Original Article

Prospects for the Use of Two-Stage Therapy in Various Variants of Vaginal Microbiota Disorders

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Abstract

This study proves the importance of determining the composition and quantitative ratio of the vaginal microbiota in bacterial vaginosis for the selection of differentiated therapy. Two-stage therapy of bacterial vaginosis, selected taking into account the molecular biological variants of vaginal dysbiosis, had a persistent treatment effect in 87.3% of cases. The additional use of probiotics of the oligosaccharide group as an anti-relapse therapy enhances the colonization of lactobacilli and ensures the stability of the vaginal biotope in 95.4% of cases.

Keywords: Bacterial Vaginosis, Treatment of Bacterial Vaginosis, Metronidazol, Clindamycin, Probiotics, Prebiotics.

INTRODUCTION

The female genital tract is a complex ecological system that includes a dynamic balance of various aerobic and anaerobic microorganisms in the vagina and cervix [1]. When the microecology of the vagina changes and the pH increases, conditions unfavorable for the growth of lactobacilli are created [2]. Many researchers have recognized that bacterial vaginosis (BV) is a polymicrobial infectious non–inflammatory syndrome, which is characterized by the growth of obligate or facultative anaerobic opportunistic microorganisms: G. vaginalis, Prevotella spp., Porphyromonas spp., Mobiluncus spp., Veilonella spp., Bacteroides spp., Peptococcus spp., Peptostreptococcus spp., with the possible participation of Mycoplasma spp. and Ureaplasma spp. [3].

The true diversity of microflora in BV was established only after the introduction of molecular biological research methods, when many new (often uncultivated) microorganisms were described, such as Atopobium vaginae, BV-associated bacteria from the order Clostridiales, Megasphaera spp., Leptotrichia spp., Dialister spp., Chloroflexi spp., Olsenella spp., Streptobacillus spp., Shuttleworthia spp., P. asaccharolytica [3]. These microorganisms accumulate, forming various communities or profiles, which indicate that BV is not a single whole, but a syndrome of variable composition, causing a variety of symptoms and different outcomes and leading to variability in responses to various antibiotic regimens.

Modern principles of BV treatment include the use of complex two-stage treatment with subsequent restoration of normal vaginal microflora. The first stage of treatment is aimed at the eradication of conditionally pathogenic microorganisms and anaerobes, while the second stage uses drugs that help restore the pH of the vagina and normal microflora [4, 5].

As it is known, the main goal of BV therapy is to ensure stable colonization of the vagina by life- and competitive lactobacilli that actively produce lactic acid and are able to form useful biofilms, which leads to the death of pathogenic microorganisms. The data obtained in vitro studies indicate the ability of lactobacilli to effectively destroy biofilms [6] and the combination of antibacterial drugs with probiotics is considered as a promising approach to the treatment of BV. However, many sources indicate a low level of survival of lactobacilli, which is explained by insufficient colonization and rapid elimination of introduced foreign bacterial strains [1]. It is assumed that the adsorption of lactobacilli is of a specific nature and depends on the correspondence of the receptors of this particular strain of lactobacilli to the receptors of the cells of the vaginal epithelium of a particular woman [1], which may be the reason for the low effectiveness of treatment and frequent recurrence of BV. At the same time, it has been established that the realization of the therapeutic effects of probiotics is carried out not only due to the presence of living bacteria in their composition, but mainly due to the products of vital activity synthesized by them – biologically active substances (lactic acid) [7].

According to many authors, the diverse bacterial structure of BV requires the use of additional antibacterial therapy or a combination of drugs capable of eliminating a wide range of possible pathogenic microorganisms [8, 9]. Special attention should be paid to the problem associated with the use of antibiotics, which in some cases leads to the aggravation of dysbiosis

and the growth of pathogens resistant to them. Antiseptics are of great interest due to their favorable characteristics in this regard [10].

It is also important to take into account that in women with vaginal microbiocenosis without the dominance of lactobacilli corresponding to CST IV, the detected diagnostic results may be mistakenly formulated as BV, which makes antibiotic therapy inappropriate in this case, and this may contribute to a clear violation of inter-microbial relations with the development of BV [8, 9, 12].

