# BRITISH MEDICAL JOURNAL

# British Medical Journal

## Volume 3, No.3, May 2023

**Internet address:** http://ejournals.id/index.php/bmj
**E-mail:** info@ejournals.id
Published by British Medical Journal
Issued Bimonthly
3 knoll drive. London. N14 5LU United Kingdom
+44 7542 987055



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# PROGNOSTIC ROLE OF CYTOKINES IL-6, IL-8, TNFa AND IL-10 IN PREDICTING BACTERIAL VAGINOSIS RECURRENCE

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*Abstract. Bacterial vaginosis - a violation of the physiological balance between lactobacilli and conditionally pathogenic microorganisms of the vagina is an urgent problem of obstetrics and gynecology due to high rates of recurrence after treatment, reaching up to 60% within a year. The revision of approaches to the diagnosis and prevention of recurrent bacterial vaginosis taking into account the level of cytokines IL-6, IL-8, TNFa and IL-10 allows predicting the course and outcomes of treatment of bacterial vaginosis.*

*Keywords: bacterial vaginosis, vaginal microbiocenosis, cytokines IL-6, IL-8, TNFa, IL-10.*

**Introduction.** The female reproductive tract contains a complex ecosystem, including a protective barrier of multilayer squamous epithelium, immune components and microorganisms [1]. Interactions between the components of the microenvironment of the female reproductive tract play an important role in the homeostasis of the genital tract, and a change in their balance can contribute to the emergence of various pathologies that violate reproductive and gynecological health [2].

Lactic acid bacteria dominate in the normal vaginal microflora, accounting for 95-98% of the total biotope [3]. The stability of the vaginal ecosystem is ensured by the ability of lactobacilli to form lactic acid and hydrogen peroxide, bacteriocins and the competitive ability of lactobacilli to nutrients and adhesion to the vaginal mucosa [4, 5].

Bacterial vaginosis (BV) disrupts the stable state of the ecosystem, accompanied by an increase in the pH of the vaginal environment of more than 4.5 due to a decrease in the number of acid-forming lactobacilli and an increase in opportunistic, primarily anaerobic bacteria [6, 7]. The most common microorganisms associated with BV are Gardnerella vaginalis, Atopobium vaginae, as well as representatives of obligate anaerobic microorganisms Sneathia/Leptotrichia/Fusobacterium spp., Megasphaera/Veillonella/Dialister spp., Lachnobacterium/Clostridium spp., etc. [8].

The urgency of the problem of bacterial vaginosis is associated with the widespread spread of this disease [9]. According to various authors, about 30% of cases of pathological discharge from the genital tract in women are associated with bacterial vaginosis, more than 50% of women with BV report relapses of the disease six months after various treatment methods, repeated cycles of which lead to a deterioration in the quality of life of women and is a major medical and social problem [10, 11]. BV is associated with a number of obstetric and gynecological pathologies, such as premature discharge of amniotic fluid, premature birth, intrauterine infections that pose a real threat to the life of the fetus and newborn, an increased risk of infection with urogenital infections, pelvic inflammatory diseases that worsen women's reproductive health [12, 13], which emphasizes the importance of a comprehensive study of this problem.

The current state of knowledge about the mechanisms by which epithelial and immune cells in the reproductive tract interact with the microflora shows that there is a bidirectional relationship between bacteria and the macroorganism, in which bacteria apparently regulate inflammation and immunity, while the host body's immune system can modulate microbiocenosis. Cytokine production is an important part of the immune response of the macroorganism and is necessary for protective immunity. For example, the reproductive tract is able to increase the concentration of interleukin-1β (IL-1β) and then IL-8 in response to the presence of pathogens, which plays an important role in activating both adaptive and innate immune response against bacteria associated with BV [14].Moreover, toll-like receptors (TLRs) on mucosal cells can bind and identify a wide range of molecular patterns associated with bacterial pathogens and initiate signaling cascades aimed at eliminating infection, if necessary [15, 16]. However, it is also suggested that sustained cytokine production can cause damage to the epithelial barrier and cause infiltration of Th-cells into the genital mucosa, which can damage the stability of microbiocenosis and lead to increased aggression of infections [17, 18]. In BV, the protective properties of the vaginal mucosa are significantly reduced, due to an increase in the level of a number of proinflammatory cytokines (IL-8, IL-1α, IL-1β, interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α)) and a pronounced imbalance of the Th1/Th2 response in contrast to women with normal vaginal microflora [19, 20].

