UDC 618+616:579.61+616.15+615.38

ROLE OF MICROORGANISMS AND THEIR METABOLITES IN THE MECHANISM OF PROTECTION OF THE VAGINAL ECOSYSTEM

Rakhmatullaeva M.M., Sharipova Sh.U.
Bukhara State Medical Institute

✓ ***Resume***

The review article presents literature data on the role of lactobacilli and their metabolites in maintaining a healthy state of the vaginal ecosystem. Changes in metabolites in the reproductive tract are not only due to the dominance of a particular microflora in vaginal dysbiosis, but are also interrelated with changes in the immune system.

The study of the interaction between microorganisms, their metabolites and the immune system in the reproductive tract will make it possible to determine the pathogenetic mechanisms of the development of bacterial vaginosis, which will lay the foundation for further research on early diagnosis and effective treatment of the disease.

Key words: lactobacilli, metabolites, vaginal microbiocenosis, Lactobacillus crispatus, Lactobacillus iners

РОЛЬ МИКРООРГАНИЗМОВ И ИХ МЕТАБОЛИТОВ В МЕХАНИЗМЕ ЗАЩИТЫ ВАГИНАЛЬНОЙ ЭКОСИСТЕМЫ

Рахматуллаева М.М., Шарипова Ш.У.
Бухарский государственный медицинский институт

✓ ***Резюме***

В обзорной статье представлены данные литературы относительно роли лактобактерий и их метаболитов в поддержании здорового состояния вагинальной экосистемы. Изменения метаболитов в репродуктивном тракте не только обусловлены с доминированием той или иной микрофлоры при дисбиозе влагалища, но и взаимосвязаны с изменениями иммунной системы.

Изучение взаимодействия между микроорганизмами, их метаболитами и иммунной системой в репродуктивном тракте позволит определить патогенетические механизмы развития бактериального вагиноза, что положит основу для дальнейших исследований по ранней диагностике и эффективному лечению заболевания.

Ключевые слова: лактобактерии, метаболиты, микробиоценоз влагалища, Lactobacillus crispatus, Lactobacillus iners

МИКРООРГАНИЗМЛАР ВА МЕТАБОЛИТЛАРИНИНГ ВАГИНАЛ ЭКОСИСТЕМА ҲИМОЯ МЕХАНИЗМИДАГИ ЎРНИ

Раҳматуллаева М.М., Шарипова Ш.У.

Бухоро давлат тиббиёт институти

✓ ***Резюме***

Ушбу шарҳда вагинал экосистемани соғлом ҳолатда сақлашда лактобактериялар ва уларнинг метаболитларининг ўрни тўғрисида адабий маълумотлар келтирилган. Репродуктив трактда метаболитларнинг ўзгариши қин дисбиозида у ёки бу микрофлоранинг устунлиги билангина боғлиқ бўлмай, балки иммун система ўзгаришлари билан ҳам ўзаро боғлиқдир.

Репродуктив трактда микроорганизмлар, уларнинг метаболитлари ва иммун системанинг ўзаро боғлиқлигини ўрганиш бактериал вагиноз патогенетик механизмларини аниқлашга имкон беради, бу эса касалликни эрта аниқлаш ва самарали даволаш бўйича кейинги тадқиқотларга замин яратади.

Калит сўзлар: лактобактериялар; метаболитлар; қин микробиоценози; Lactobacillus crispatus; Lactobacillus iners

Relevance

The vaginal ecosystem includes microorganisms, metabolites and immune components, and the balance of interactions between them plays an important role in maintaining homeostasis and the health of the female reproductive tract [17].

The total bacterial mass (microbial contamination) in the vaginal mucosa in clinically healthy women of reproductive age ranges from 106 to 108.5 GE / ml. The proportion of lactobacilli in the microbiocenosis ranges from 90–99% and ranges from 105.4 to 108.5 GE / ml [16]. In normal vaginal microbiocenosis, the growth of various communities of opportunistic flora should not exceed 104 GE / ml, and the total number of transient microorganisms should not exceed 15–20% of the total pool of microorganisms [2].