As can be seen from the presented data, the issue of effective treatment of bacterial vaginosis remains debatable. Adequate and effective medical correction of vaginal dysbiosis should take into account the polymicrobial etiology of BV, the peculiarities of its pathogenesis, and the reserve abilities of lactobacilli. In this regard, the search for the most optimal treatment methods that contribute to having positive effects on the vaginal microflora with long-term remission of the disease seems relevant.

The Purpose of the Study

To determine the effectiveness of different methods of treatment of bacterial vaginosis by dynamic assessment of the composition of the vaginal microbiota and immunological parameters.

Materials and Methods of Research

A clinical and laboratory examination of 110 women with BV was conducted, a gynecological history was carefully collected, and the features of extragenital pathology were studied.

In order to develop an effective treatment method, we conducted a differentiated approach to two-stage therapy based on the molecular biological characteristics of the vaginal microbiota. To do this, the patients were divided into 3 groups: In group I, consisting of 38 patients in whom the state of the vaginal microbiota corresponded to moderate dysbiosis, therapy was carried out with metronidazole drugs, 500 mg per os 2 times a day for 7 days. In group II, 40 patients with severe anaerobic dysbiosis were treated with clindamycin for 7 days. In group III there were 32 patients in whom pronounced aerobic-anaerobic dysbiosis was verified, taking into account the presence of both aerobic and anaerobic representatives of the microbiota, treatment was carried out with antiseptic agents (chlorhexidine) for 7 days. With an increased content of Candida fungi, antimycotic therapy was simultaneously prescribed.

At the 2nd stage of treatment, depending on the initial number of lactobacilli, prebiotics were prescribed – an acid-containing drug or probiotics.

After the therapy, a follow-up examination was carried out after 1 month, and then an assessment of the effectiveness of treatment after 3 and 6 months, as well as upon presentation of complaints.

The presence of specific vaginal secretions (liquid and homogeneous) was determined; an amine test was performed; the pH of the vaginal fluid was measured and a microscopy analysis of vaginal smears for "key cells" was performed. Molecular biological typing of the vaginal microbiota was performed using the Femoflor-16 test system.

Results and their Discussion

The study of vaginal microbiocenosis includes all microorganisms related to the endogenous microflora of the vagina, and only quantitative accounting of them gives a complete picture of its condition at the time of the study.

Moderate dysbiosis was detected in 34.5% of women (moderate anaerobic dysbiosis – 20.0%; moderate aerobic-anaerobic dysbiosis – 14.5%). The proportion of pronounced dysbiosis was 65.5% (pronounced anaerobic dysbiosis – 36.4% and pronounced aerobic-anaerobic dysbiosis – 29.1%). Then, taking into account the profile of the vaginal microbiota, we formed subgroups of women. Group I included 38 women with moderate anaerobic and aerobic-anaerobic dysbiosis, group II included 40 women with severe anaerobic dysbiosis and group III included 32 women with severe aerobic-anaerobic vaginal dysbiosis. This conditional division into subgroups was aimed at an adequate selection of differentiated therapy.

At a follow-up visit a month after treatment, complaints and clinical symptoms of the disease were absent in women of all three groups. In group I, the pH of vaginal discharge decreased from the initial 6.04±0.03 to 4.07±0.06, in groups II and III – from 6.33±0.05 to 4.18±0.05 and from 6.17±0.05 to 4.19±0.05, respectively ($p<0.001$). There were no significant differences between the groups in the number of leukocytes, desquamated epithelial cells and microbial cells in smears ($p>0.05$). The effectiveness of therapy against Gardnerella vaginalis, Bacteroides spp., gram-negative and gram-positive cocci was comparable and statistically significant in all three groups ($p<0.001$), which confirms our adequate choice of various treatment methods taking into account molecular biological variants of vaginal dysbiosis.

