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ASSOCIATION BETWEEN VITAMIN D DEFICIENCY AND BACTERIAL VAGINOSIS AND VAGINITIS IN WOMEN

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Normal vaginal microbiocenosis is essential for maintaining reproductive health, while its disruption may contribute to bacterial vaginosis and nonspecific vaginitis. The study included 132 women of reproductive age, including healthy participants and patients with bacterial vaginosis or nonspecific vaginitis, with normal or reduced vitamin D concentrations. Vitamin D deficiency was detected in 58.3 % of the examined women, 41.7 % had normal serum vitamin D concentrations. The findings demonstrated that vitamin D deficiency was associated with more pronounced disturbances of the vaginal microbiocenosis and local immune response. Women with vitamin D deficiency showed greater changes in immunoglobulin levels and pro-inflammatory cytokine concentrations than patients with normal vitamin D levels, emphasizing the potential role of vitamin D deficiency in the pathogenesis of bacterial vaginosis and nonspecific vaginitis. Comparative assessment of diagnostic approaches indicated that molecular detection methods provide greater diagnostic value for microbiome-associated vaginal disorders than conventional microscopy-based criteria.

Key words: vaginal microbiocenosis, vitamin D deficiency, bacterial vaginosis, nonspecific vaginitis, local immunity, reproductive health.

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ЗВ'ЯЗОК МІЖ ДЕФІЦИТОМ ВІТАМІНУ D ТА БАКТЕРІАЛЬНИМ ВАГІНОЗОМ І ВАГІНІТОМ У ЖІНОК

Нормальний вагінальний мікробіоценоз має важливе значення для збереження репродуктивного здоров'я, тоді як його порушення може сприяти розвитку бактеріального вагінозу та неспецифічного вагініту. До дослідження було включено 132 жінки репродуктивного віку, серед яких були здорові учасниці, а також пацієнтки з бактеріальним вагінозом або неспецифічним вагінітом із нормальною або зниженою концентрацією вітаміну D. Дефіцит вітаміну D було виявлено у 58,3 % обстежених жінок, тоді як у 41,7 % концентрація вітаміну D у сироватці крові перебувала в межах норми. Отримані результати показали, що дефіцит вітаміну D асоціювався з більш вираженими порушеннями вагінального мікробіоценозу та місцевої імунної відповіді. У жінок із дефіцитом вітаміну D спостерігалися більш значні зміни рівнів імуноглобулінів та концентрації прозапальних цитокінів порівняно з пацієнтками з нормальним рівнем вітаміну D, що підкреслює потенційну роль дефіциту вітаміну D у патогенезі бактеріального вагінозу та неспецифічного вагініту. Порівняльна оцінка діагностичних підходів показала, що молекулярні методи мають вищу діагностичну цінність при захворюваннях піхви, пов'язаних із порушенням мікробіома, порівняно з традиційними мікроскопічними критеріями.

Ключові слова: вагінальний мікробіоценоз, дефіцит вітаміну D, бактеріальний вагіноз, неспецифічний вагініт, місцевий імунітет, репродуктивне здоров'я.

Infectious and inflammatory diseases of the female genital tract associated with opportunistic microorganisms remain an important problem in gynecological and obstetric practice. Bacterial vaginosis (BV) is characterized by disruption of the normal vaginal microbiota, reduction in protective lactobacilli, and proliferation of anaerobic and facultatively anaerobic microorganisms [1, 7, 13]. Nonspecific and aerobic vaginitis are also associated with alterations in vaginal microbiota; however, unlike BV, they are usually accompanied by a more pronounced inflammatory response [5, 6].

Disruption of the vaginal microbiocenosis is clinically important because it has been associated with adverse reproductive and obstetric outcomes. Vaginal dysbiosis, bacterial vaginosis, and aerobic vaginitis have been linked to an increased risk of pregnancy complications and adverse pregnancy outcomes [6]. In addition, bacterial vaginosis represents a substantial global health burden and is associated with considerable clinical and economic consequences [12]. Among women with infertility, bacterial vaginosis has also been associated with adverse reproductive outcomes [16].

