VAGINAL MICROBIOTA

(collections of articles)

DNA-TECHNOLOGY

INTRODUCTION

The term vaginal microbiota is generally used in reference to all microbial taxa on the surface of the vaginal epithelium. This biotope is of particular interest to doctors and researchers due to its influence on the female reproductive health and its role in the establishment of a new-born baby's microbiome. In addition, the imbalance of the vaginal microbiota — dysbiosis — is strongly associated with an increased risk of developing urogenital infections and with a number of pregnancy complications.

In recent decades, great advances were made in understanding composition and functioning of the vaginal microbiota in women of reproductive age. This became possible because of the possibility to study microbial communities using molecular techniques: genome sequencing, polymerase chain reaction, and DNA hybridization. These methods allowed researchers to overcome the limitations of the culture-based method, which for many years was the "gold standard" in the study of human microbiome. The introduction of molecular genetic research methods in the study of human microbiota culminated in the "Human Microbiome Project", launched in 2008. As a result, new microorganisms were discovered, the taxonomy of many commensal microbes were revised. Moreover, new microbes associated with various pathological conditions were identified, and the interpretation of the "normal" vaginal microbial community was revised.

This selection of scientific articles to presents the current concepts about vaginal microbiota, modern techniques for its studying and diagnosing dysbiosis.

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Vaginal Microbiota in the Context of Quantitative PCR: What is Norm?

E. S. Voroshilina, L. V. Tumbinskaya, A. E. Donnikov, E. A. Plotko, L. V. Khayutin

Vaginal microbiota is a complex dynamic system, dependent upon many endogenous and exogenous factors. These include age, reproductive behavior, obstetric and gynecological history, hormonal state, the use of hormonal and antimicrobial drugs, and extragenital diseases. Cultural and social factors such as national traditions, advertisements for feminine hygiene products, etc. should also be taken into account. Nevertheless, when it comes to norm in terms of vaginal microbiota, it is first of all based on the clear idea about quantitative and qualitative composition of the vaginal microbial community. In order to introduce molecular genetic methods into clinical practice, it is vital to determine normal and pathological characteristics of vaginal microbiota in different groups of women. The aim of this study was to analyze vaginal microbiota of clinically healthy women of reproductive age, as well as perimenopausal and postmenopausal women, using RT-PCR method (Femoflor-16, DNA-Technology LLC).

Materials and methods

604 asymptomatic women (ages17–73) without any complaints of pathologic discharge or discomfort in the vulvovaginal area were included in this study. Exclusion criteria were clinical or/and microscopical signs of vaginitis, bacterial vaginosis (BV).

The 1st group comprised of 230 nonpregnant women of the reproductive age (26–36) who came in for a routine checkup or for pregnancy spacing. The 2nd group included 283 pregnant women (ages 25–33) of which 140 were examined in the I trimester (68 of them were going to terminate their pregnancy), 50 were examined in the II trimester, and 93 — in the III trimester. The 3rd group had 65 perimenopausal women (ages 48–52), and 26 postmenopausal women (ages 57–60) comprised the 4th group.

Vaginal microbiota was evaluated by means of microscopy and RT-PCR. All samples met the microscopic criteria of normocenosis: the number of WBC (white blood cells) ranged from 0 to 5, and lactobacilli prevalent in microflora.

Vaginal microbiota was analyzed using RT-PCR (Femoflor-16 kit, DNA-Technology LLC).Total bacterial load (TBL), the proportion of lactobacilli, and the proportion of each group of opportunistic microorganisms were calculated, using special software.

The proportion of normal flora, facultative anaerobic microorganisms and anaerobic microorganisms was determined as well.

Statistical data processing was performed using SPSS Statistics v.17.0 (Inc., Chicago, USA).

Results and discussion

The following types of vaginal microbiota were discriminated when analyzing RT-PCR results. The first type is normocenosis when the proportion of lactobacilli in the TBL is more than 80%, the proportion of opportunistic microorganisms (facultative and obligate anaerobes) is less than 10%, and the quantity of associated microorganisms (*Candida spp., Mycoplasma spp.*) is less than 10^4 GE/ml. The variant of normocenosis where the quantity of *Candida spp.* and *Mycoplasma spp.* is higher than 10^4 GE/ml is determined as conditional normocenosis. The second type was determined as moderate aerobic or anaerobic dysbiosis — the proportion of lactobacilli is lower (20 to 80% of the TBL), and the proportion of facultative and obligate anaerobes is higher. And the third type — apparent dysbiosis — when the proportion of lactobacilli is less than 20% of the TBL and the proportion of facultative and obligate anaerobes is more than 80% of TBL.

TBL and the number of lactobacilli in nonpregnant women of reproductive age ranged from $10^{5.4}$ to $10^{8.5}$, nevertheless, the proportion of lactobacilli in 90% of the examined women ranged from 55.5% to 99.9%. The number of aerobic and anaerobic microorganisms differed from the number of lactobacilli by two orders, and their proportion was 0.03% and 0.26% in TBL.