Repeated examination 3 months after treatment revealed no typical BV complaints and clinical symptoms in group I women. Women of groups II and III had complaints of pathological discharge with an unpleasant odor in 7.5% and 6.2% of cases, respectively. The number of cases of detection of borderline pH values of vaginal discharge (pH= 4.5) was higher in group III

(25.0%), as well as exceeding the threshold value (pH > 4.5) – 6.2% of cases. "Key cells" were found in 2.5% and 3.1% of women, the contamination of heterogeneous vaginal microflora increased in 7.5% and 12.5% of smears of groups II and III, respectively. Absolute normocenosis was established in 39.5%, 30.0% and 28.1%, conditional – in 55.3%, 52.5% and 53.2%, moderate dysbiosis – in 5.2%, 17.5% and 18.7% of patients of groups I, II and III, respectively (Fig. 1). Cases of severe dysbiosis were absent in all groups.

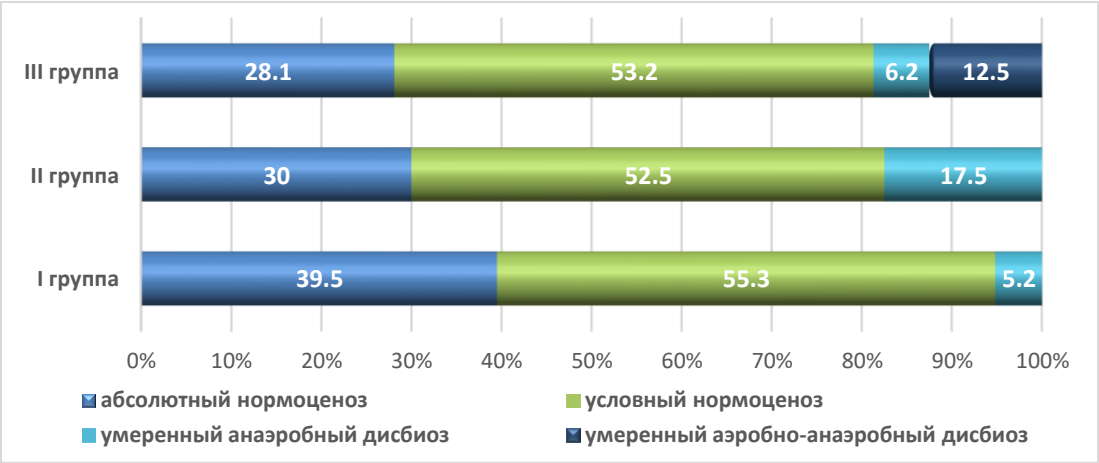

Fig. 1. Variants of the vaginal microbiota in groups 3 months after treatment

As can be seen, after treatment in groups with severe dysbiosis, compared with group I, cases of restoration of the vaginal microbiota to moderate normocenosis prevail, which shows the dependence of the results of correction of vaginal dysbiosis on the initial state of the mucosal microbiota. Decrease in the frequency of occurrence of the group Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. It was more pronounced in group I (from 90.2% to 42.1% – 2.2 times) compared with group II (from 100% to 52.5% – 1.9 times) and group III (from 90.6% to 56.2% – 1.6 times) (Fig. 2).

Frequency of occurrence of Sneathia/Leptotrichia/Fusobacterium spp., Megasphaera/Veillonella/Dialister spp. and Lachnobacterium/Clostridium spp. It was sharply reduced in all groups, but this trend was clearly manifested in groups with severe dysbiosis (up to 5 times in group II and up to 3 times in group III).