**The purpose of the study:** to study the clinical role of IL-6, IL-8, TNFa and IL-10 in predicting the outcomes of treatment of bacterial vaginosis.

**Materials and methods of research.** A prospective randomized case-control study involving 125 women was conducted at the following clinical bases: Bukhara Regional Perinatal Center and Bukhara Regional Center for Reproductive Health of the Population.

The main group consisted of 110 women with BV and a group of clinical and laboratory control - 15 practically healthy women with vaginal normocenosis. Depending on the molecular biological profile of the vaginal microbiocenosis of the women of the main group, subgroups were formed: group 1 – patients with pronounced anaerobic dysbiosis (with the predominance of facultative anaerobes Gardnerella vaginalis and Atopobium vaginae in the microbiocenosis; n=38), group 2 - with pronounced anaerobic dysbiosis (with the predominance of obligate anaerobes in the microbiocenosis; n=40) and group 3 – with pronounced aerobic-anaerobic dysbiosis (n=32).

Criteria for inclusion in the main group: reproductive age; absence of systemic and local antibacterial therapy for 1 month prior to this examination; exclusion of sexual contact in the last 72 hours before the examination; exclusion of STIs; clinical and microscopic confirmation of the diagnosis of bacterial vaginosis, including the identification of positive diagnostic criteria proposed by R. Amsel.

Criteria for inclusion in the control group: reproductive age; absence of systemic and local antibacterial therapy for 1 month prior to this examination; clinical and microscopic confirmation of the normal state of the vaginal microflora; absence of complaints.

During the clinical examination, the general condition, somatic and gynecological status of women were assessed. The cervix and vagina were examined using gynecological mirrors, and the presence of Amsel criteria was determined. The pathological nature of vaginal discharge, the pH of the vaginal discharge is more than 4.5, a positive amine test, the presence of "key cells" in the smears together make up the Amsel test. The presence of three signs out of four shows that the patient suffers from bacterial vaginosis. The state of vaginal microbiocenosis was assessed by microscopic examination of vaginal discharge by Gram and molecular biological PCR-RV using a set of Femoflor-16 reagents. Concentrations of cytokines IL-6, IL-8, TNFa and IL-10 in vaginal flushes were determined by solid-phase enzyme immunoassay.

The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2003 software package, including the use of built-in statistical processing functions. The methods of variational parametric and nonparametric statistics were used with the calculation of the arithmetic mean of the studied indicator (M), the mean square deviation (σ), the standard error of the mean (m), relative values (frequency, %), the statistical significance of the measurements obtained when comparing the average values was determined by the Student's criterion (t) with the calculation of the probability of error (p) when checking the normality of the distribution (by the kurtosis criterion) and the equality of the general variances (Fischer's F – criterion). The confidence level $p<0.05$ was taken as statistically significant changes. The relationship of the parameters was determined using Pearson pair correlation.

**Results and discussion.** The average age of women in the main group was 35.5±0.68 years, in the control group -32.6± 1.01 years. The analysis of the social status of women in the main group showed that 46.4% of women were employees, 27.3% were workers, 24.5% were unemployed (housewives), and 1.8% were students. The main and control groups were comparable in the main parameters, including social status, somatic diseases, menstrual and reproductive history, contraceptive methods used, etc.

