

Types of Vaginal Lactobacilli as Biomarkers of the Physiological State of Microbiocenosis

Rakhmatullaeva Makhfuza Mubinovna

Bukhara State Medical Institute, Bukhara, Uzbekistan

Abstract The review article presents data on the significance of various types of lactobacilli in maintaining the vaginal microbiocenosis in dynamic balance. Literature data on the proportion of different types of lactobacilli in the vaginal microbiocenosis in normal and dysbiosis are compared, and their protective functions are taken into account. The constancy of the vaginal microbiocenosis is associated with the dominance of *Lactobacillus crispatus*, which has more pronounced protective properties, while the detection of *Lactobacillus iners* indicates the instability of the composition of the vaginal microbiocenosis and the possibility of its shift towards dysbiosis.

Keywords Lactobacilli, Vaginal microbiocenosis, Types of lactobacilli

1. Introduction

Vaginal microflora is one of the most important factors in the stability of the microbiocenosis of the lower part of the reproductive tract. Colonization resistance of the vagina is determined by the ability of lactobacilli to maintain an acidic environment by producing lactic acid, forming highly active oxygen derivatives that have a detrimental effect on transient anaerobic microorganisms, bacteriocins, and the presence of pronounced adhesion to the epithelial cells of the vagina [1].

The total bacterial mass (microbial contamination of the vagina) in clinically healthy women of reproductive age ranges from 10^6 to $10^{8.5}$ GE/ml. The proportion of lactobacilli in the microbiocenosis ranges from 90-99% and ranges from $10^{5.4}$ to $10^{8.5}$ GE/ml [2].

To date, about 120 species of the genus *Lactobacillus* have been identified, and more than 20 representatives of lactobacilli have been described for vaginal microbiocenosis. The genomes of vaginal lactobacilli are significantly smaller in comparison with the genomes of lactobacilli of other microbiocenoses [3]. This phenomenon may contribute to the fact that vaginal *Lactobacillus species* show a certain degree of adaptation to a lifestyle that depends on the macroorganism [3], which may ultimately ensure the dynamism of the vaginal microbiocenosis.

Most often, the basis of normal vaginal microbiocenosis in women of reproductive age consists of 4 species: *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri* and *Lactobacillus iners* [2,4]. It was found that each of the four types of vaginal lactobacilli is unique due to the possession of their genome by various

families of numerous proteins. These specific changes in the form of increase and loss of genes occurred during the evolution of vaginal lactobacilli and thus developed a unique set of proteins for a specific species [3].

Different types of lactobacilli play an important role in maintaining the stability of the physiological microbiocenosis, but their share in this does not have the same degree. Each woman has her own individual species composition of lactobacilli, but stable codominance of multiple species of lactobacilli in a single microbiota is observed infrequently, and as a rule, it is dominated by only one or two species [3,5].

2. Materials and Methods

Differences in the composition of vaginal microbiocenosis are due to a combination of cultural, behavioral, genetic, and possibly other factors. The species composition of lactobacilli primarily depends on the geographical area of residence and ethnicity of women. *L. crispatus* is more often identified among European women (50.6%), while *L. iners* – among women of Asian, African and Latin American groups (53,2-58,3%) [4]. These data also echo the results of a later study, which showed that the overall occurrence of lactobacilli among African women is significantly lower than in Asian and European women, and the species *L. vaginalis* is the most common species among African women [6]. Studies by Budilovskaya OV. et al. [5] showed that the frequency of detection of *L. crispatus* in normocenosis (53%) is almost 2 times higher than in moderate dysbiosis (29%) and 13 times higher in severe dysbiosis (4%).

To date, attempts are continuing to determine the microbiological criteria for the "norm" of vaginal

microbiocenosis. However, research in recent years has shown that this is not the case for most women. Studying the vaginal microbiocenosis in healthy women of reproductive age, Ravel J. et al. [4] identified five main types (clusters) of vaginal microbiocenosis, the so-called community state types (CST). In 26.2% of the study population of clinically healthy women of reproductive age, CST I was detected, characterized by the dominance of *L. crispatus*. 6.3% of women were found to have CST II with a predominance of *L. gasseri*, 34.1% had a predominance of *L. iners* (CST III) and 5.3% – *L. jensenii* (CST V). These types were found in European and Asian women. CST IV detected among Latin American women and African-American women was not dominated by lactobacilli and was represented by the polymicrobial association of strict and facultative anaerobes.