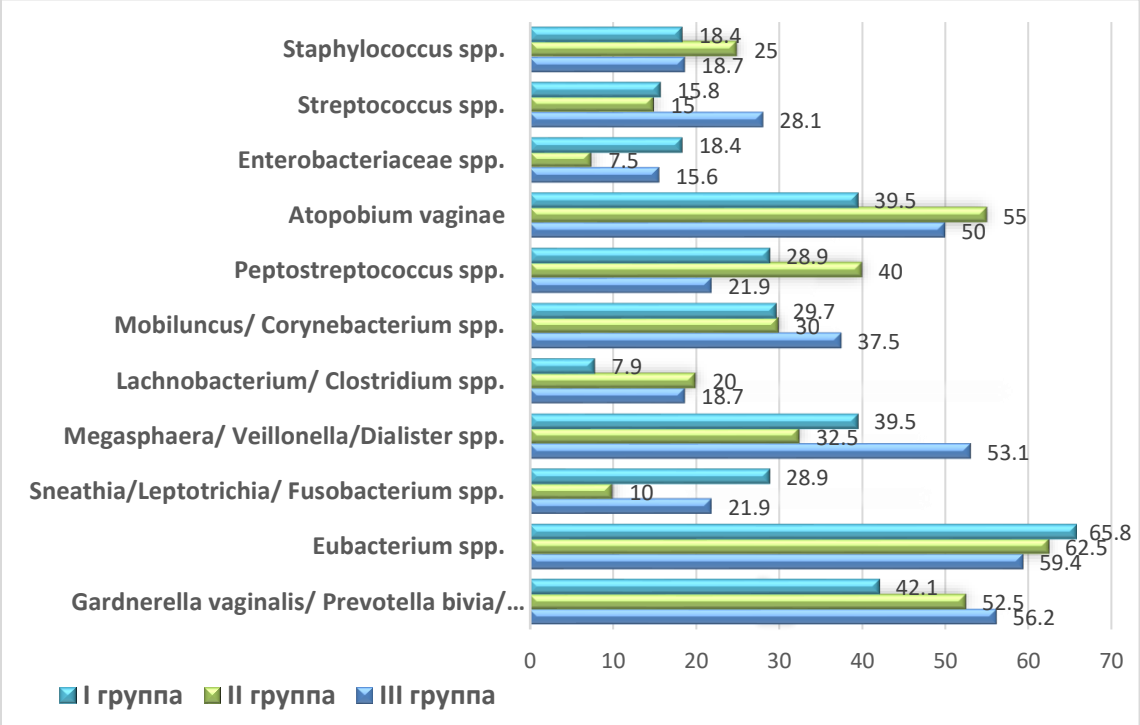

Fig. 2. Frequency of detection of microorganisms in diagnostically significant amounts in groups 3 months after treatment

The picture of the increase in the content of lactoflora took the following character: in group I, the average concentration of lactobacilli increased from 6.61 ± 0.19 to 7.09 ± 0.12, in group II – from 3.92± 0.22 to 6.82± 0.14 and in group III – from 4.82± 0.41 to 6.64 ± 0.18. As can be seen, more pronounced dynamics was observed in the last two groups where probiotics were used at the second stage of treatment.

Despite this, an increase in the content of opportunistic flora was detected in 5.3%, 17.5% and 18.7% of samples in women of groups I, II and III, respectively. When analyzing the quantitative content of the identified microorganisms, it was found that Gardnerella vaginalis is found most often in almost all associations of microorganisms – up to 96%, then Atopobium vaginae. When monitoring treatment after 3 months, Atopobium vaginae was detected in 1/3 of patients in association with other microorganisms. Eubacterium spp. It was stably determined in approximately 20% of BV cases after treatment. Peptostreptococcus spp. and Mobiluncus spp. were somewhat less common – 17-20%.

To correct the existing disorders, at the same time, to prevent the aggravation of dysbiosis, we used means that improve the microbiota of the mucous membranes as a whole. For this purpose, prebiotics of the oligosaccharide group (inulin) were used. Prebiotics are substances or functional food products that contain substances that are not digested in the upper gastrointestinal tract. Passing through the intestines, they are a nutrient base for a certain group of microorganisms, mainly for bifidobacteria and lactobacilli, inhabiting the human colon and positively affecting, in turn, the composition of the microbiota. The most well—known prebiotics are oligofructose, inulin, galacto-oligosaccharides, lactulose (preparations "Dufalak", "Normase", etc.), breast milk oligosaccharides, etc. There is a growing body of evidence that taking substances such as inulin and fructo-oligosaccharides as probiotics can stimulate the growth of certain strains of lactobacilli and bifidobacteria in the intestine, potentially altering their protective anti-infective function. However, the subsequent effect of these supplements on the vaginal microbiota and the course of the dysbiotic process remains unexplored.