The most frequent groups of opportunistic bacteria were *Gardnerella vaginalis/ Prevotellabivia/Porphyromonas spp.* and *Eubacterium spp.* which were detected in the majority of samples in quantities ranging from 10^2 to 10^5 GE/ml/. *Atopobium vaginae* (marker of bacterial vaginosis) was detected significantly less often, which implies that its presence in the vaginal microbiota is less typical in the clinically healthy women compared to *Gardnerella vaginalis*.

In the 1st group normocenosis was detected in 25.8% of the patients, conditional normocenosis — in 63.9% of the patients, apparent anaerobic dysbiosis — 0.5%, moderate anaerobic dysbiosis — 4.6%, moderate aerobic dysbiosis — 3.6%.

Ureaplsma spp. in the quantities of more than 10^4 GE/ml was detected in 21.1% of all samples, while *Mycoplasma spp*. was virtually nonexistent in the vaginal microbiota of healthy women.

Candida spp. in the quantities of more than 10^3 GE/ml was detected in 52% women in the 1st group. *Candida spp*. exists in the same conditions as lactobacilli, and it

is not sensitive to the changes in the pH and does not compete with the normal flora. The study showed that significant quantities of *Candida spp*. do not necessarily lead to the symptoms of urogenital candiasis, which means that it is necessary to take into account clinical data of a patient.

In the 2nd group (pregnant women) normocenosis was detected in 36.3% of the cases, conditional normocenosis — in 59.8% of the cases, apparent anaerobic dysbiosis — 0.5% of the cases, moderate anaerobic dysbiosis — 3.4% of the cases.

Structure of conditional normocenosis in pregnant women is very similar to that in nonpregnant women. In 18.2% of the cases it was due to the presence of *Ureaplasma spp*., in 59.9% — due to the presence of *Candida spp*., in 21.9% both species were detected.

There were no statistically significant differences in the composition of the vaginal microbial community between pregnant and nonpregnant women, which suggest that qualitative and quantitative composition of vaginal microbiota does not undergo significant changes during pregnancy. Lactobacilli make up more than 99% of all the detected microorganisms in the vaginal microbiota of a clinically healthy woman.

During perimenopause and in the postmenopause vaginal microbiota undergoes significant changes mainly due to the decrease in the level of estrogens. The use of standard approach to result interpretation in the evaluation of vaginal microbiota by means of RT-PCR led to pathological findings in the majority of this group of women.

Criteria of absolute normocenosis met 10.7% samples in the 3rd group (perimenopause) and in 15.4% of samples in the 4th group (postmenopause). 21.5% and 11.5% of patients in the 3rd and 4th groups respectively met the criteria of conditional normocenosis. Thus, the criteria for the normal vaginal microbiota should be different for older women compared to the women of reproductive age.

As menopause progresses, the TBL decreases. In the 3rd group, the proportion of lactobacilli also was lower, and it was 10 times lower than the TBL. The proportion of obligate anaerobes (particularly *Gardnerella vaginalis* and *Eubacterium spp*.) increased, while the quantity of *Ureaplasma spp.* and *Candida spp*. was similar to that of women of the reproductive age.

With the advance of menopause, the absolute number of lactobacilli continued to decrease, and they were being substituted by obligate anaerobic microorganisms. This process continues in the postmenopause (group 4), moreover, aerobic and mixed aerobic-anaerobic microbial associations begin to play a significant role. *Ureaplasma spp.* and *Mycoplasma spp*. were not detected in most cases. The lack of clinical manifestations of vaginal inflammatory processes suggests that this might be a variant of the norm for this age group.

Enterobacteria in menopausal patients were detected in the quantities of up to 10^3 GE/ml, which is less than in women of the reproductive age. In some of cases enterobacteria were not detected at all (women in the I trimester).

Staphylococci were detected in the quantities of up to 10^3 GE/ml in menopausal women (10^2 GE/ml in women with atrophy). In women of the reproductive age these microorganisms were detected in higher quantities.

G. vaginalis spp., Eubacterium spp., Mobilincus spp., Clostridium spp. were detected in the majority of women in all the groups. *G. vaginalis* and *Eubacterium spp.* were typically detected in higher quantities.

Clostridium spp. and *Peptostreptococcus spp.* were found in most women of reproductive age, as well in menopausal women in moderate quantities. *Megashpaera spp.* was found less often in the women of reproductive age; in menopausal women its proportion was higher.

Mycoplasma spp. was rarely detected in women of all the age groups, which indicates that it is rarely present in the vaginal microbiota of clinically healthy women. Ureaplasma was found in women of all age groups, often in significant quantities.

Candida spp. was also present in the majority of examined women in the quantities of more than 10^3 GE/ml. This suggests the need to revise existing views on the acceptable amount of *Candida spp*. in the vaginal microbial community.