The polymicrobial nature of BV complicates its laboratory diagnosis. Clinical and microscopic approaches, particularly the Amsel criteria and Nugent scoring system, remain widely used; however, their diagnostic performance may differ depending on the composition of the vaginal microbiota and the characteristics of the examined population [2]. Modern studies emphasize that BV cannot be explained by the presence of a single microorganism, including *Gardnerella vaginalis*, and should instead be considered a complex polymicrobial dysbiosis involving multiple bacterial taxa [1, 13]. Therefore, molecular-genetic methods may provide additional diagnostic value in cases with inconclusive clinical or microscopic findings.

Another important issue is the possible relationship between vitamin D status and vaginal microbiota disorders. Vitamin D deficiency and insufficiency are common among women of reproductive age [3, 4]. Several studies have investigated the association between serum vitamin D concentration and bacterial vaginosis, although their findings are not completely uniform. A meta-analysis of observational studies showed that vitamin D

deficiency was associated with an increased risk of bacterial vaginosis during pregnancy, while case-control and population-based studies have also examined the relationship between serum 25-hydroxyvitamin D levels and bacterial vaginosis [8–10, 11, 14].

The potential biological basis of this association may be related to the immunomodulatory properties of vitamin D. Vitamin D is involved in the regulation of innate and adaptive immune responses and may influence the production and activity of inflammatory mediators [15, 17]. Consequently, vitamin D deficiency may contribute to changes in local immune defense and potentially facilitate disturbances of the vaginal microbiocenosis. Considering the possible interaction between vitamin D status, vaginal dysbiosis, and local immune responses, evaluation of immunological markers in women with bacterial vaginosis and nonspecific vaginitis may provide additional insight into the pathogenesis of these conditions [9, 10, 17].

The purpose of the study was to determine the importance of vitamin D deficiency in the pathogenesis of BV and NSV, to study the etiopathogenesis of these diseases, and to apply the principles of modern approaches in their diagnosis.

Materials and methods. The study was conducted from January 2022 to December 2024 and included 132 women of reproductive age examined at the Educational-Surgical Clinic of Azerbaijan Medical University (AMU), Maternity Hospital No. 5 named after Sh. Alasgarova, and the “Referans” clinic.

132 women of reproductive age were examined at the Educational-Surgical Clinic of AMU, Maternity Hospital No. 5 named after Sh. Alasgarova, and the “Referans” clinic, and examination material was taken from them. Of the examined women, 26 were healthy, 54 were patients with BV (vitamin D concentration was normal in 26 women, low in 28), and 52 were patients with NSV (vitamin D concentration was normal in 25 women, low in 27). Examination of the taken pathological materials was carried out in the laboratory of the Department of Medical Microbiology and Immunology of AMU, in the clinical-biochemical laboratory, in the Teaching Surgery Clinic of AMU, and in the laboratory of the “Referans” clinic.

The research was conducted in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association and generally accepted international principles for biomedical research involving human participants. The study did not involve experimental therapeutic interventions, and the clinical and laboratory examinations were performed within the scope of the diagnostic procedures required for the assessment of the participants. All participants were informed about the purpose and procedures of the study, the confidentiality of the information obtained, and the

voluntary nature of participation. Written informed consent for participation in the study and the use of biological materials and clinical data for scientific purposes was obtained from each participant prior to inclusion in the study. Personal data were anonymized and used exclusively for research purposes.

The study material was blood serum, smears from the vagina, cervix and urethra, cervicovaginal washings. The study used complex microbiological, immunological, biochemical, molecular-genetic methods.

The patients were examined with a gynecological mirror for the condition of the mucous membrane of the walls of the vagina, and examination material was taken from the cervical canal, the middle 1/3 of the vagina and the urethra with a sterile swab. A pH meter (“HANNA”, Germany) was used to measure vaginal pH, and 1–2 drops of 10 % KOH solution were used for the Amine test. The taken smears were stained by the Gram method and subjected to microscopy, revealing vaginal epithelial cells, leukocytes, “key cells”, Dederlein rods, pathogenic bacteria, and pseudomycelia of *Candida* fungi. The examination materials were evaluated according to the Amsel and Nugent criteria.

To obtain a pure culture of bacteria, materials from the posterior arch of the uterus were taken with a sterile cotton swab and cultured in a carrier nutrient medium (BioMerioux), and then meat-peptone agar, blood agar and egg yolk-salt agar, *Enterococcus* agar (Serva), Endo medium, *Gardnerella vaginalis* agar, Saburo medium were used. Gas-generating packages were used in an anaerostat to create anaerobic conditions. Berge nomenclature and EUKAST standards were used to identify the obtained pure cultures.