Bacterial vaginosis (BV) is characterized by an increase in the diversity of the vaginal microflora, a decrease in the number of Lactobacillus spp. in the vagina and an increase in the number of anaerobes and microaerophilic bacteria associated with BV [26]. BV-associated bacteria mainly include Gardnerella vaginalis, Atopobium vaginae, Mycoplasma hominis, Megasphaera spp., Mobiluncus spp., Roseburia spp., Dialister spp., Sneathia spp. and Prevotella spp. [6].

Many of the bacteria associated with bacterial vaginosis (bacterial vaginosis-associated bacteria - BVAB) are common representatives of the normal vaginal microbiota [26], however, to date, little has been studied about the synergistic or antagonistic effects between them, the metabolic characteristics of microbiocenosis with the domination of various communities of vaginal microflora [6].

Protective role of lactobacilli in the vaginal ecosystem

In contrast to the wide variety of the gastrointestinal tract, the microbiocenosis of the female genital tract is less diverse and changes dynamically during the menstrual cycle [7]. The genomes of vaginal lactobacilli are much smaller in comparison with the genomes of lactobacilli microbiocenoses of other mucous membranes. This phenomenon may contribute to the fact that the vaginal species of lactobacilli demonstrate a certain degree of adaptation to the lifestyle, depending on the macroorganism [22], which may ultimately provide the dynamism of the vaginal microbiocenosis.

Most often, the basis of normal vaginal microbiocenosis in women of reproductive age is made up of 4 species: Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus gasseri and Lactobacillus iners [16]. It has been established that each of the four types of vaginal lactobacilli is unique due to the possession of their genome by one or another family of numerous proteins. These specific changes in the form of an increase or loss of genes occurred during the evolution of vaginal lactobacilli and thus, a unique set of proteins for a particular species was developed [22].

Each woman has her own individual species composition of lactobacilli, however, stable codominance of multiple species of lactobacilli in a single microbi- cenosis is rarely observed, and as a rule, only one or two species dominate in it [4, 22].

However, several studies in clinically healthy women of different populations have revealed the presence of this type of microbiocenosis, in the structure of which there is an absence of lactobacilli or the presence of certain microorganisms, such as Gardnerella vaginalis, or various species of Peptostreptococcus, Prevotella, Pseudomonas, Streptococcus and / or Corynebacterium, which may not be a pathological condition [1, 10]. Thus, Ravel J. et al. (2011) identified five different bacterial communities - community state types (CST) in clinically healthy women [26]. CSTs are grouped as CSTs I, II, III, IV, V, respectively, with each CST being dominated by L. crispatus, L. gasseri, L. iners, polymicrobial flora, including Lactobacillus and bacteria associated with bacterial vaginosis, and L jensenii [12, 26]. While CST I, III, and IV have been extensively studied and are commonly found in women, CST II and V are rare [9]. Studies have shown that differences in the composition of the bacterial community between subjects may be related to race, diet, age, sexual behavior, immunity, and genetic polymorphism [8, 21].

Thus, the predominance of non-lactobacillary microflora is not always associated with the state of bacterial vaginosis; therefore, it can be assumed that the state of the disease or its absence is not only determined by the number of bacteria, but includes a much wider aspect involving the internal environment of the macroorganism and the microenvironment of the vagina.

Metabolites in the female reproductive tract are substrates, intermediates, and byproducts of

biochemical reactions caused by the interaction of human nutrients with bacteria, reflecting subsequent gene expression events [6].