In this connection, after 3 months of anti-relapse therapy, we re-evaluated the microscopic and microbiological picture of the vagina. The occurrence of lactobacilli increased in all smears compared to similar indicators detected 3 months ago (from 92.5% in group II and 90.6% in group III to 100% after 6 months). Both morphotypes of Gardnerella vaginalis, Bacteroides spp., Mobiluncus spp., and gram-negative and gram-positive cocci were detected in single smears.

The vaginal microbiota has become more stable after anti-relapse therapy. Absolute normocenosis was recorded in 44.7%, 42.5% and 37.5% of cases, conditional – 50.1%, 52.5% and 53.2% of cases in groups I, II and III, respectively (Fig. 3). The frequency of detection of associations of aerobic and anaerobic microorganisms in the vaginal discharge of women of the three groups significantly sharply decreased and did not differ from each other a friend (p>0.05).

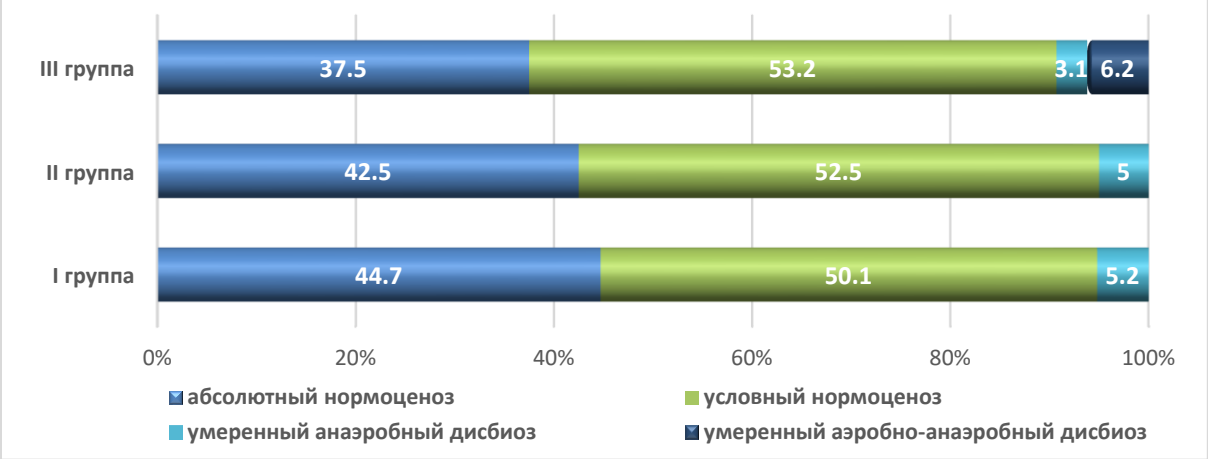

Fig. 3. Frequency of detection of microorganisms in diagnostically significant amounts in groups 6 months after treatment

In general, in all three groups, within 6 months after two-stage therapy, it was possible to maintain a decrease in colonization of the vaginal epithelium by anaerobic microorganisms associated with BV and to strengthen the representation of lactobacilli there. However, both quantitatively and qualitatively, the use of prebiotics of the oligosaccharide group as anti–relapse therapy had advantages: the average number of lactobacilli turned out to be an order of magnitude higher, and the species representation was better compared to groups where the anti-relapse stage of therapy was not used.

One of the most important functions of the cytokine system is to ensure the coordinated action of the immune, endocrine and nervous systems in the development of an inflammatory reaction. The cytokine network is represented by a variety of proteins or glycoproteins produced by lymphocytes, macrophages and other types of leukocytes, as well as fibroblasts, endothelial, epithelial and other somatic cells.