Although microscopic and bacteriological examination methods are widely used in clinical practice in the diagnosis of inflammatory diseases of the uterus, sometimes it is difficult to identify pathogenic microbes that play a major role in the etiology of these diseases. Therefore, during the study, Real-Time PCR (RTq-PCR) was used in cases of suspected diagnosis of BV. For this purpose, for the first time in Azerbaijan, the “Allplex TM” BV test panel was developed at the “Referans” Clinical Laboratory Center. The panel includes the main bacteria that play a role in the etiology of BV, such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera type-1*, *Mobiluncus spp.*, *Bacteroides fragilis*.

To study the effect of vitamin D (Bioactiva Diagnostic, Germany) on the pathogenesis and immunological aspects of BV and NSV, enzyme-linked immunosorbent assay (ELISA) was used on cervico-vaginal washes and blood serum (total immunoglobulin concentration; cervico-vaginal TNF- α , IL-1- β , IL-6 cytokine levels).

Results of the study. Of the examined women, 26 were practically healthy, 54 were BV, and 52 were

diagnosed with NSV. Microscopic examination of the vaginal mucosa of practically healthy women revealed no pathological conditions, the Amin test (–) was negative, and the vaginal pH was 3.8–4.5. The Nugent score was evaluated as 0–3 points (normal) in 19 examination materials, and 4–6 points (moderate form) in 7. There were no signs of inflammation in the vagina in women with BV, whitish-gray foul-smelling discharge was observed, the Amine test was negative in 8 patients, and positive (pH 5.0–7.0) in 46. During microscopy, leukocytes, epithelium were detected in small quantities (up to 10), “key cells” and *G. vaginalis* (in most smears), and lactobacteria were detected very little or not at all. According to the Amsel criteria, the diagnosis was assessed as BV in 36 patients. The Nugent score was assessed as 7–10 points (BV) in 34 examination materials, 4–6 points (intermediate condition) in 17, and 0–3 points (normal) in 3.

Thus, according to the joint analysis of the results of both the Amsel and Nugent criteria, the diagnosis of BV was confirmed in 29 women, and a suspicious diagnosis was found in 25 women.

Signs of inflammation – redness, edema and pathological discharge – were detected in the mucous membrane of the vagina of women with

NSV, the Amine test (–) was negative, and the vaginal pH was above 4.5. In the examination materials of patients with vaginitis, epithelium, leukocytes were high (30–60), and lactobacteria were detected in small quantities or not at all. In the evaluation of vaginal microocenosis according to Nugent criteria, the examination material of 1 person was evaluated with a score of 0–3 (normal), 11 with a score of 4–6 (intermediate microflora), and 40 with a score of 7–10. In total, 106 (80.3 %) of the examined women had a confirmed diagnosis, of which 25 were practically healthy, 38 were patients with bacterial vaginosis, and 43 were patients with nonspecific vaginitis. According to the Nugent criteria, it was possible to obtain information about the microocenosis of the vagina in 93 (70.5 %) of whom 19 were practically healthy, 34 were patients with bacterial vaginosis, and 40 were patients with nonspecific vaginitis.

Thus, the results of the assessment of patients with BV according to both the Amsel and Nugent criteria were compared, and in some examination materials, different results were obtained according to both diagnostic criteria. The results are shown in Table 1.

Table 1

Comparison of diagnostic results using the Nugent and Amsel criteria among patients with bacterial vaginosis

Parameter	Amsel criteria	Nugent criteria
Diagnostic results		
Bacterial vaginosis (+), sign (+)	38	34
Bacterial vaginosis (–), sign (+)	1	7
S, sign (+)	39	41
Bacterial vaginosis (+), sign (–)	16	20
Bacterial vaginosis (–), sign (–)	25	19
S, sign (–)	41	39
Bacterial vaginosis (+), S	54	54
Bacterial vaginosis (–), S	26	26
Total, S	80	80
Agreement indicators		
po	0.788	0.663
Pe	0.496	0.504
K	0.579	0.319
Sk	0.091	0.107
T	6.3817	2.991
P	<0.001	0.004
Diagnostic performance indicators		
Se, %	70.4±6.2	63.0±6.6
Sp, %	96.2±3.8	73.1±8.7
PPV, %	97.4±2.5	82.9±5.9
NPV, %	61.0±7.6	48.7±8.0
LR+	18.30 – Excellent	2.34 – Average
LR–	0.31 – Average	0.51 – Not useful
GDV, %	78.8±4.6	66.3±5.3

Note: Statistical agreement was assessed using Cohen's Kappa criterion. Diagnostic performance was evaluated using sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratios (LR+ and LR–), and gross diagnostic value (GDV). A *p* value <0.05 was considered statistically significant.