Grouping of microbial communities of the vagina was continued in the works of a number of researchers. Research by Dolls JA. et al. [7] two clusters were identified in healthy women: one dominated by *L. iners*, the other by *L. crispatus*. There was an almost complete negative correlation between *L. crispatus* and *L. iners*. In women with bacterial vaginosis (BV), bacterial communities were grouped into three clusters. One of them was characterized by the dominance of *Gardnerella vaginalis* and *Leptotrichia amnionii*, the second – by the dominance of representatives of the *Lachnospiraceae* family. The third cluster had a high level of species diversity, but the dominant species were *Sneathia sanguinegens* and *Gardnerella vaginalis*.

In studies by Nazarova VV. et al. [8] bacterial communities of disturbed vaginal microflora were studied. All bacterial communities of the vagina were grouped into four clusters. Cluster I included cases where the vaginal microflora consisted mainly of lactobacilli. Cluster II included the cases of dominance of aerobic microorganisms: *Enterobacteriaceae*, *Streptococcus* and *Staphylococcus*. Clusters III and IV were associated with BV and included cases of dominance of facultative anaerobic (*Gardnerella vaginalis*, *Atopobium vaginae*) and obligate anaerobic (*Sneathia/ Leptotrichia/ Fusobacterium, Megasphaera/ Veillonella/ Dialister, Lachnobacterium/ Clostridium*) microflora, respectively. The clusters differed significantly in vaginal pH, with the highest values observed for cluster IV.

The detection of vaginal microbiocenosis corresponding to CST IV changed the idea of a "healthy" vaginal microbiocenosis, since women in this group did not show the dominance of lactobacilli in the vaginal microbiocenosis, as well as signs of an infectious process in the lower reproductive tract [4]. The normal functioning of the vaginal microbiocenosis, which is represented by the polymicrobial association of strict and facultative anaerobes, can also be maintained with a deficiency of *Lactobacillus species* due to the ability of genus of bacteria *Atopobium vaginae*, *Megasphaera species*, *Leptotrichia species*, and *Corynebacterium species* to produce lactic acid [9]. Therefore, the absence of lactobacilli or the presence of certain microorganisms, such as *Gardnerella vaginalis*, or

various species of *Peptostreptococcus*, *Prevotella*, *Pseudomonas*, *Streptococcus* and/or *Corynebacterium* in the structure of microbial communities in different populations may not be a pathological condition [10,11]. In this case, it is assumed that these microorganisms play a role in protecting the vaginal biotope from pathogenic microorganisms.

Gaier P. et al. [10] noted that in some women, vaginal types change from one to another, while in others they remain relatively stable. It turns out that the vaginal microbiocenosis dominated by *L. crispatus* often passes into *L. iners* - the dominant or mixed biotope (CST III), and rarely into CST IV. In turn, vaginal microbiocenosis with a predominance of *L. iners* is twice as likely as to pass into CST IV than *L. crispatus*-dominant (CST I). Data on the transformation of vaginal microbiocenoses associated with lactobacilli of other species (*L. gasseri*, *L. jensenii*, *L. vaginalis*) are rare and this issue requires more detailed study [10]. Lactobacilli belonging to CST IV do not provide adequate protection for vaginal microbiocenosis, so this type of microbiocenosis can easily turn into vaginal dysbiosis [12].

It is obvious that the species *L. iners* is often found both in the physiological state of microbiocenosis and in vaginal dysbiosis. This species has a more pronounced adaptive ability compared to other lactobacilli by regulating the expression of its genes [13]. It was found that the existence of *L. iners* in conditions of dysbiosis is mediated by increased production of cholesterol-dependent cytolysin, which destroys cell walls, and enzymes that break down mucin and glycerol. Such high adaptability of *L. iners* and survival in different conditions (including after antimicrobial therapy) can be considered as a significant factor that ensures the restoration of lactobacilli [13]. However, it is also suggested that due to the relative absence of antagonism between *L. iners* and BV-associated anaerobes, the predominance of this type of lactobacilli in the vaginal microflora increases the risk of developing BV [4,14]. This may be due to the fact that *L. iners* has several phenotypes, some of which contribute to the maintenance of physiological microbiocenosis, while others are associated with the occurrence of dysbiosis [5].