Despite the differences in the bacterial composition of vaginal communities, the acidification of the vaginal environment occurs through the formation of metabolites of organic acids, mainly lactic acid [12, 26]. Lactobacilli form lactic acid by anaerobic glycolysis of glycogen, which is released due to the constant rejection and cytolysis of the superficial cells of the vaginal epithelium. The contents of the vagina contain L- and D-isomers of lactic acid. The L-isomer of lactic acid can be synthesized not only by lactobacilli, but also by vaginal epithelial cells, while the D-isomer is synthesized only by lactobacilli. According to Linhares IM. et al. (2011), the content of D-lactic acid in the vaginal contents is higher when lactobacilli are dominant, with the exception of L. iners [18]. This is explained by the fact that L. iners is unable to synthesize the D-isomer of lactic acid, since the genome of the microorganism lacks genes responsible for its synthesis [18, 30]. Lactic acid, which forms 110 mmol, acidifies the vaginal medium to an average pH of 3.5 [23]. In women with lactobacilli dominance, the vaginal pH is closely correlated with the concentration of lactic acid in the vaginal fluid [23].

L. crispatus and L. jensenii have similar metabolic patterns [27], while L. crispatus and L. iners have different metabolic characteristics [24]. For example, studies have shown that the L. crispatus genome is almost twice as large as the L. iners genome [10]. However, the carbon metabolism of L. iners is fermented with fewer compounds than L. crispatus [24]. When the dominant bacteria are L. crispatus and / or L. jensenii, most of the metabolites in the vagina are amino acids and dipeptides, with higher levels of ornithine being determined lysine, glycylproline, phenylalanine [27]. L. iners, like BV associated flora, correlate with amino acid catabolites such as proline, threonine, aspartate, serine, and valinyl glutamate, the level of which in the vaginal fluid is higher than in other substrates [27]. As the researchers explain, the lack of a mechanism for the synthesis of essential amino acids in L. iners forces it to rely heavily on exogenous amino acids obtained from the macroorganism [11]. Its limited metabolic profile and dependence on the nutrients of the microorganism make it highly sensitive to environmental changes [11].

Due to the structure of its genome, L. iners has the ability to quickly adapt to changing environmental conditions [19], switching its metabolism and using glycerol of the phospholipids of the membranes of vaginal epithelial cells as a nutrient substrate instead of glycogen. L. iners produces a toxin - cholesterol-dependent cytolysin, similar in properties to vagolysin Gardnerella vaginalis, and under conditions of insufficient acidity (at pH 4.5-6.0) its production is 6 times more active than at pH less than 4.5 [25].

The mucous layer on the surface of epithelial cells of the female reproductive tract plays an important role as a first line of defense against microbial invasion. Under conditions of damage to the epithelial barrier or exposure to microbes or their metabolites, epithelial cells are activated. When microorganisms break through the line of defense, epithelial cells use recognition receptors to identify microorganisms that produce inflammatory factors and recruit immune cells to resist microbial invasion and colonization [5].

Dominance of Lactobacillus spp. in the genital tract is essential because it suppresses pathogens and maintains immune balance. Studies have shown that the concentration of pro-inflammatory factors in the vagina is very low when L. crispatus and L. jensenii are dominant [3, 14].

Lactic acid as a metabolite produced mainly by Lactobacillus spp. Is also associated with the immunity of the reproductive tract [9]. L-lactic acid produced by Lactobacillus spp. can induce an anti-inflammatory response and suppress the production of proinflammatory cytokines and chemokines induced by the toll-like receptor (TLR) in the epithelial cells of the cervix and vagina at low pH [9]. In addition, lactic acid can induce the secretion of the anti-inflammatory cytokine interleukin IL-10, decrease the production of the pro-inflammatory cytokine IL-12 in dendritic cells, and reduce the cytotoxicity of natural killer cells [13].

The anti-inflammatory activity of lactic acid also requires the presence of organic acids produced by microorganisms to maintain vaginal health, mainly by increasing the production of the anti-inflammatory cytokine IL-1RA, inhibition of the pro-inflammatory signal of the cytokine IL-1, while the production of pro-inflammatory cytokines IL-6 and macrophage inflammatory protein-3α (MIP-3α) decreases slightly [9]. Therefore, the interaction between normal microflora, metabolites and the immune system in the reproductive tract is very important for maintaining a healthy vaginal ecosystem.

