THE EFFECT OF PROBIOTIC THERAPY ON THE VAGINA MICROBIOTA AND THE HUMORAL LINK OF IMMUNITY IN BACTERIAL VAGINOSIS

The aim of the research is to determine the effect of the probiotic preparation «Dialak» (dietary supplement), which includes the strain Lactobacillus casei IMV B-7280, on the vaginal microbiota and humoral immunity in women with bacterial vaginosis (BV). Methods. 40 female patients aged 20—45 years with disturbed vaginal microbiota and 10 healthy individuals were examined. The verification of 3 types of vaginal biocenosis states, namely normocenosis, intermediate type, and vaginal dysbiosis, was carried out on the basis of the Recommendations for the Treatment of Sexually Transmitted Infections Weekly Morbidity and Mortality Report (2021) and laboratory diagnostic methods according to the well-known criteria proposed by R. Amsel. Female patients with an intermediate type of BV (group 1) received suppositories and capsules of the probiotic (once daily) for 10 days. Women with vaginal dysbiosis (group 2) received metronidazole in a dosage of 500 mg twice a day for 7 days during the first stage, and then 1 suppository at night and oral capsules of the probiotic in the morning for 10 days during the second stage. The studied vaginal secretion was stained by the Gram method in the Kopeloff modification and also sown on nutrient media to determine facultatively anaerobic and obligately anaerobic microorganisms. Microorganism identification was carried out on the basis of morphological, cultural, biochemical, and antigenic properties according to the classification of D. H. Bergey (2009). The activity of humoral immunity was determined by evaluating the number of B-lymphocytes in the peripheral blood of patients using flow cytometry, as well as the levels of serum Ig A, M, and G before treatment and after 1 month using the immunoturbidimetric method and the Cobas 6000 test system from Roche Diagnostics (Switzerland).

Results. When analyzing the vaginal microbiota in two groups of patients before treatment, a decrease in the number of Lactobacillus spp. and Bifidobacterium spp. and a significant increase in the number of obligate anaerobic microorganisms, including Gardnerella vaginalis, were found compared to the control group. Before treatment, the number of Lactobacillus spp. in women of group 2 was lower compared to group 1. In patients with vaginal dysbiosis before treat-
The normal vaginal microbiota is a dynamic population of various microorganisms, with a predominance of lactobacilli, which provide colonization resistance of the vaginal mucosa as they perform antimicrobial, fermentative, immunoregulatory functions, and more. The vaginal microbiota includes various anaerobic and aerobic microorganisms, such as *Lactobacillus* spp., *Bacteroides* spp., *Prevotella* spp., *Mobiluncus* spp., *Veillonella* spp., *Peptostreptococcus* spp., *Atopobium vaginale*, and others [1, 2]. According to the International Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10), and sexually transmitted infections (STIs), opportunistic members of the urogenital microbiota have significant clinical significance. Changes in the vaginal microbiota composition can clinically manifest themselves in various nosological forms of diseases, such as BV, nonspecific vaginitis, and vaginal candidiasis. It is known that in associations, the pathogenicity and virulence of microorganisms are enhanced, so it is practically impossible to determine which microorganism plays a leading role in the current stage of an infectious-inflammatory disease. Therefore, it is difficult to detect specific clinical symptoms of the disease [1, 3, 4, 7].

To diagnose BV according to the European guidelines for the management of patients with vaginal discharge (2018), developed by the International Union Against Sexually Transmitted Infections and the World Health Organization, a modern microscopic test using the Hay-Ison criteria (4 grades) has been proposed. Grade 0: vaginal microbiota does not belong to BV, only epithelial cells are found under microscopy with no lactobacilli, indicating recent antibiotic therapy. Grade 1 (normal flora): *Lactobacillus* morphotypes predominate. Grade 2 (intermediate): mixed flora with some lactobacilli, but *Gardnerella* or *Mobiluncus* morphotypes are also present. Grade 3 (BV): predominantly *Gardnerella* and/or *Mobiluncus* morphotypes, with key cells; lactobacilli are low or absent. Grade 4: vaginal microbiota does not belong to BV, only gram-positive cocci are found, without lactobacilli (flora corresponds to aerobic vaginitis).

Two groups of BV markers are distinguished: low-specificity markers, such as *G. vaginalis*, *Mobiluncus* spp., and *Leptotrichia* spp., which are present in both healthy women and those with BV, and high-specificity markers, such as *A. vaginae*, vaginosis-associated bacteria (*Clostridium phylum*, *G. vaginalis*, and *A. vaginae*), prone to...
forming biofilms and resistant to many modern antibacterial drugs [2, 3, 4, 6, 7, 8].