In addition, according to some data, there is a massive increase in the number of non-resident vaginal *Lactobacillus species* that are not found in normocenosis [15,16]. Thus, Melkumyan AR. et al. [16] in their studies isolated lactobacilli in normocenosis in a high titer ($>10^7$ CFU/ml) and more often in monoculture, whereas in bacterial vaginosis titers were always low ($<10^5$ CFU/ml) and often isolated 2-3 types of lactobacilli. According to the authors, the absolute titer of these lactobacilli remained high in 85% of patients with BV with a relatively low proportion in the total bacterial mass ($<10\%$) ($>10^7$ GE/sample).

These data echo the results of the study by Shishkova YuS. et al. [17], according to which, dysbiotic processes of the vagina of pregnant women in the first trimester are accompanied not only by a decrease in the number of lactobacilli in the vaginal discharge, but also by a large

heterogeneity of the species composition of lactobacilli, while significantly increasing their biofilm-forming ability. With a significant decrease in the proportion of normal flora in some samples of non-pregnant women, the dominant species is *L. vaginalis*. It is also interesting that samples without lactobacilli were detected in dysbiotic processes – their share was 9% in BV [18].

Lactic acid synthesis by lactobacilli and epithelial cells provides oxidation of the vaginal environment (the pH of the vaginal contents of most healthy women of reproductive age varies between 3.8 and 4.5), which creates unfavorable conditions for the life of opportunistic and pathogenic microorganisms. Many *Lactobacillus* species also produce hydrogen peroxide (H_2O_2), but recent data indicate that lactic acid has a greater bactericidal activity against BV-associated bacteria compared to H_2O_2 [19].

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 epithelial cells of the vagina, and the D-isomer is synthesized only by lactobacilli. According to Linhares IM. et al. [20] the content of D-lactic acid in the vaginal contents is higher when lactobacilli dominate, with the exception of *L. iners*. This is due to the fact that *L. iners* is not able to synthesize the D-isomer of lactic acid, since the genome of the microorganism lacks the genes responsible for its synthesis [20,21]. The D-isomer of lactic acid, determined in high concentrations in the vaginal discharge when *L. crispatus* dominates, allows inhibiting the production of extracellular matrix metalloproteinase inducer. This inducer causes the synthesis of metalloproteinase 8, which creates prerequisites for ascending genital infection, which in turn can cause premature birth [21].

Immunomodulatory effects of *L. crispatus* have been noted, for example, the *L. crispatus* strain ATCC 33820 suppresses the growth of *Candida albicans* 27 in vitro by modulating the expression of toll-like receptors 2 and 4, interleukin 8, and β -defensin 2 and 3 in epithelial cells [22]. As regards study by Ghartey JP. et al. [23] that demonstrated the ability of the *L. crispatus* strain ATC 33197 to inhibit activity against *E. coli*.

The association of *L. iners* dominance in the lactoflora with a light proinflammatory background, similar to changes in BV, was revealed [18]. According to a number of authors, the mRNA expression levels of the IL-8, TLR4, IL-10, CD69, and CD45 genes were significantly increased and IL-18 was reduced in the *L. crispatus* dominated samples, which partly explains the increased risk of developing BV in women with this type of microbiocenosis. The tendency to increase proinflammatory factors in CST IV also indicates that the formation of a proinflammatory state associated with the absence or low number of lactobacilli may be the ground for the development of BV [24].

Thus, despite the fact that *L. iners* is normally present in the vaginal microbiocenosis quite often, its protective role in ensuring normocenosis remains controversial, and many authors even believe that *L. iners* plays a negative role and is involved in the pathogenesis of bacterial vaginosis [13].

3. Results and Discussion

The protective role of the other most common lactobacilli (*L. jensenii*, *L. gasseri*) in maintaining the vaginal microbiocenosis is doubted, since when *L. jensenii* and *L. gasseri* dominate, the vaginal environment becomes less acidic (pH=4.7±0.4 and pH=5.0±0.7, respectively) than when *L. crispatus* dominates (pH=4.0±0.3) [4]. The dominance of *L. jensenii* is associated with a decrease in lactate and succinate in the vaginal discharge and an increased risk of preterm birth, since this species produces only D-lactic acid, and therefore has a lower protective potential compared to *L. crispatus* [25]. *L. jensenii* has a protective potential against *E. coli* colonization through the synthesis of an adhesive exoprotein [26]. In addition, *L. jensenii* suppresses the expression of pro-inflammatory mediators in the presence of toll-like receptor agonists [27]. The species *L. gasseri* is often found in dysbiosis: it dominates in the group of moderate dysbiosis and ranks second in frequency after *L. iners* in the group of severe dysbiosis [5]. It was found that in the group of women with BV, the dominance of *L. gasseri* in the normal flora was significantly more frequent – 30% compared to conditionally healthy women – 8% [18].