Metabolites of BV-Associated Bacteria

BV is closely related to metabolites in the vaginal fluid. The metabolites of amines, organic acids, short-chain fatty acids, amino acids, nitrogenous bases, and monosaccharides in women with BV differ significantly from those in women without BV [29]. In women with BV, the metabolite profile is characterized by lower concentrations of amino acids and dipeptides, accompanied by higher levels of amino acid and polyamine catabolites [26]. BVABs increase vaginal pH by creating short-chain fatty acids and amines, and are also able to use lactic acid as an energy source [19, 28].

Many BVABs are known to produce several acid metabolites; for example: Bacteroides spp. produce succinate, Peptococcus spp. - butyrate and acetate, Gardnerella vaginalis - acetate and succinate, clostridia and gram-positive cocci - caproate and Dialister spp. - propionate. Typically, acetate is the predominant metabolite in the vaginal fluid of women with BV compared to elevated concentrations of propionate, butyrate, and succinate [27].

Studies show that in BV metabolites better reflect the disease phenotype than the microorganisms themselves. Before the onset of disease symptoms, the appearance or disappearance of certain metabolites in the vagina has a positive or negative correlation with the metabolic function of certain microorganisms [28]. Changes in maltose, kynurenine, nicotinate, malonate, acetate, and nicotinamide adenine dinucleotide (NAD +) reflect the occurrence of BV and can be used as metabolic biomarkers to distinguish BV from the healthy state of the vagina [29]. When BV is healed, the metabolites associated with BV are significantly reduced [27]. In addition, metabolic analysis of secretions from the genital tract plays an important role in the diagnosis of BV. McMillan A. et al. (2015) found that an increase in the level of 2-hydroxyisovalerate and γ-hydroxybutyrate and a decrease in lactic acid and tyrosine in the vagina are the most sensitive and specific indicators for the diagnosis of BV. Therefore, not only the vaginal microflora, but also their metabolites can be used as effective reference indicators for clinical diagnostics.

Vaginal microflora in BV can activate the immune response of the reproductive tract of a macroorganism, while not causing obvious symptoms of inflammation. The reason may be associated with the effect of microorganisms associated with BV and their metabolites on immunity. Delgado-Diaz DJ. et al. (2019) found that long-term action of organic acids, metabolites of vaginal microbiocenosis, associated with BV, leads to impaired regulation of the immune response of cervical and vaginal epithelial cells in vitro [9]. Short-chain fatty acids can attract and activate innate immune cells of the female reproductive tract, such as neutrophils and monocytes [29]. In addition, the succinic acid produced by Prevotella spp. and Mobiluncus spp. in BV, it can also suppress leukocyte chemotaxis and regulate the immune response [20, 29].

Thus, the conducted studies have shown that the interaction between microflora, metabolites and the immune system in BV is of great importance for understanding the clinical symptoms and signs of the disease. However, to confirm the effect of metabolites on the vaginal microflora and the immune system of the microorganism in BV, additional studies of the mechanism of these interactions are needed.

Bacterial metabolites as disease markers

Research Gajer R. et al. (2012) showed that the existence of a transitional composition of the vaginal community is associated with the production of lactic acid by members of this community, and that only long-term changes in the composition of the microbiocenosis are associated with changes in metabolite profiles [12]. In addition, Laghi L. et al (2014) evaluated the metabolomic composition of vaginal fluid in women with BV and found that if the vaginal fluid contains a high content of lactic acid in comparison with short-chain fatty acids, then in these cases spontaneous remissions will occur in the absence of treatment indicating that these cases cannot be considered true cases of BV. In this regard, the authors believe that different profiles of short-chain fatty acids detected in dysbiosis can be useful as biomarkers and supplement existing methods for diagnosing BV by identifying true cases of the disease [15].

According to Aldunate M. et al (2015), the use of metabolites as markers of BV will allow determining the likelihood of spontaneous remission in women whose microbiocenosis is in a transitional state, which prevents the unnecessary use of antibacterial drugs that can negatively affect the indigenous microflora of the vagina, contributing to the development of BV. and form antibiotic resistance [5]. Evaluation of metabolites in the vaginal fluid can also be used to determine the effectiveness of BV treatment and / or the likelihood of BV recurrence [15].