A significant number of traditional schemes proposed for the treatment of BV patients have low therapeutic efficacy, as prolonged use of antibiotics often causes destabilization of the vaginal and intestinal ecosystems, with the disruption of their microbiota’s qualitative and quantitative composition, immune dysfunction, and a significant increase in the number of antibiotic-resistant microorganisms, accompanied by the emergence of side effects and disease recurrence [5, 7, 10, 11, 15]. As already known, there is a close relationship between the quantitative and qualitative composition of the vaginal and intestinal microbiota and immune system dysfunction [9, 10]. Representatives of the microbiota of the lower genital tract in healthy women, which are mainly represented by L. crispatus, L. jensenii, and L. iners, metabolize glycogen secreted by the vaginal epithelium and produce lactic acid, which is largely responsible for the vagina pH when it exhibits virucidal activity against pathogens [6, 15—17]. The cervix secretions contain factors of innate immunity: complement, defensins, and immunoglobulins (Igs) in high concentrations, which are the first line of defense against pathogens (Kozlowski et al., 1999). Women with BV have been found to have an increased vaginal pH, which inhibits the vitality of lactobacilli, destroys the protective mucous layer, and promotes the fixation of pathogens to epithelial cells [6, 16], as well as the disruption of the humoral immune system of the mucous membrane, as evidenced by a decrease in the content of IgA and IgM in vaginal washes and an increase in the concentration of IgM in serum. It should be noted that changes in the indicators of the humoral immune system are more often found in women who have been suffering from BV for a long time (more than 5 years) [1, 3] and are associated with the production of certain exometabolites by pathogens [15]. Thus, bacterial colonization of the vagina leads to the accumulation of metabolic products, polyamines, and enzymes (sialidases, collagenases, proteases, etc.). Sialidase, which is more commonly produced by G. vaginalis, can cause immunosuppression and promote the recurrence of BV. Other hydrolytic enzymes, such as vaginolysin, also suppress IgA production [6, 15, 17]. At the same time, high levels of IgA against G. vaginalis hemolysin prevent adverse outcomes in BV development, especially during pregnancy [16] as the innate immunity of the vaginal mucosa is closely related to the adaptive immunity development.

The mechanisms of immune response development in BV remain insufficiently studied, and the recurrence of this disease is a serious burden on healthcare worldwide [16]. Many studies have confirmed that BV can increase the risk of gonorrhea, chlamydia, HIV, herpes virus, and papillomavirus infections, and thus, cervical cancer [3, 4, 17]. To effectively treat patients with BV, probiotics with a defined multifactorial mechanism of action (antimicrobial, anti-inflammatory, and immunomodulatory) are used alone or in combination with etiologic or pathogenetic therapy, which can normalize the vaginal microbiota, the state of the immune system, and inflammation [5, 10, 11, 15]. Thus, probiotics affect the activity of innate immune factors, Toll-like receptor expression, and cytokine production; prevent cytokine-mediated damage to the vaginal mucosa; enhance trophic and proliferative reactions of epithelial cells, and more. Therefore, the search for and study of the therapeutic effectiveness of new probiotic cultures that can be used in BV, taking into account the level of dysbiotic and immune disorders, as well as the nature of the course and severity of a particular pathological process, is timely and relevant.

The aim of the study was to determine the effect of the probiotic preparation «Dialak» (dietary supplement), which contains the strain Lactobacillus casei IMV B-7280, on the vaginal
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Materials and methods. A total of 40 women aged 20—45 years with BV and 10 clinically healthy persons of the same age were examined in the Ternopil Communal Non-Commercial Enterprise «Mother and Child» (Ukraine). All patients with BV and clinically healthy women provided voluntary consent to participate in the study. It should be noted that the patients were motivated to participate in these studies because they had the right to receive their results, based on the analysis of which the tactics of their individual treatment were developed. The inclusion criteria for women with BV in clinical trials were the exclusion of using any antibacterial drugs for 1 month prior to the examination and the absence of venereal and inflammatory processes of the reproductive system. The probiotic (dietary supplement «Dialak»), which includes the strain of lactobacilli L. casei IMV B-7280 in the form of capsules for oral use and suppositories, was used in the study. The probiotic strain L. casei IMV B-7280 is deposited in the Ukrainian Collection of Microorganisms of the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine.

The treatment of BV patients was carried out in accordance with the Order of the Ministry of Health of Ukraine No. 417 (2011) «On the organization of outpatient obstetric and gynecological care in Ukraine».

The clinical-laboratory examination of female patients included analysis of complaints and evaluation of clinical signs of the disease course and duration. In the diagnosis of BV, the Kira classification (2012) was used and confirmed by bacterioscopic and bacteriological studies. Our study included women who had 3 types of vaginal bioecological status: normocenosis, intermediate type, and vaginal dysbiosis. The presence of the fourth type, i.e., vaginitis, was a criterion for their exclusion from the study. All the women were subject to a comprehensive examination using the determination of the pH reaction of the vaginal content and the amine test. The intensity of odor when using the amine test is evaluated on a scale from 1 to 4 pluses. With a negative test, the odor is present at a distance of up to 20 cm as a weak odor, ++ — an odor that is felt at a distance of up to 50 cm, +++ — a pronounced odor that is felt at a distance of up to 1 m, and ++++ — an intense odor that is felt at a distance of more than 1 m.