The analysis of the complaints presented showed that the most frequent symptom in the women of the main group was the presence of pathological secretions from the genital tract (83.6%). Subjective signs of vaginal infections in the form of itching, burning in the genital area and pain during urination were noted by 20.9% and 10.9% of women in the main group. The pH of vaginal discharge was 6.2±0.12 in the main group, 4.1±0.31 in the control group ($p<0.001$). A positive amine test, defined as the smell of "rotten fish" when 10% potassium hydroxide was added to vaginal secretions, was noted in 82.3% of cases in the main group.

Microscopy of vaginal smears revealed the extinction of the leukocyte reaction in the form of up to 1-2 cells in the field of view, a large number of epithelial cells with more than 5 "key cells" in the field of view, a massive number of bacterial cells belonging to the morphotypes of anaerobic bacteria. The nature of the vaginal microbiota in 70.9% of cases corresponded to the profile of pronounced anaerobic dysbiosis and in 29.1% of cases – pronounced aerobic-anaerobic dysbiosis. In the control group, absolute normocenosis was detected in 26.7% of cases and conditional normocenosis – in 73.3% of cases.

The content of lactoflora in the women of the main group was low and amounted to 3.92±0.22 lg GE/ml (Fig. 1). A significantly high content of Gardnerella vaginalis ($p<0.001$), Eubacterium spp. ($p<0.005$), Sneathia/Leptotrichia/Fusobacterium spp. and Megasphaera/Veillonella/Dialister spp. ($p<0.001$), Lachnobacterium/Clostridium spp. ($p=0.013$), Mobiluncus/Corynebacterium spp. ($p=0.004$), Peptostreptococcus spp. and Atopobium vaginae ($p<0.001$) in women with BV. Of the commensal microorganisms, only Mycoplasma hominis significantly exceeded the control parameters ($p=0.007$).

Unlike the Streptococcus spp. and Staphylococcus spp. groups, the concentration of Enterobacteriaceae was high in the group with BVb, while this facultative anaerobe was not detected in the control group.

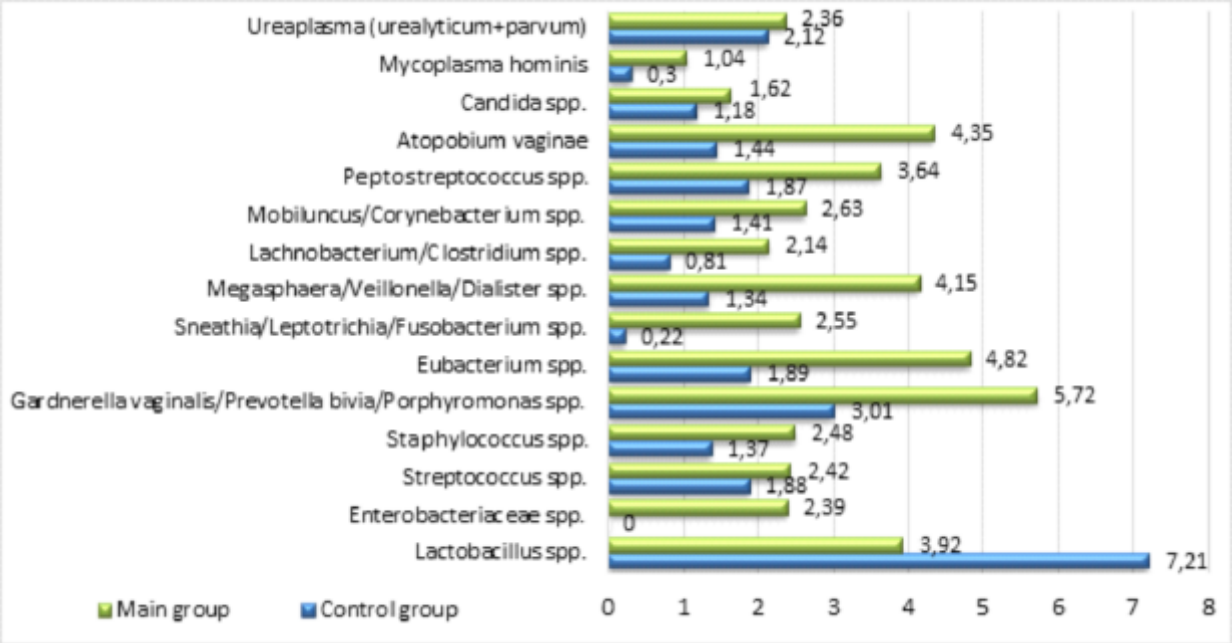


Fig. 1. Quantitative composition (lg GE/ml) of representatives of the vaginal microbiota in the main and control groups