Findings

Thus, analysis of literature data shows that the mechanisms of maintaining the vaginal ecosystem and the development of bacterial vaginosis remain not fully understood. The degree of protection provided by various communities of the vaginal ecosystem is to be formulated by studying the metabolomic characteristics of bacteria and the influence of their metabolites on intermicrobial relations in the community and on immune defense mechanisms. Disclosure of the pathogenetic mechanisms of bacterial vaginosis is important for improving approaches to the treatment and prevention of bacterial vaginosis.

LIST OF REFERENCES:

1. Gladysheva I.V., Cherkasov S.V. Corynebacteria of the vaginal microbiome - potential pathogens or promising probiotics? // Bulletin of the Orenburg Scientific Center of the Ural Branch of the Russian Academy of Sciences. - 2019. - No. 3. - S. 1-20.
2. Nazarova V.V., Shipitsyna E.V., Shalepo K.V., Savicheva A.M. Bacterial communities that form the vaginal microecosystem in normal conditions and in bacterial vaginosis // Journal of Obstetrics and Women's Diseases. - 2017. - T. 66, No. 6. - P. 30–43.
3. Rakhmatullaeva M.M. The importance of the level of pro-inflammatory cytokines in the development of bacterial vaginosis in women with contraception // Materials of the IV All-Russian scientific-practical conference with international participation "Socially significant and especially dangerous infectious diseases." - Sochi, 2017. - pp. 196-198.
4. Rakhmatullaeva M.M., Navruzova N.O. Micro biocenosis of the vagina: the role of lactobacilli in its maintenance // Problems of Biology and Medicine. - 2020. - No. 5 (122). - S.269-272.
5. Aldunate M, Srbinovski D, Hearps AC, Latham CF, Ramsland PA, Gugasyan R, et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. Front Physiol. 2015; 6: 164.
6. Ceccarani C, Foschi C, Parolin C, D'Antuono A, Gaspari V, Consolandi C, et al. Diversity of vaginal microbiome and metabolome during genital infections. Sci Rep. 2019; 9(1):14095.
7. Chen Y, Bruning E, Rubino J, Eder SE. Role of female intimate hygiene in vulvovaginal health: global hygiene practices and product usage. Womens Health (Lond.). 2017; 13(3): 58-67.
8. Crann SE, Cunningham S, Albert A, Money DM, O'Doherty KC. Vaginal health and hygiene practices and product use in Canada: a national cross-sectional survey. BMC Womens Health. 2018; 18(1) :52.
9. Delgado-Diaz D.J., Tyssen D, Hayward J.A., Gugasyan R., Hearps A.C., Tachedjian G. Distinct immune responses elicited from cervicovaginal epithelial cells by lactic acid and short chain fatty acids associated with optimal and non-optimal vaginal microbiota. Front Cell Infect Microbiol. 2020; 9:446.
10. Doyle R., Gondwe A., Fan Y-M., Maleta K., Ashorn P., Klein N., et al. A Lactobacillus-deficient vaginal microbiota dominates postpartum women in rural Malawi. Appl Environ Microbiol. 2018; 8410-17.
11. France M.T., Mendes-Soares H., Forney L.J. Genomic comparisons of Lactobacillus crispatus and Lactobacillus iners reveal potential ecological drivers of community composition in the vagina. Appl Environ Microbiol. 2016;82: 7063-73.
12. Gajer P., Brotman R.M., Bai G., Sakamoto J., Schütte U.M., Zhong X., et al. Temporal dynamics of the human vaginal microbiota. Sci Transl Med. 2012; 4(132): 132-152.
13. Ilhan Z.E., Laniewski P., Thomas N., Roe D.J., Chase D.M., Herbst-Kralovetz M.M. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine . 2019; 44: 675-690.
14. Kyongo J.K., Jespers V., Goovaerts O., Michiels J., Menten J., Fichorova R.N., et al. Searching for lower female genital tract

soluble and cellular biomarkers: defining levels and predictors in a cohort of healthy Caucasian women. PLoS One. 2012; 7(8): P 439-51.