The laboratory investigations included a microscopic examination of vaginal smears using the Kopeloff modification and bacterial cultures on nutrient media to identify facultative anaerobic and obligate anaerobic microorganisms. The diagnosis was verified based on the Recommendations for the Treatment of Sexually Transmitted Infections (Weekly Morbidity and Mortality Report (2021)). The well-known criteria for BV proposed by R. Amsel (1983) were also taken into account: the presence of homogeneous secretions, clue cells in them, microorganisms stained by the Gram method, pH of secretions (> 4.5), and so on. Scrapings (samples) were taken from the vaginal mucosa of the affected women before and after treatment, and the number of microorganisms (lactobacilli and other microorganisms), epithelium, and leukocytes was determined after Gram staining. When analyzing the results obtained, it was taken into account that a large number of epithelium and leukocytes, from 5 to 10 in the field of view, indicate the development of inflammation and disturbance of homeostasis, which is one of the BV signs. Scrapings from the mucous membrane of the cervical canal of the cervix and the posterior fornix of the vagina were used as material for determining pH. The amine test of vaginal content was used as an express diagnostic method for detecting volatile amine isonitrile produced by the BV-associated bacteria G. vaginalis and A. vaginae. The biomaterial was applied to a glass slide, and a drop of potassium hydroxide was added. A positive result was indicated by an unpleasant odor of «rotten fish.»
The sick women who took part in the study were divided into 2 groups. Group 1 consisted of sick women with an intermediate type of BV (n = 20) who received suppositories (1 suppository in the morning and at night) and a capsule form of probiotic (once a day) for 10 days. Group 2 included women with vaginal dysbiosis (n = 20) who were treated with a tablet form of metronidazole at a dose of 500 mg twice a day for 7 days as the first stage of treatment. At the second stage of therapy, these women received 1 suppository at night and a capsule form of probiotic orally in the morning for 10 days. A separate control group consisted of 10 clinically healthy individuals.

After 17 days of treatment, the dynamics of the disease clinical picture and the subjective sensations of the women were evaluated. Microbiological studies were also conducted. Thus, before and 17 days after treatment, material from the mucous membranes of the vagina and cervix was collected using a sterile standardized swab and immediately immersed in a sterile tube with transport medium Amies (Violife Italiana S.r.I., Italy). Sowing on dense nutrient media was carried out using the quantitative method of sectoral sowing according to Gold. The following nutrient media were used to study the vaginal microbiota: blood agar (Violife Italiana S.r.I., Italy), saline agar (Violife Italiana S.r.I., Italy), sugar broth and serum agar (Violife Italiana S.r.I., Italy), medium for lactobacilli (Violife Italiana S.r.I., Italy), Bifidum medium (FARMAKTIV LLC, Ukraine), and thioglycolate medium for anaerobes (Violife Italiana S.r.I., Italy). Sabouraud medium (FARMAKTIV LLC, Ukraine) was used for fungi of the Candida genus, and Endo medium (Violife Italiana S.r.I., Italy) was used for enterobacteria. Samples introduced into Wilson-Blair medium and thioglycolate broth were incubated at 37 °C for 48 h under anaerobic conditions in sealed GENbox boxes (VioMerieux, France) using gas-generating packages GENboxanaer (VioMerieux, France) to create anaerobic conditions.

Samples that were introduced into other nutrient media were incubated in a thermostat at 37 °C for 24 h and in Sabouraud’s medium for almost 5 days. After cultivation, colony growth was evaluated, and their morphology, coloring, consistency, size, and presence of hemolysis were studied. Microorganisms were stained using Gram staining. Quantitative counting was performed by determining colony-forming units (CFU) in one gram of vaginal secretion. Microorganism identification was carried out on the basis of morphological, cultural, biochemical, and antigenic properties according to the classification of D. H. Bergey (2009).

One month before and after the treatment of female patients, indicators characterizing the state of humoral immunity were investigated. Peripheral blood was collected, and surface antigens of B lymphocytes were determined using flow cytometry. The content of immunoglobulins G, A, and M in the serum was investigated using a widely accepted immunoturbidimetric method.

To test the normality of the distribution of the studied variables, the Shapiro-Wilk test was used with the Statistica 10.0 program. The probability of the results was estimated by the variational-statistical method of analysis with the determination of the arithmetic mean (M) and the standard error of the mean (m). The Mann-Whitney U test was used to compare observations in independent samples. The Wilcoxon T-test was used to compare indicators determined under two different conditions in the same sample. A probability of p < 0.05 was considered significant, which was equal to or greater than 95.0% (0.95), i.e., the risk of error was less than 5.0% (0.05). In all cases, differences were interpreted as significant at p < 0.05.