A study of the local cytokine level showed a significant increase in the content of pro- and anti-inflammatory cytokines in groups of women with BV in relation to their production in women with vaginal normocenosis ($p<0.001$) (Fig.2).

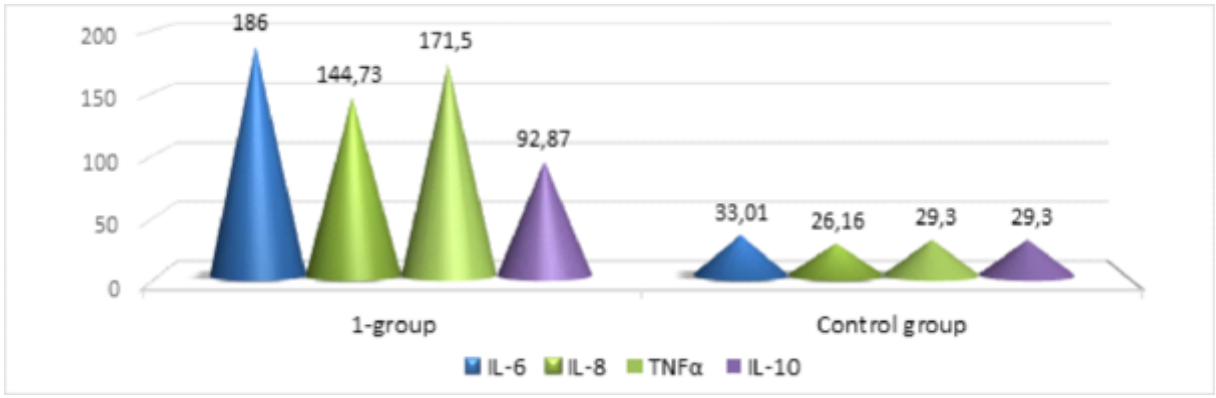


**Fig. 2. The level of cytokines in the vaginal discharge of women of the studied groups**

IL-6 is a cytokine with both pro- and anti-inflammatory properties, and its release into biological fluids is an early marker of infectious load. It exhibits various biological effects, including activation of B- and T-lymphocytes, induction of acute phase protein synthesis in the liver, modulation of hematopoiesis. IL-8 induces chemotaxis in T-lymphocytes, stimulates differentiation of T-helper cells, growth and activity of T-lymphocytes and killer cells, and an increase in lactoferrin levels. The main role in the regulation of the Th1 response is played by TNFa, which is one of the pleiotropic cytokines affecting all cells.Anti-inflammatory cytokines in the early stages of inflammation limit damage to healthy tissue and provide a balance between necessary and pathological inflammation. The key anti-inflammatory cytokine is IL-10, which counteracts the effect of the main pro-inflammatory cytokines by inhibiting the production of TNFa, IL-1β and IL-6 [16].

As can be seen, in BV, there is a repeated increase in the level of pro- and anti-inflammatory cytokines, the main purpose of which is to form an adequate immune response to the growth of opportunistic flora. But at the same time, there is a significant imbalance in non-specific protective reactions, expressed in the activation of the pro-inflammatory link over the anti-inflammatory cytokine link.

Next, we conducted a two-stage therapy of bacterial vaginosis with the use of antibacterial drugs at the first stage of treatment and then probiotics or prebiotics.

A control microscopic examination of vaginal smears a month after treatment showed that the microscopic parameters corresponded to the parameters of a normal smear, which was considered a criterion of cure.