15. Laghi L., Picone G., Cruciani F., Brigidi P., Calanni F., Donders G., et al. Rifaximin modulates the vaginal microbiome and metabolome in women affected by bacterial vaginosis. Antimicrob. Agents Chemother. 2014; 58(6): 3411–20.
16. Lamont R.F., Sobel J.D., Akins R.A., Hassan S.S., Chaiworapongsa T., Kusanovic J.P., et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG. 2011; 118: 533-549.
17. Li H., Zang Y., Wang C., Li H., Fan A., Han C., Xue F. The interaction between microorganisms, metabolites, and immune system in the female genital tract microenvironment. Front Cell Infect Microbiol. 2020; 10: 609 488.
18. Linhares I.M., Summers P.R., Larsen B., et al. Contemporary perspectives on vaginal pH and lactobacilli. Am. J. Obstet. Gynecol. 2011; 204(2): 120. P1-5.
19. Macklaim J.M., Fernandes A.D., Di Bella J.M., Hammond J., Reid G., Gloor G.B. Comparative meta-RNA-seq of the vaginal microbiota and differential expression by Lactobacillus iners in health and dysbiosis. Microbiome. 2013; 1:12.
20. McMillan A., Rulisa S., Sumarah M., Macklaim J.M., Renaud J., Bisanz J.E., et al. A multi-platform metabolomics approach identifies highly specific biomarkers of bacterial diversity in the vagina of pregnant and non-pregnant women. Sci Rep. 2015; 5:14174.
21. Mehta S.D., Nannini D.R., Otieno F., et al. Host genetic factors associated with vaginal microbiome composition in Kenyan women. Msystems. 2020; 5(4):e00502-20.
22. Mendes-Soares H., Suzuki H., Hickey R.J., Forney L.J. Comparative functional genomics of Lactobacillus spp. Reveals possible mechanisms for spezialization of vaginal lactobacilli to their environment. /Journal of Bacteriology. 2014; 196:1458-1470.
23. O'Hanlon D.E., Moench T.R., Cone R.A. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. PLoS One. 2013: 8;e80074.
24. Pruski P., Lewis H.V., Lee Y.S., Marchesi J.R., Bennett P.R., Takats Z., et al. Assessment of microbiota: host interactions at the vaginal mucosa interface. Methods. 2018; 149:74-84.
25. Rampersaud R., Planet P.J., Randis T.M., Kulkarni R., Aguilar J.L., Lehrer R.I., et al. Inerolysin, a cholesterol-dependent cytolysin produced by Lactobacillus iners. J Bacteriol. 2011; 193(5):1034-1041.
26. Ravel J., Gajer P., Abdo Z., Schneider G.M., Koenig S.S., McCulle S.L., et al. Vaginal micro biome of reproductive-age women. //Proc Natl Acad Sci USA. 2011; 108:4680-4687.
27. Srinivasan S., Morgan M.T., Fiedler T.L., Djukovic D., Hoffman N.G., Raftery D., et al. Metabolic signatures of bacterial vaginosis. mBio. 2015;6(2):e00204-15.
28. Yeoman C.J., Thomas S.M., Miller M.E., Ulanov A.V., Torralba M., Lucas S., et al. A multi-omic systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. Plops 1. 2013; 8:e56111.
29. Vitali B., Cruciani F., Picone G., Parolin C., Donders G., Laghi L. Vaginal microbiome and metabolome highlight specific signatures of bacterial vaginosis. //Eur J Clin Microbiol Infect Dis. 2015; 34(12):2367-76.
30. Witkin S.S., Mendes-Soares H., Linhares I.M., et al. Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. 2013. M Bio 4(4):e00460-13.

Entered 09.01.2021