**Results.** Upon initial examination of women with intermediate-type BV and vaginal dysbiosis, attention was drawn to the presence of a large amount of vaginal discharge, itching, burning, and discomfort in the genital area...
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(Table 1). In contrast to vaginal dysbiosis, no thick yellow-green discharge from the vagina was detected in women with intermediate-type BV. Prior to treatment, the amine test was positive in significantly fewer cases in women with BV compared to those with vaginal dysbiosis (p < 0.05). After treatment, a statistically significant improvement in the condition of women in both comparison groups was observed (p < 0.05). Thus, in all women with BV, the discharge with a «fishy» odor during the amine test, discomfort in the genital area, as well as itching and burning sensation disappeared after treatment. In some women in this comparison group, white vaginal discharge was observed. In a small number of women with vaginal dysbiosis, after treatment, pathological white discharge, discharge with a «fishy» odor during the amine test, and discomfort in the genital area were detected, but itching and burning completely disappeared. After using the probiotic during the follow-up examination of the women, no signs of vaginal mucosa damage or any complaints of allergic nature were found, indicating its safety.

It has been found that the pH of vaginal secretions in women from both comparison groups ranged from 3.8 to 6.3, with the normal range being from 3.8 to 4.5. In most patients, an increase in the pH level was observed before treatment (Table 1). The pH level of vaginal secretions before treatment was higher in a significantly larger number of cases with vaginal dysbiosis than in cases with intermediate BV (p < 0.05). As shown in Fig. 1, the mean pH values increased in both comparison groups compared to clinically healthy women. After treatment, an increased pH level was detected in significantly fewer cases in both groups compared to the pre-treatment level (Table 1). Our findings indicate that an increased pH level was observed in a larger number of women with vaginal dysbiosis who received treatment (p < 0.05) compared to cured female patients with intermediate BV. After treatment, a decrease in the mean pH value of vaginal secretions was observed in the group of

<table>
<thead>
<tr>
<th>Table 1. Clinical signs of the disease in patients with BV (1st group) and vaginal dysbiosis (2nd group) before and after treatment</th>
</tr>
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<tbody>
<tr>
<td>Indicators</td>
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<tr>
<td>White or gray vaginal discharge</td>
</tr>
<tr>
<td>Thick discharge, yellowish-green in color</td>
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<tr>
<td>Discharge with a «fishy» smell when performing an amino test</td>
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<tr>
<td>Discomfort in the genital area</td>
</tr>
<tr>
<td>Itching and heartburn</td>
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<tr>
<td>pH of vaginal discharge</td>
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*statistically significant difference between the study groups (p < 0.05)
women with vaginal dysbiosis compared to the pre-treatment level \(p < 0.05\), while in the group of women with intermediate BV, there was a tendency toward a decrease in pH after treatment, but it was not statistically significant (Fig. 1).

The results of microbiological research on vaginal secretions of patients with intermediate BV and vaginal dysbiosis before and after treatment are presented in Table 2. The amount of *Lactobacillus* spp. in the vagina of women in both comparison groups before treatment was significantly lower than in the control group. After treatment, the number of these bacteria in the vagina of women with intermediate BV and dysbiosis increased compared to pre-treatment levels. However, the number of these bacteria in the vagina of patients with dysbiosis remained lower than in patients with BV \(p < 0.05\).

Before treatment, patients with intermediate BV and dysbiosis showed a significant increase in the number of anaerobic microorganisms, namely *G. vaginalis, Fusobacterium* spp., *Veillonella* spp., *Mobiluncus* spp., *Peptococcus* spp., and *Bacteroides* spp., in the vagina compared to the control group \(p < 0.05\). In patients in group 2, the number of such microorganisms as *G. vaginalis, Fusobacterium* spp., *Veillonella* spp., *Mobiluncus* spp., *Peptococcus* spp., and *Bacteroides* was higher than in patients in group 1 \(p < 0.05\), except for *Eubacterium* spp. \(p > 0.05\). After treatment, normalization of the number of these anaerobic microorganisms in the vagina of patients in both comparison groups was observed, except for *Veillonella* spp. \(p > 0.05\) (Table 2). The amount of *Bifidobacterium* spp., which also creates a low pH in the vagina, was reduced in both groups before treatment. After treatment, these indicators were normalized compared to the control group.