The calculation is performed according to standard formulas for assessing sensitivity (Se), specificity (Sp), positive and negative prognostic value (PPV, NPV), etc. As can be seen from the table, Amsel's criteria are characterized by significantly higher specificity (Sp=96.2 %), sensitivity (Se=70.4 %), positive prognostic value (pPV=97.4 %) and negative prognostic value (nPV=61.0) compared to Nugent's criteria (Sp=73.1 %, Se=63.0 %, pPV=82.9 % and nPV=48.7), respectively. Since the sensitivity of both methods is relatively low (Amsel criteria–70.4 %, Nugent criteria–63.0 %), negative results do not completely exclude the disease. The even lower negative predictive value of the Nugent criteria (48.7 %) indicates that they are not a reliable

exclusion tool. Thus, although examination using the Amsel and Nugent criteria is useful, other laboratory examinations are needed to detect relevant pathologies.

In addition to microscopic analysis, a culture study was conducted, during which microorganisms were identified and quantified. Pure cultures were isolated from the examined culture materials, and the identified microorganisms were identified using generally accepted microbiological methods, guided by C. Berger's nomenclature and EUCAST standards.

In all materials taken from practically healthy women, *Lactobacillus* spp. were found in large quantities, 10^7 – 10^9 CFU/ml. *G. vaginalis* was detected in 4 patients, in insignificant concentrations (Table 2).

Table 2

Vaginal microbiota of healthy women and patients with bacterial vaginosis

Microorganisms	Reference interval	Control group (26)	Bacterial vaginosis (54)		Non-specific vaginitis (52)	
	Q (CFU/ml)	Detection (Q/%)	Detection (Q/%)	χ^2/p	Detection (Q/%)	χ^2/p
<i>Lactobacillus</i> spp.	$\geq 10^7$	26/100	-	75.507/<0.001*	-	73.565/<0.001 *
<i>Bifidobacterium</i> spp.	$\leq 10^4$	3/11.5	15/27.8	1.805/0.179	5/9.6	0.017/0.895
<i>G. vaginalis</i>	$\leq 10^4$	1/3.8	40/74.0	31.890/<0.001*	6/11.5	0.490/0.484
<i>Prevotella</i> spp.	$< 10^4$	-	9/16.7	3.356/0.067	11/21.2	4.776/0.029*
<i>Porphyromonas</i> spp.	$\leq 10^3$	-	-	-	3/5.8	0.390/0.532
<i>Bacteroides frag.</i>	$\leq 10^3$	-	20/37	10.940/0.01*	10/19.2	4.144/0.042*
<i>Fusobacterium</i> spp.	$\leq 10^3$	-	2/3.7	0.053/0.819	3/5.8	0.390/0.532
<i>Veillonella</i> spp.	$\leq 10^3$	-	3/5.6	0.356/0.551	5/9.6	1.309/0.253
<i>Cutibacterium</i> spp.	$\leq 10^4$	9/34.6	17/31.5	0.079/0.779	7/13.5	4.757/0.029*
<i>Staphylococcus</i> spp.	$\leq 10^3$	4/15.4	14/25.9	1.118/0.290	33/63.5	16.068/<0.001*
<i>Streptococcus</i> spp.	$\leq 10^3$	5/19.2	5/9.3	1.595/0.207	11/21.2	0.039/0.843
<i>Enterococcus</i> spp.	$\leq 10^3$	2/7.7	6/11.1	0.006/0.937	10/19.2	0.997/0.318
<i>Enterobacteriaceae</i> spp.	$\leq 10^3$	2/7.7	15/27.8	3.116/0.078	17/32.7	4.601/0.032*
<i>Candida</i> spp.	$\leq 10^3$	4/15.4	8/14.8	0.004/0.947	19/36.5	3.730 /0.053
Total		56	154		140	

Note: * - $p < 0.001$ corresponds to a high degree of significance of the results. Q-Quantity.