To identify the relationship between microbiological and immunological parameters in the possible recurrence of B C, we further re-evaluated the above parameters 3 and 6 months after treatment.

When assessing the composition of the vaginal microflora after a three-month interval, absolute normocenosis was established in 7 (18.4%) women of group 1, conditional – in 19 (50.0%). 2 (5.6%) women had severe anaerobic dysbiosis, 9 (23.7%) had moderate anaerobic dysbiosis. In group 2, absolute normocenosis was recorded in 17.5% (7 women) of cases, conditional normocenosis was detected in 42.5% (17 women) of cases. 5 (12.5%) women had severe anaerobic dysbiosis, 11 (27.5%) had moderate anaerobic dysbiosis. In women of group 3, in 12.5% and 43.8% of cases, the state of vaginal microbiocenosis corresponded to absolute and moderate normocenosis, respectively, but there were also cases of pronounced (18.7% – in 6 women) and moderate aerobic-anaerobic dysbiosis (25.0% – in 8 women).

As can be seen, 3 months after treatment, the majority of women in the study groups noted the preservation of the stability of vaginal microbiocenosis in the form of absolute and conditional microbiocenosis. However, 7.9%, 12.5% and 18.7% of cases in groups 1, 2 and 3 had cases of severe dysbiosis, which is estimated as a recurrence of BV. It should be noted that the highest recurrence rate of BV was detected in group 2 - in women with a high proportion of obligate–anaerobic microflora in smears and in group 3 - in women with mixed dysbiosis (with the presence of both anaerobic and aerobic groups of microorganisms). This fact can be explained by the fact that, despite the treatment, deeper violations of microbiocenosis with the participation of obligate anaerobes in group 2 cases and the presence of a multi-aggressive microflora in group 3 cases may not allow a full restoration of vaginal microbiocenosis to normocenosis. In our opinion, the study of immunological parameters in this contingent of women can give a more accurate explanation of the course of these events.

Analysis of the results of studying the level of cytokines IL-6, IL-8, TNFa and IL-10 3 months after treatment in group 1 showed that their concentration in the vaginal contents was 53,93±18,13, 43,04±5,98, 28,7±2,1 and 31.44±5.23 pkg/ml, respectively, and were almost approaching control data (Table 1). There was a slight increase in the activity of the local cytokine status in group 2, which was significantly expressed in relation to the concentration of IL-6 and TNFa (60.79±11.35 and 44.57±8.69 pkg/ml) versus the control data (33.01±6.70 and 25.3±1.0 pkg/ml), respectively ($p<0.05$). In group 3, normalization of the level of the studied cytokines was noted in most cases, however, significantly high values of IL-6 and IL-10 (58.28±10.12 and 37.39±6.33 pkg/ml) were detected against control data (33.01±6.70 and 22.72±3.29 pkg/ml), respectively ($p<0.05$).

**Table 1**
**Cytokine levels in the groups 3 months after treatment**

| Indicator | 1-group | 2-group | 3-group | Control group | p1–4; p2–4; p3–4 |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| IL-6 | 37,93±8,13 | 60,79±11,35 | 58,28±10,12 | 33,01±6,70 | 0,643; **0,043; 0,046** |
| IL-8 | 26,14±5,98 | 41,89±9,82 | 49,13±13,78 | 23,16±9,48 | 0,792; 0,179;0,131 |
| TNFα | 27,8±3,1 | 44,57±8,69 | 38,16±12,68 | 25,3±1,0 | 0,448; **0,035;** 0,320 |
| IL-10 | 29,44±5,23 | 42,71±9,78 | 37,39±6,33 | 22,72±3,29 | 0,285; 0,061; **0,048** |

As can be seen from the table. 1 the tendency to decrease and normalize cytokine indices persists after a three-month period after treatment in all groups, but in some cases there is a failure of the established equilibrium. It should be noted that in violation of the microbiocenosis of the vagina, there is a "paradox" of a constant immune response against latent infection. Activation of conditionally pathogenic microflora always occurs against the background of violations in the cytokine profile. Excessive growth of opportunistic microflora stimulates the secretion of a number of cytokines by various producing cells. At the same time, cytokines play a different role in the occurrence and course of bacterial vaginosis: Th1-type cytokines help to reduce bacterial reproduction and accelerate clinical recovery, while Th2-type cytokines have the opposite effect [2].