To confirm the change in the quantity of *Lactobacillus* spp. before and after treatment of the patients, we additionally used the Shapiro-Wilk W-test to check the distribution normality for the indicators and to select a parametric method (Fig. 2). The Shapiro-Wilk test is used to determine the normality of distribution and study continuous variables. The null hypothesis for this test is that the data are normally distributed. The Prob < W value in the output data is the p-value. If the chosen alpha level is 0.05 and

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**Fig. 1.** pH indicators of vaginal secretion in women with intermediate BV (1st group) and vaginal dysbiosis (2nd group) before and after treatment.
the p-value is less than 0.05, then the null hypothesis that the data are normally distributed is rejected. If the p-value is greater than 0.05, then the null hypothesis is not rejected. As the absence of normal distribution of *Lactobacillus* spp. indicators was found in the patients of both comparison groups, the Mann-Whitney U-test was used for independent samples, whereas the Wilcoxon T-test was used for dependent samples. When analyzing the vaginal microbiota in patients of the first and second groups before treatment using these statistical research methods, a decrease in the number of *Lactobacillus* spp. compared to the control group (p < 0.05) was confirmed as well. It should be noted that before treatment, the number of *Lactobacillus* spp. in women with vaginal dysbiosis was significantly lower than in women with an intermediate type of BV (p < 0.05). After treatment of the patients, an increase in the number of *Lactobacillus* spp. in the vagina of both comparison groups was also confirmed (p < 0.05), but the number of these bacteria in the vagina remained higher in patients with an intermediate type of BV than in those with vaginal dysbiosis (p < 0.05).

**Table 2. Number of microorganisms in the vagina of women with BV (1st group) and vaginal dysbiosis (2nd group) before and after treatment, lg CFU/mL, M ± m**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Control group n = 10</th>
<th>1st group n = 20</th>
<th>2nd group n = 20</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>9.06±0.37</td>
<td>6.80±0.23*</td>
<td>9.20±0.78**</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>7.00±0.21</td>
<td>4.70±0.25*</td>
<td>5.78±0.21**</td>
</tr>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>2.25±0.25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>G. vaginalis</em></td>
<td>2.00±0.41</td>
<td>4.47±0.24*</td>
<td>1.33±0.17**</td>
</tr>
<tr>
<td><em>Eubacterium</em></td>
<td>2.00±0.32</td>
<td>5.00±0.45*</td>
<td>2.00±0.00**</td>
</tr>
<tr>
<td><em>Veillonella</em></td>
<td>1.33±0.21</td>
<td>3.89±0.51*</td>
<td>2.00±0.41**</td>
</tr>
<tr>
<td><em>Mobiluncus</em></td>
<td>1.33±0.33</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Fusobacterium</em></td>
<td>1.50±0.29</td>
<td>4.14±0.64*</td>
<td>2.00±0.00**</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>3.58±0.31*</td>
<td>2.16±0.64**</td>
<td>4.83±0.53*</td>
</tr>
<tr>
<td><em>Peptococcus</em></td>
<td>—</td>
<td>—</td>
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</table>

* — significance of the investigated groups compared to the control group before treatment (p < 0.05); ** — significance within the investigated groups after treatment (p < 0.05); p1 — significance of group 1 compared to group 2 before treatment.
**Fig. 2.** Histograms of Lactobacillus spp. indices in the vagina of clinically healthy women (control group, A), as well as those for intermediate BV (group 1, B) and vaginal dysbiosis (group 2, C) before and after treatment to test for normal distribution using the Shapiro-Wilk W-test.
Having compared the *Lactobacillus* spp. before and after treatment in the group of women with vaginal dysbiosis, we can conclude that there is a statistically significant difference with \( p < 0.05 \) (\( p = 0.000089 \)), indicating the diagnostic value of the *Lactobacillus* spp. as an index for classifying (distinguishing, separating) patients before and after treatment. To evaluate the effectiveness of the applied BV treatment methodology, it is necessary to compare the control group and the post-treated-with- *Lactobacillus* spp. group. Since the samples under study are independent and not normally distributed, the non-parametric Mann-Whitney test should be used for their comparison. Analyzing the results of the comparison of the control group and women with vaginal dysbiosis after treatment with *Lactobacillus* spp., we revealed no statistically significant difference with \( p > 0.05 \) (\( p = 0.37 \)) that could confirm the effectiveness of the treatment.

The results of our research have shown that the normalization of the vaginal microbiota in female patients under probiotic therapy is associated with changes in some indicators of the humoral immune system, involved in protecting the mucous membranes from pathogenic and opportunistic microorganisms. It was found that the relative number of B lymphocytes (CD19+ cells) in the peripheral blood of the control group was 8.80 ± 0.80%. The number of these cells in the peripheral blood of patients in groups 1 (8.80 ± 0.61%) and 2 (9.70 ± 0.57%) did not change before treatment compared to the control group. After treatment, the number of CD19+ cells in the peripheral blood of patients in both comparison groups did not change either and was 8.40 ± 0.60 and 9.40 ± 0.65%, respectively.