Thus, compared with other examination groups and normal values (reference interval), in patients with BV, *Lactobacillus* spp. were isolated at insignificant concentrations in very few examination materials or were not detected at all, while *G. vaginalis* and *Bacteroides frag.* were detected in large concentrations in 74.0 % and 37.0 % of patients, respectively ($P < 0.05$). In patients with NSV, facultative anaerobic bacteria were detected in large quantities (10^5 – 10^8 CFU/ml) and lactobacilli (normal $\geq 10^7$ CFU/ml) in small quantities and in insignificant concentrations. The most frequently detected bacteria in patients with NSV were *Staphylococcus* spp. (63.5 %), *Enterobacteriaceae* spp. (32.7 %), *Prevotella* spp. (21.2 %) and others ($P < 0.05$). The detection of *Candida* spp. $\leq 10^3$ CFU/ml in the examined women, the absence of pseudomycetes and

clinical signs denied the diagnosis of vulvovaginal candidiasis.

In the next stage, using the Allplex™ Bacterial Vaginosis Test panel, qualitative and quantitative analysis of the main etiological agents of BV and *Lactobacillus* spp. in vaginal secretions was performed using RT qPCR, and the diagnosis of BV was denied in 5 out of 30 examination materials, and confirmed in 25.

In accordance with the purpose of the study, the concentration of vitamin D in the blood was determined at a later stage.

In the control group, the average value the concentration of vitamin D was 38.8 ± 0.04 ng/ml. In 26 patients with BV and 25 patients with NSV, the level of 25-OH vitamin D was 37.2 ± 0.3 ng/ml and 38.1 ± 0.3 ng/ml, respectively, which did not differ significantly from the indicators in the control group.

On the contrary, in 28 women with BV and 27 patients with NSV, a significant ($P<0.05$) decrease in the concentration of this vitamin was observed, which was associated with vitamin D deficiency or deficiency. Thus, it was at the level of 20.7 ± 1.0 ng/ml and 19.4 ± 1.1 ng/ml, respectively.

To determine the effect of vitamin D deficiency on the pathogenetic properties of BV and NSV, all examined patients were divided into five groups depending on the level of vitamin D in their body. Group I consisted of 26 practically healthy women with normal vitamin D. Among patients with BV, two groups were distinguished depending on the content of vitamin D: 26 women with normal vitamin D levels (group II) and 28 with a vitamin D concentration below 30 ng/ml (group III). Among patients with NSV, two groups were also distinguished. So, one of them consisted of 25 women with a normal amount of vitamin D in their body (group IV), and the other – 26 women with vitamin D deficiency or deficiency (group V).

We investigated the dependence of the accumulation of different classes of immunoglobulins in the blood and vaginal secretion in patients with BV and NSV on the amount of vitamin D in the body. First, the concentration of the main classes of immunoglobulins in cervico-vaginal washings was determined.

In group I, the amount of SIgA was 63.2 ± 0.6 mg/l. In all patients with BV, its concentration was below the physiological norm. In patients from group II, who had normal serum vitamin D, the concentration of this immunoglobulin (52.5 ± 0.2 mg/l) was lower than in the control group. When the amount of vitamin D in BV patients decreased, the level of SIgA decreased slightly, and in patients in group III it was 47.5 ± 0.3 mg/ml. The level of SIgA in cervicovaginal washings of patients with NSV was lower than in both the control group I and the corresponding groups of patients with BV. Thus, in patients in group IV, it was observed at concentrations of 42.2 ± 0.4 mg/l, and in patients in group V – 34.2 ± 0.4 mg/l ($P<0.001$). IgM was almost not detected in cervicovaginal washes of patients with group I and BV. Among patients with NSV, the level of this immunoglobulin was 5.0 ± 0.1 mg/l in group IV, and 7.0 ± 0.1 mg/l in group V ($P<0.001$, $P>0.05$). The concentration of IgG in group I was 151.4 ± 0.5 mg/l. In patients with BV in groups II and III, the concentration of this immunoglobulin was 152.1 ± 0.6 mg/l and 159.4 ± 0.7 mg/l, respectively. The highest level of IgG among patients was in NSV patients, especially in those with vitamin D deficiency: 189.4 ± 0.7 mg/l in group IV, 248.5 ± 0.5 mg/l in group V ($P<0.001$). The level of monomer IgA was 214.3 ± 0.6 mg/l in group I, and 209.9 ± 0.5 mg/l and 184.9 ± 0.9 mg/l in patients with BV (in groups II and III). It was found at a level of 163.2 ± 1.3 mg/l in group

IV patients, and at a minimum level of 145.0 ± 1.1 mg/l in group V patients ($P<0.001$).