The increase in the content of conditionally pathogenic microflora in the groups we identified was associated with high values of IL-6, TNFa and IL-10, i.e. in cases where the imbalance of Th1/Th2 cytokines after BV treatment was not fully eliminated, a high content of conditional pathogens was noted, mainly of the Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. and Atopobium vaginae, somewhat less frequently – Sneathia/Leptotrichia/Fusobacterium spp., Megasphaera/Veillonella/Dialister spp. and Lachnobacterium/Clostridium spp.

Thus, 3 months after treatment in groups 2 and 3, in most cases, the normalization of the cytokine profile to control values is observed, which reflects the restoration of nonspecific local protection and the maintenance of adequate colonization resistance. But in some cases, the cytokine reaction remains elevated, even in those women whose state of vaginal microbiocenosis met the criteria of moderate dysbiosis. In our opinion, a long-term imbalance of the cytokine link can create a threat of recurrence of BV, therefore, we prescribed additional anti-relapse therapy (ART) not only to women with pronounced dysbiosis, but also moderate dysbiosis in whom the concentration of cytokines was high during the study 3 months after treatment.

It seemed especially important to us to evaluate not only the effect of anti-relapse therapy on vaginal microbiocenosis, but also to identify the duration of the therapeutic effect for 6 months. Therefore, the study groups were divided into the corresponding subgroups of women with and without anti-relapse therapy. As an anti-relapse therapy, probiotics were used intravaginally for 10 days in combination with peros prebiotics.12 women of group 1, 16 women of group 2 and 14 women of group 3 who used pro- and prebiotics as anti-relapse therapy were included in groups 1, 2 and 3 with ART, respectively.

Vaginal microbiocenosis after anti-relapse therapy has become more stable. Absolute normocenosis was recorded in 43.8%, 44.4% and 42.9% of cases, conditional - 56.2%, 55.6% and 57.1% of cases in groups 1, 2 and 3 with ART, respectively.

The study of the dynamics of cytokine levels in groups 1, 2 and 3 of women receiving anti-relapse therapy showed that the concentration of IL-6, IL-8, TNFa and IL-10 in the vaginal contents practically did not differ from the control data (p>0.05) (Table 2).

**Table 2**

**Cytokine levels in the groups 6 months after treatment with additional anti-relapse therapy**

| Indicator | 1- group (with ART) (n=12) | 2- group (with ART) (n=16) | 3- group (with ART) (n=14) | Control group | p1–4; p2–4; p3–4 |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| IL-6 | 35,41±5,86 | 37,13±5,79 | 35,23±5,62 | 33,01±6,70 | 0,789; 0,645; 0,802 |
| IL-8 | 32,24±7,62 | 28,76±8,91 | 24,65±8,08 | 23,16±9,48 | 0,461; 0,670; 0,906 |
| TNFα | 25,42±4,88 | 29,16±3,38 | 26,71±4,38 | 25,3±1,0 | 0,981; 0,282; 0,756 |
| IL-10 | 25,63±4,73 | 26,43±5,68 | 23,44±3,57 | 22,72±3,29 | 0,285; 0,576; 0,883 |