In the serum of patients in both comparison groups, we found an increase in the level of IgM compared to the control group (\( p < 0.05 \); Fig. 3). At the same time, as shown in Fig. 2, we found
a decrease in the serum IgA concentration of in female patients in group 2 compared to the control group. On the other hand, the concentration of IgA in the serum of female patients in group 1 remained at a normal level. The concentration of IgG in the serum (Fig. 3) decreased in all patients compared to the control group (p < 0.05). After treatment, an increase in the IgG levels in the blood serum of patients in both groups was observed compared to pre-treatment values (p < 0.05). However, its concentration remained lower in patients of the 1st group (p < 0.05) compared to the control group. The concentrations of serum IgA in women of the 2nd group also increased after treatment to the level of the control group. At the same time, the level of IgM in the blood serum of patients in both groups normalized. Thus, after complex treatment of patients with intermediate-type BV and vaginal dysbiosis, a tendency toward normalization of most of the studied parameters of humoral immunity was observed.

**Discussion.** We examined sick women with disturbed vaginal microbiota, who were diagnosed with an intermediate type of BV and vaginal dysbiosis. For the treatment of intermediate-type BV, we used the capsule form of «Dialak» and suppositories, and for dysbiosis — the antibiotic metronidazole and the probiotic «Dialak» to restore the microbiota. As known, BV is a polymicrobial disease that arises from the depletion of normal vaginal flora, primarily with *Lactobacillus* spp., and an increase in anaerobic bacteria. As a result, the characteristics
of vaginal fluid change, in particular viscosity and odor [20].

We noted the presence of a large amount of discharge from the vagina, itching, burning, and discomfort in the genital area of the examined women with intermediate-type BV and vaginal dysbiosis. As for vaginal dysbiosis, a thick yellowish-green discharge was found in the vagina. Before treatment, the amine test was positive in women from both groups, but in group 1, it was + and ++, while in women with vaginal dysbiosis, it was +++ and ++++. According to other researchers’ data, patient complaints of BV showed few symptoms: only 62.67% of those surveyed complained of a change in the discharge nature, the practically equal number indicated itching in the vagina and an unpleasant odor of discharge, 19.89% of patients were concerned about dysuric manifestations, and 15.10% reported dyspareunia [11]. Before treatment, the amine test was positive in 91.30% of BV patients. The degree of severity of the positive amine test was as follows: 1st degree (+) — in 19.05% of women, 2nd degree (++) — in 33.33% of women, and 3rd degree (+++) — in 47.61% of women [12].

During the analysis of the vaginal microbiota in the examined patients of both groups, a decrease in the number of Lactobacillus spp. was detected prior to treatment. However, in group 2, a lower level of Lactobacillus spp. was observed compared to group 1 prior to treatment. Additionally, we found that in cases of intermediate BV and vaginal dysbiosis, there was a significant increase in the number of anaerobic microorganisms, including G. vaginalis, Mobiluncus spp., Veillonella spp., Fusobacterium spp., Peptostreptococcus spp., and Bacteroides, which was higher in patients with vaginal dysbiosis than in patients with BV, except for Eubacterium spp. Bacterial vaginosis is primarily caused by G. vaginalis, which decreases lactic acid production, alters pH (> 4.5), and suppresses the growth of lactobacilli. As a result, favorable conditions for facultative anaerobic microorganisms are created.

According to [2, 12], in BV patients, the most commonly isolated facultative anaerobes in vaginal secretions were G. vaginalis in 38 (95%) women, followed by E. coli in 17 (42.5%), Corinebacterium spp. in 11 (27.5%), and S. epidermidis in 10 (25.0%). S. saprophytics, S. aureus, Streptococcus spp., and Proteus mirabilis were isolated in less than 10% of women each. Among the obligate anaerobes, the most commonly isolated microorganisms in women with bacterial vaginosis were Peptostreptococcus spp., Peptococcus spp., Bifidobacterium spp., Eubacterium spp., and Veillonella spp.

Note that disruption of vaginal microbiota caused by BV creates a more favorable environment for HIV infection and increases the risk of genital herpes virus (HSV-2) transmission by 2.3 times [17]. Literature sources confirm that the reduction of colonization resistance of indigenous vaginal microbiota and its colonization by opportunistic microorganisms (enterococci, staphylococci, etc.), which dominate over lactobacilli and other normal flora, lead to a decrease in the immunostimulatory activity of lactobacilli, and this is the reason for the development of vaginal dysbiosis, its resistance to therapy, and susceptibility to relapse in this population of patients [27]. Therefore, probiotic therapy for these patients is an important alternative method of treatment and is widely used in clinical practice. Live lactobacilli from probiotic preparations and their metabolic products are capable of maintaining the optimal acidity of the vaginal environment, functioning of local immunity, anti-inflammatory response, and competing with external pathogens [9].