Interleukin 6 (IL-6) is a growth and differentiation factor of B lymphocytes, an activator of polyclonal production of

immunoglobulins, supports the proliferation of T cells, is one of the mediators of acute phase inflammatory reactions.

Interleukin 8 (IL-8) is a chemokine of the CXC family, provides chemotaxis of neutrophils and T-lymphocytes, activates neutrophils, promotes angiogenesis, inhibits the release of histamine by basophils, regulates the synthesis of IgE by B cells.

Interleukin 10 (IL-10) is an anti–inflammatory cytokine, has immunosuppressive activity against type I T helper cells and antigen-presenting cells, suppresses the production of proinflammatory cytokines, however, it stimulates proliferation, differentiation and functional activity of B lymphocytes.

The positive effect of probiotics on the vaginal microbiota was also reflected in the normalization of the local concentration of pro- and anti-inflammatory cytokines. As can be seen from Table 1, a significant decrease in cytokine concentration after 3 months compared with the baseline data before treatment was observed in almost all groups ($p<0.001$). The level of IL-6 in group I and IL-8 in group III remained somewhat high. After additional pre-relapse therapy with prebiotics, after another 3 months, a decrease in IL-6 levels was revealed by 2 times in all groups, IL-10 by more than 2 times in groups with severe dysbiosis. As can be seen, the pre-relapse therapy led to the complete elimination of the imbalance between the pro- and anti-inflammatory cytokine links.

Table 1: Comparison of local cytokine concentrations in the study groups before, 3 and 6 months after treatment

Indicator	before treatment	3 months after treatment	p	6 months after treatment	p
I группа					
IL-6	163,66±19,70	133,93±18,13	0,273	59,74±6,19	0,000
IL-8	140,67±11,14	93,04±5,98	0,001	75,18±7,49	0,069
IL-10	119,00±14,66	31,44±5,23	0,000	32,54±4,26	0,871
II группа					
IL-6	181,99±17,35	91,79±15,35	0,000	55,23±8,11	0,042
IL-8	175,46±9,19	114,89±11,05	0,000	78,10±7,54	0,009
IL-10	135,80±15,09	41,39±6,33	0,000	28,89±3,78	0,098
III группа					
IL-6	217,74±24,76	100,28±12,00	0,000	57,18±8,36	0,006
IL-8	115,56±14,08	123,13±13,78	0,703	85,93±8,53	0,028
IL-10	110,98±10,98	72,71±9,78	0,014	31,87±4,02	0,000

In 2 (5.3%) women of group I, in whom the profile of the vaginal microbiota corresponded to moderate dysbiosis, it was noted that this condition persisted after 6 months. Given the absence of clinical symptoms of the disease, the absence of a pro-inflammatory cytokine response, it can be assumed that this microbiological profile may be a variant of the norm for these women. Given this circumstance, the question arises about the expediency of correcting the state of moderate dysbiosis in this case. Our observations have shown that the vaginal microbiota in these women, without additional medical interventions, can spontaneously turn into a conditional normocenosis over time. At the same time, the vaginal microbiota is characterized by a frequent temporary transition to a conditional normocenosis and vice versa.

Thus, the formation of the vaginal microbiota after treatment depended both on the nature of the therapy and on the initial state of the vaginal microbiota. The state of the vaginal microbiota remained more stable for 6 months after treatment in group I. In groups with initially formed as pronounced dysbiosis (groups II and III), there is a tendency to increase the diversity and concentration of opportunistic microflora by 3 months after treatment. Therefore, repeated clinical microscopic and, if necessary, molecular biological control of the vaginal microbiota with anti-relapse therapy is a preventive measure of deep dysbiotic disorders of this biotope.

Conclusion

According to the results of the study, it can be concluded that a two-stage treatment regimen for BV, selected taking into account the molecular biological variants of vaginal dysbiosis, had a persistent treatment effect in 87.3% of cases. The additional use of probiotics of the oligosaccharide group as an anti-relapse therapy enhances the colonization of lactobacilli and ensures the stability of the vaginal biotope in 95.4% of cases.

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