The study of the dynamics of cytokine levels in women after 6 months in groups without anti-relapse therapy showed that the concentration of IL-6, IL-8, TNFa and IL-10 in the vaginal contents of women in group 1 was 57,74±6,19, 55,18±7,49, 36,34±3,73 and 32.54±4.26, respectively, while the concentration of IL-6 and IL-8 significantly differed from similar control data (p<0.05) (Table 3). Activation of the cytokine link of immunity was noted in group 2 without anti-relapse therapy, which is expressed by a significant increase in the concentration of IL-6, IL-8, TNFa and IL-10 (64,22±9,16, 78,10±7,54, 45,74±5,58 and 46.89±3.78 pkg/ml, respectively) versus control data (p<0.05).In group 3 without anti-relapse therapy, significantly high values of IL-6, IL-8, TNFa and IL were also detected-10 (57,18±8,36, 65,93±8,53, 54,37±6,54 and 37.87±4.02 pkg/ml, respectively) relative to the control data (p<0.05). The detection of such an imbalance in the local concentration of the studied cytokines was associated with an increase in the content of conditional pathogens in smears. It is obvious that 6 months after treatment, an unbalanced local protection of the vagina contributes to the manifestation of a recurrence of BV.

**Table 3**

**Cytokine levels in the groups 6 months after treatment without additional anti-relapse therapy**

| Indicator | 1- group (without ART) (n=26) | 2- group (without ART) (n=24) | 3- group (without ART) (n=18) | Контроль | p1–4; p2–4; p3–4 |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| IL-6 | 57,74±6,19 | 64,22±9,16 | 57,18±8,36 | 33,01±6,70 | **0,010; 0,042; 0,031** |
| IL-8 | 55,18±7,49 | 78,10±7,54 | 65,93±8,53 | 23,16±9,48 | **0,012; 0,001; 0,002** |
| TNFα | 32,34±3,73 | 45,74±5,58 | 54,37±6,54 | 25,3±1,0 | 0,097; **0,001;<0,001** |
| IL-10 | 32,54±4,26 | 46,89±3,78 | 37,87±4,02 | 22,72±3,29 | 0,076; **0,001; 0,007** |

Thus, excessive growth of opportunistic microflora in BV triggers a local immune response in the form of a cytokine cascade. The revealed orientation and severity of immune shifts indicate an important pathogenetic role of immune mechanisms in the development and course of BV. Laboratory studies have shown that the dysbiotic state of the vaginal biotope is caused by a sharp increase in the number of obligate and facultative anaerobic conditionally pathogenic microorganisms and a sharp decrease or disappearance of lactobacilli. Functional disorders in the work of the immune system contribute to a malfunction in the established microbial equilibrium, in which lactobacilli remain smaller and their functional capabilities decrease, and conditionally pathogenic microorganisms become activated and become able to overcome the barriers of the body's defense, which causes the clinical manifestation of the disease.

It is important to note that the use of additional preventive therapy has a beneficial effect on the indicators of nonspecific local protection. Moreover, the use of drugs in combination with probiotics that improve the microbiocenosis of the mucous membranes as a whole contributes to a significant normalization of the local level of cytokines in the vagina. Studies conducted 6 months after BV treatment showed that the established favorable background associated with the strengthening of nonspecific local immune protection in groups of women with preventive therapy contributes to the preservation of vaginal normocenosis and the strengthening of the dominant number of lactobacilli. In groups of women who did not receive preventive therapy, versatile fluctuations in the level of the studied humoral protection factors were revealed. The failure of the immune system was combined with an increase in the growth of opportunistic microflora, which can serve as a risk of recurrence of BV.

Conclusion. The conditions of pronounced anaerobic dysbiosis (with a predominance of obligate anaerobes) and pronounced mixed dysbiosis are more prone to an imbalance of the TH1/Th2 response 3 months after treatment, in contrast to cases with pronounced anaerobic dysbiosis (with a predominance of facultative anaerobes). Thus, in clinical

practice, it is of great importance to determine the local level of cytokines after the treatment of bacterial vaginosis. In accordance with the key role of the Th1/Th2 imbalance of the immune response in BV, our results show that the dysfunction of the pro- and anti-inflammatory link of cytokine protection plays an important role in the formation of recurrent forms of bacterial vaginosis and can be used as a marker of this pathology, which is clinically significant for the management of these patients.

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