In the patients examined, complaints of discharge, itching, and burning were absent in group 1 in 95% of cases after treatment, but in 5% of cases, complaints of white discharge remained. In group 2, after treatment, pathological white discharge was detected in 10% of cases, and discomfort was reported in 20% of cases. Prior to treatment, the amine test was positive.
in 25% of cases in group 1 and in 100% of cases in group 2. After treatment, the amine test became negative in most women in group 1 and was positive in only 25% of cases among women in group 2. The pH of vaginal secretions ranged from 3.8 to 6.3, with a norm of 3.8 to 4.5. Both groups of women showed an increase in pH compared to the control group. After treatment, they showed a change in pH level, but there was a slight increase in vaginal secretion pH in 15% of cases in group 1 and 25% of cases in group 2. After treatment, both groups of patients showed an increase in the number of Lactobacillus sp. in the vagina, but a higher number of these bacteria was identified in group 1 after treatment compared to group 2. In patients receiving treatment, normalization of the number of anaerobic microorganisms G. vaginalis, Mobiluncus spp., Veillonella spp., Fusobacterium spp., Peptostreptococcus spp., Bacteroides spp., and Bifidobacterium spp. was also observed in the vaginal flora of both groups of patients, but the number of Veillonella spp. increased. It is likely that the probiotic «Dialak» promotes the regeneration of the mucous membrane and synthesis of lactic acid, which leads to a change in the pH of vaginal secretions and promotes an increase in the population level of lactobacilli. However, additional research is needed to confirm this point of view.

Probiotic therapy of the patients examined resulted in a change in the indicators of the humoral immune system. The immune defense of the female body during infections of the genital tract involves cellular and humoral mechanisms of forming immunological resistance, where the main function of the immune system is to maintain antigenic homeostasis through recognizing, inactivating, and destroying all genetically foreign structures, as well as providing immunological surveillance [21]. As demonstrated by literary sources, the state of local humoral immunity significantly differs from the norm in etiologically-close to BV non-specific inflammatory diseases of the cervix and vaginal mucosa. Herein, a comparative analysis of the content of immunoglobulins of classes G, A, and M revealed an increase in the concentration of IgG and IgA in cervical secretions as well as the appearance of IgM with simultaneous depression of the synthesis of secretory slgA, in contrast to the findings in healthy patients, indicating a violation of the mechanisms of synthesis of the secretory component by epithelial cells [21, 23, 26]. BV is most often caused by depression of local immunity and inhibition of the activity of local immune defense factors, primarily by reducing the production of secretory immunoglobulin A (slgA), whose main functional property is to protect the mucous membranes from microbial aggression [21]. Also, it was shown that the level of IgA in the peripheral blood does not differ from normocenosis in cases of dysbiosis. The level of IgM, unlike IgA, increased as the severity of dysbiosis increased and significantly exceeded the indicators in normocenosis. The level of IgG in the blood increased to a small extent with an increase in the degree of dysbiosis by 1.2—1.3 times [1, 13].

In patients undergoing examination before treatment, we also observed an increase in the level of IgM in serum, which may be due to the growth of anaerobic flora since IgM is known to be bound to endotoxins of gram-negative bacteria (lipopolysaccharides) [19]. On the other hand, the concentration of serum IgG decreased, which may reflect their susceptibility to the development of immunodeficiency due to secondary activation of the humoral immune response in the context of BV. These changes can be interpreted as a compensatory reaction of the immune system to the disruption of the vaginal microbiota. A decrease in IgG content, which is the most resistant to the action of proteolytic enzymes and strongly bound to antigens, is often accompanied by a decrease in IgA [18], as was found in our studies. Thus, we found a decrease in the concentration of serum IgA in patients in
group 2 compared to controls, but its level remained normal in the case of BV. Our data have shown that the probiotic «Dialak» has an immunomodulatory effect aimed at normalizing the indicators of the humoral immune response. After treatment, we observed an increase in the levels of IgG and IgA, while the concentration of IgM decreased simultaneously.

**Conclusions.** High therapeutic efficacy of the probiotic «Dialak» in the form of vaginal suppositories and capsules for intermediate-type BV and vaginal dysbiosis has been established, as evidenced by the gradual disappearance of clinical symptoms of the disease such as discharge from the vagina of white, gray, or yellowish-green color with a «fishy» odor, itching, discomfort in the genital area, and burning. The restoration of vaginal microbiota was confirmed by an increase in the number of *Lactobacillus* spp. and *Bifidobacterium* spp. as well as a decrease in the number of obligate anaerobic microorganisms, including *G. vaginalis*. After using the probiotic «Dialak», normalization of humoral immunity indicators was observed: the level of serum IgA and IgG increased while the level of IgM in serum decreased due to proper treatment.