In the next stage, the concentration of immunoglobulins was also studied in blood serum. The concentration of IgA in the blood serum was 2.46 g/l in group I, 2.20 ± 0.01 g/l in group II, 2.24 ± 0.01 g/l in group III, 2.35 ± 0.03 g/l in group IV, and 2.47 ± 0.02 g/l in group V. IgM was almost not detected in the blood serum of healthy women (group I) and BV patients (groups II, III). Among patients with NSV, the level of this immunoglobulin was between 3.0 ± 0.1 g/l in group IV, and 3.8 ± 0.1 g/l in patients in group V. The concentration of IgG was 8.0 ± 0.07 g/l in group I, and 8.1 ± 0.07 g/l and 8.2 ± 0.08 g/l in groups II and III. This indicator reached 10.0 ± 0.12 g/l in group IV, and 10.9 ± 0.07 g/l in group V – in patients with NSV with vitamin D deficiency.

In the next step, we examined the levels of some key pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) in cervicovaginal lavage.

The concentration of TNF- α in the cervico-vaginal wash of practically healthy women was 5.1 ± 0.1 pg/ml. In patients with BV, it was 23.6 ± 0.4 pg/ml and 35.2 ± 0.5 pg/ml in patients in groups II and III, respectively. In group IV, it was detected at a level of 52.9 ± 0.7 pg/ml, in group V, 103.3 ± 0.7 pg/ml. During the study of IL-1 β concentration, it was determined that in group I it was 22.8 ± 0.3 pg/ml, in group II it was 43.0 ± 1.9 pg/ml. In group III it was 73.2 ± 1.5 pg/ml, and in group IV and V patients it was 138.2 ± 0.8 pg/ml and 151.5 ± 0.3 pg/ml, respectively. The amount of IL-6 was 10.7 ± 0.2 pg/ml in group I. The highest concentration of this cytokine was determined in group V patients with vitamin D deficiency in their body, 146.4 ± 0.7 pg/ml.

At the same time, the dependence of the concentration of cytokines we studied in cervico-vaginal secretion on the amount of vitamin D was also examined in blood serum. Compared to group I (1.2 ± 0.08 pg/ml), the concentration of TNF- α in the blood serum of women with BV was 2.1 ± 0.07 pg/ml and 3.3 ± 0.09 pg/ml in groups II and III, respectively. In the blood serum of women with NSV, TNF- α was 30.7 ± 0.78 pg/ml in group IV, while in group V this indicator was 46.3 ± 1.27 pg/ml. In group I, IL-1 β was observed at the lowest concentration – 1.7 ± 0.06 pg/ml. In group IV, it was observed at the highest concentration, 7.2 ± 0.9 pg/ml, and in group V, 55.1 ± 0.89 pg/ml. When IL-6 was examined in the blood serum, the same pattern was observed. Thus, the highest level was found in patients NSV with vitamin D deficiency, group V, with 58.9 ± 1.03 pg/ml ($P<0.001$).

The aim of the study was to examine the relationship between the RT q-PCR – Allplex TM test panel for BV and serum vitamin D levels. Of the 25 patients with BV who used RT q-PCR, 9 had normal vitamin D levels (≥ 30 ng/ml), while 16 had vitamin D deficiency (≤ 30 ng/ml).

Of the 25 samples with a confirmed diagnosis of BV, 7 had monoinfections as the etiologic agent, and 18 had associations of two or three microorganisms. Among patients with normal serum vitamin D concentrations, *G. vaginalis* was detected in 6 patients, *B. fragilis* monoinfection was detected in 1 patient, and other pathogens were detected in association with *G. vaginalis* in 18 patients (Fig. 1).