Thus, individual species of lactobacilli have unique characteristics that give them specific competitive advantages over other species. At the same time, different types of lactobacilli differ in protein expression in response to any changes in the metabolism of the macroorganism, and changes in the ratio of other representatives of the vaginal microbiocenosis may affect which type of lactobacilli will dominate [3].

The total number of transient microorganisms normally does not exceed 15-20% of the total pool of microorganisms [8]. Normally, the vaginal microbiocenosis is represented by anaerobic/aerobic flora, which includes more than 300 species. Microorganisms of various types enter into certain interactions of synergy or competition through certain metabolites, information molecules [28]. Such connections (quorum sensing) largely determine the state of the vaginal microecosystem, namely, the norm, or conditions are created for the formation of a pathological process.

Components of the normal vaginal microbiological association can be *Gardnerellavaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, yeast-like fungi of the genus *Candida*, and anaerobic bacteria (genus *Mobiluncus* and others) [8] in small quantities. Thus, *Gardnerella vaginalis* is present in the vagina of 14-69% of women who do not have signs of BV, *Bacteroides* spp. detected in 9-13% of women, *Fusobacterium* spp. in 14-40%, *Porphyromonas* spp. in 31%, *Prevotella* spp. in 60%. In rare cases (0-5%), *Mobiluncus* species are found in the vaginal discharge [2].

In normal vaginal microbiocenosis, the growth of various communities of opportunistic flora should not exceed 10^4 CFU/ml. The frequency of occurrence of *Candida* fungi in the vagina of healthy women, according to various researchers, is 15-30%, the level of contamination is up to

10^4 CFU/ml [29]. However, *Candida spp.* persist in the vast majority of women of fertile age and the frequency of their actual presence in the vaginal normocenosis may be significantly higher. Fungi of the genus *Candida* are similar to lactobacilli metabolic needs. This feature predisposes them to occupy the vacant ecological niche with a decrease in the total number of lactobacilli and the manifestation of vulvovaginal candidiasis.

Despite the fact that the number of individual groups of microorganisms, such as *Gardnerella vaginalis*, *Prevotella bivia*, *Porphyromonas spp.*, *Eubacterium spp.*, can reach quite high values (up to 10^6 GE/ml), the total proportion of obligate anaerobic microorganisms in clinically healthy women does not exceed the proportion of normal flora. The proportion of groups of aerobic microorganisms is 10 times less than the proportion of obligate-anaerobic microorganisms [5].

The qualitative and quantitative composition of the vaginal microflora of most women in different periods of life is quite stable. However, the ratio of obligate and opportunistic vaginal microflora can constantly change in response to exogenous and endogenous factors. The composition of the vaginal microbiocenosis of women of reproductive age is influenced by hormonal changes occurring during the menstrual cycle, pregnancy and postmenopausal period, lifestyle, sexual education and hygiene skills, occupational hazards, the use of antibacterial drugs, hormonal and intrauterine contraceptives [30, 31]. However, in most cases, the changes are temporary and the physiological state of the vaginal microbiocenosis is quickly restored by natural protective mechanisms involving lactobacilli.

The struggle for dominance among the vaginal microflora is a continuous process. It is known that correction of impaired vaginal microbiocenosis and BV therapy is a difficult problem, since 50% of women have relapses within a year after treatment [32]. The level of lactobacilli after a course of antibacterial therapy is crucial to guarantee long-term remission. When identifying species associated with BV immediately after treatment, the probability of relapse is 90%, and on the contrary, women who have a 96% share of lactobacilli in the microbiocenosis immediately after treatment have a 74% chance of remaining in remission [14].

4. Conclusions

Thus, the physiological microbiocenosis of the vagina is supported by the species and quantitative composition of various lactobacilli, the degree of participation of which in ensuring its stability is not the same, which requires further study of this issue in order to understand the pathogenetic mechanisms of bacterial vaginosis and improve methods of its therapy and prevention.

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