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ВПЛИВ ПРОБІОТИКОТЕРАПІЇ НА МІКРОБІОТУ ПІХВИ ТА ГУМОРАЛЬНУ ЛАНКУ ІМУНІТЕТУ ПРИ БАКТЕРІАЛЬНОМУ ВАГІНОЗІ

Інфекційні захворювання піхви, на частку яких припадає понад половини гінекологічних патологій, тісно пов’язані з дисбіотичними порушеннями в мікробіомі вагіни, що проявляються зниженням або повною відсутністю Lactobacillus spp. та різким збільшенням кількості анаеробних мікроорганізмів, що призводить до розвитку одного з типів інфекцій — бактеріального вагінозу (ВВ). Метою нашої роботи було визначити вплив пробіотичного препарату «Діалак» (дієтична добавка), до складу якого входить штам Lactobacillus casei IMB В-7280, на мікробіоту піхви та гуморальну ланку імунітету у жінок з ВВ. Методи. Обстежено 40 хворих жінок віком 20—45 років з порушенням мікробіоценозу піхви та 10 клінічно здорових осіб того ж віку. Верифікація 3 типів стану біоценозу піхви: нормоценоз, проміжний тип та дисбіоз здійснювалась на підставі рекомендацій з лікування інфекцій, що передаються статевим шляхом (Weekly Morbidity and Mortality Report (2021). Враховували також загальновідомі критерії ВВ, запропоновані R. Amsel (1983). Хворі жінки з проміжним типом ВВ отримували супозиторії та капсульну форму пробіотика (один раз
на добу) протягом 10 діб. Жінки з дисбіозом піхви на першому етапі отримували метронідазол у кількості 500 мг 2 рази на добу протягом 7 діб, а на другому — 1 супозиторій на ніч та перорально капсульну форму пробіотика протягом 10 днів. Досліджуваний вагінальний секрет фарбували за методом Грама в модифікації Kopeloff, а також висівали на живильні середовища для визначення факультативно-анаеробних і об- лігатно-анаеробних мікроорганізмів. Ідентифікацію мікроорганізмів проводили на основі морфологічних, культуральних, біохімічних та антигеннихластостей згідно класифікації Д.Х. Берджі (2009). Активність гуморальної ланки імунітету визначали, оцінюючи кількість В лімфоциті у периферійні крої ніхор за допомогою проточної цитофлюорометрії, а також за вмістом сироваткових IgA, IgM та IgG за допомогою імунотурбідиметричного методу та тест системи Cobas 6000 (Roche Diagnostics, Швейцарія).

**Результати.** Встановлено, що в піхві жінок із БВ суттєво зменшувалась кількість Lactobacillus spp. та Bifidobacterium spp. порівняно з клінічно здоровими жінками (контрольна група). Водночас у піхві хворих жінок обох груп порівняння підвищувалась кількість анаеробних мікроорганізмів: Gardnerella vaginalis, Fusobacterium spp., Veillonella spp., Mobiluncus spp., Peptococcus spp., Bacteroides на тлі появи у великій кількості піхвових виділень (із високим рН), а також свербіжу, печії та дискомфорту в області геніталій. Кількість Lactobacillus spp. при дисбіозі піхви була нижчою, ніж при проміжному типі БВ. У хворих із дисбіозом піхви кількість анаеробних мікроорганізмів була вищою, ніж у хворих з проміжним типом БВ, за винятком Eubacterium spp. Амінотест був позитивним у значно меншої кількості випадків серед хворих жінок із проміжним типом БВ, ніж серед хворих жінок із дисбіозом піхви. Про порушення показників гуморальної ланки імунітету у хворих жінок обох груп порівняння свідчило підвищення рівня IgM у сироватці крові, що може бути пов’язано з розвитком анаеробної мікробіоти, а також зниження концентрації сироваткових IgA і IgG (при дисбіозі піхви) та IgG порівняно з показниками в контролі. Кількість В лімфоцитів (CD 19+ клітин) у периферійні крові хворих з проміжним типом БВ і дисбіозом піхви зберігалась на рівні контролю. Після лікування хворих жінок обох груп порівняння кількість Lactobacillus spp. та Bifidobacterium spp. у піхві збільшувалась порівняно з показниками до лікування. Також спостерігали нормалізацію в піхві кількості анаеробних мікроорганізмів, крім Veillonella spp., динамічне зниження клінічних ознак захворювання та зниження рН піхвових виділень (у жінок із дисбіозом піхви). Концентрація сироваткового IgM після лікування хворих жінок із проміжним типом БВ та дисбіозом піхви зменшувалась, а IgA — підвищувалась (при проміжному типі БВ) порівняно з показниками до лікування. Рівень сироваткового IgG підвищувався у хворих обох груп порівняння, але у хворих із проміжним типом БВ він залишався нижчим, ніж у контролі.

**Висновки.** Під впливом пробіотика «Діалак» у хворих із БВ нормалізувалась піхвова мікробіота, що підтверджувалось підвищенням кількості Lactobacillus spp., Bifidobacterium spp. та зниженням кількості анаеробних мікроорганізмів, у тому числі G. vaginalis, на тлі динамічного зниження клінічних ознак захворювання, а також відновлення до рівня норми показників гуморальної ланки імунітету. Отримані дані свідчать про ефективну лікувальну дію пробіотика «Діалак» при БВ.

**Ключові слова:** бактеріальний вагіноз, факультативні анаероби, гуморальний імунітет.