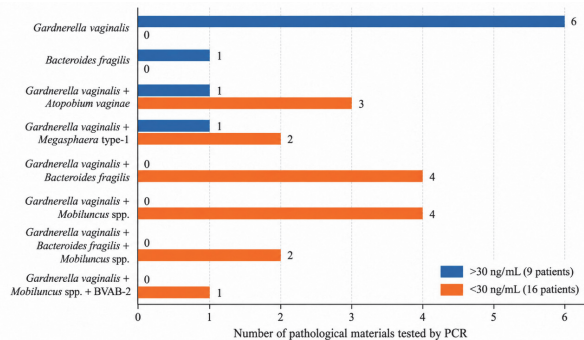


Fig. 1. The effect of vitamin D levels on the Allplex™ RTq-PCR test panel results. Note: $P < 0.001$ ($\chi^2 = 17.28$ df=1, $P < 0.001$; Yates=13.19; Fisher $P = 0.0002$) is statistically significant.

Associations were more common in patients with vitamin D deficiency (<30 ng/ml).

Discussion. The present study demonstrated that the use of a combination of microbiological, molecular-genetic, and immunological approaches improves the detection of vaginal microbiocenosis disorders in women with bacterial vaginosis and nonspecific vaginitis. Among 39 examination materials with a questionable diagnosis after microscopic assessment, culture examination additionally confirmed the corresponding diagnosis in one practically healthy woman, three patients with bacterial vaginosis, and five patients with nonspecific vaginitis. Overall, culture-based examination enabled the diagnosis of 54 % of bacterial vaginosis cases and 100% of nonspecific vaginitis cases. These findings support previous observations that bacterial vaginosis is a polymicrobial condition characterized by substantial alterations in vaginal microbiota and that conventional diagnostic approaches may not fully reflect the complexity of vaginal dysbiosis [1, 6, 7, 13].

Although the Amsel and Nugent criteria remain widely used, discrepancies between clinical and microscopic findings may lead to false-positive or false-negative results. Similar limitations and differences in diagnostic performance between the Amsel criteria and Nugent scoring have been reported by Bhujel R. et al. (2021) [2]. In the present study, real-time quantitative polymerase chain reaction was therefore used in 30 women with uncertain findings, confirming bacterial vaginosis in 25 cases (83.3 %) and excluding it in 5 cases (16.7 %). This supports the usefulness of molecular methods for identifying the polymicrobial patterns characteristic of bacterial vaginosis, particularly when routine diagnostic findings are inconclusive [1, 13].

Vitamin D deficiency was detected in 58.3 % of the examined women, whereas 41.7 % had normal serum vitamin D concentrations. The observed association between reduced vitamin D levels and vaginal microbiocenosis disturbances is consistent with published evidence indicating that vitamin D deficiency may increase the risk of bacterial vaginosis and with case-control data examining the relationship between serum 25-hydroxyvitamin D and bacterial vaginosis [9, 10]. The importance of vitamin D status in bacterial vaginosis has also been evaluated in pregnancy and population-based studies, while vitamin D insufficiency remains common among women of reproductive age [3, 14, 15].

Changes in local immunity were most pronounced in patients with vitamin D deficiency, particularly in women with nonspecific vaginitis. These alterations included changes in immunoglobulin concentrations and increased pro-inflammatory cytokine activity. This finding is biologically plausible because vitamin D has well-established immunomodulatory properties and participates in the regulation of innate and adaptive immune responses, which was noted in the work of Zughaier SM et al. (2020) [17]. Thus, vitamin D deficiency may contribute not only to vaginal microbiota imbalance but also to enhanced local inflammatory responses, potentially aggravating the course of bacterial vaginosis and nonspecific vaginitis.

Conclusions

1. There are cases where the results of BV diagnostics using the Amsel and Nugent criteria do not coincide. The Amsel criteria are more suitable for diagnosing bacterial vaginosis in clinical practice. The Nugent criteria reflect a laboratory approach but are inferior to the Amsel criteria in sensitivity and specificity.
2. Despite the importance of each diagnostic method, in vaginal diseases accompanied by a disruption of the microbiome, such as BV, only qualitative and quantitative determination of the dominant microorganisms provides a more accurate diagnosis.
3. The most effective method for diagnosing BV is RTq-PCR.
4. Changes in local immune status, characterized by changes in the concentration of immunoglobulins and pro-inflammatory cytokines, have been found in patients with BV and NSV.
5. The observed changes in immune status were more common in patients with the corresponding pathologies, including vitamin D deficiency.

Further studies should focus on larger prospective cohorts to clarify the causal relationship between vitamin D deficiency, vaginal microbiocenosis disturbances, and local immune responses, as well as to evaluate whether correction of vitamin D deficiency can improve the prevention and treatment of bacterial vaginosis and nonspecific vaginitis.

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