and others. Systemic and local levels of regulation of immunopathogenetic processes in patients with external genital endometriosis // J. Obstetrics and female diseases.-2005.-T.LIV, №. 1.-P.20-28.
4. Burney, R.O. The genetics and biochemistry of endometriosis / R.O. Burney // Curr. Opin. Obstet. Gynecol. – 2013. – Vol. 25. – P. 280–286.
5. Endometriosis: an inflammatory disease with a Th2 immune response component / S. Podgaec [et al.] // Human Reproduction. – 2007. – Vol. 22, №5. – P. 1373-1379.
6. Endometriosis markers: immunologic alterations as diagnostic indicators for endometriosis / H.C. Bohler [et al.] // Reproductive sciences. – 2007. – Vol. 14, № 6. – P. 595-604.