RT-PCR is a sensitive method for the analysis of vaginal microbiota. The groups of microorganisms analyzed above form vaginal microbiota of women of different age groups. Data obtained in this study confirms existing views on the structure of vaginal microbiota and improves our knowledge of qualitative and quantitative composition of vaginal microbiota.

Conclusion

The type of vaginal microbiota is determined by the quantitative relationship between normal flora (the predominant lactobacilli in the microbiota of women of the reproductive age) and opportunistic microorganisms rather than by the absolute quantities. In perimenopause and postmenopause anaerobic flora prevails.

The study has shown that *Atopobium vaginae*, unlike *Gardnerella vaginalis*, is rarely found in vaginal samples which meet the criteria of normocenosis. Obligate anaerobes may be present in the microbiota of clinically healthy women of all age groups in small quantities, while facultative anaerobes (streptococci and staphylococci) are detected significantly less often.

Ureaplasma in the quantity of more than 10^4 GE/ml was detected in every fifth vaginal sample from clinically healthy asymptomatic women of the reproductive age.

Yeast-like fungi (more than 10^4 GE/ml) were detected in 10% of clinically healthy women of the reproductive age. Given the lack of clinical manifestations of inflammatory processes, this could be considered as a variant of normal state of microbiota, however it is necessary to study the stability of such microbiocenos throughout a long period of time and evaluate its susceptibility to external influences.

Bacterial Communities Forming Vaginal Microbiota in the Norm and During Bacterial Vaginosis

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Bacterial vaginosis (BV) is a vaginal microbiota disorder characterized by the decrease in the proportion of lactobacilli and an increase in the proportion of anaerobic bacteria and by a vaginal discharge. Recent studies have shown that BV is not just an innocuous growth of anaerobic microorganisms easily treatable with metronidazole or clindamycin, but a disease associated with a number of women's urogenital tract disorders, and it can lead to pregnancy complications. In 60% of women, BV recurs within 12 months after treatment.

Despite significant progress in studying etiology, pathogenesis and ways of transmission of BV, many questions remain open due to the fact BV is associated with a broad range of bacteria. These bacteria belong to four bacterial types: *Firmicutes, Actinobacteria, Bacteriodetes, Fusobacteria*, and they significantly differ in their biochemical, morphological, tinctorial characteristics, as well as in their sensitivity to antimicrobial drugs. This diversity significantly complicates not only determining pathogenetic mechanisms of the disorder but also finding effective ways of diagnosing and treating BV. One of the approaches to studying an aspect of the disorder involves grouping all bacterial communities into different types of microbiota and analyzing their clinical and diagnostic significance.

The aim of this study was to describe and compare clusters of bacterial communities forming vaginal microbiota in the norm and during BV.

Materials and Methods

This study included 280 women of the reproductive age (20–45 years old) with complaints of discomfort and/or discharge from the genital tract. Exclusion criteria were pregnancy and the use of antimicrobial drugs within the period of 4 weeks before the examination.

BV was diagnosed by analyzing vaginal discharge using the Nugent score.

Cluster analysis inclusion was based on Amsel diagnostic criteria (vaginal pH, vaginal discharge, positive amine test, presence of "clue" cells detected by microscopy).

Vaginal microbiota was analyzed by means of quantitative PCR in real time (Femoflor-16, DNA-Technology, LLC). The test determines total bacterial load and absolute quantity and proportion of the following microorganism genus/species: *Lactobacillus, Enterobacteriaceae, Streptococcus, Staphylococcus, Gardnerella*

vaginalis/Prevotella bivia/Porphyromonas, Eubacterium, Sneathia/Leptotrichia/Fusobacterium, Megasphaera/Veillonella/Dialister, Lachnobacterium/Clostridium, Corynebacterium/Mobiluncus, Peptostreptococcus, Atopobium vaginae. Absolute quantities of Mycoplasma hominis, Ureaplasma and *Candida* are estimated as well.

Femoflor-16 results of 10 samples were invalid and excluded from the analysis. Thus, only 270 samples were included in cluster analysis.

Statistical data processing was conducted using GraphPad Prism (GraphPad Software, Inc.) and SPSS (IBM).

Two-step non-hierarchical cluster analysis was used to group bacterial communities into clusters.

Results and Discussion

According to the Nugent score, 172 samples were scored as normal, 27 — intermediate, 81 — BV.

Non-hierarchical clustering requires an assumption about the number of clusters. So, we assumed that all the examined cases could be divided into 4 clusters (types of microbiota), depending on the prevalence of microorganism groups: with the prevalence of lactobacilli, of aerobic microorganisms (*Enterobacteriaceae* family, staphylococci, streptococci), of facultative anaerobic microorganisms, and of obligate anaerobic microorganisms.

Candida and *Ureaplasma* genera were not included in the cluster analysis, because our previous study on the development of diagnostic criteria for BV using Femoflor-16 (performed on the same set of patients), has shown that there was no correlation between these microorganisms and the quantitative parameters of BV (Nugent score, the number of positive Amsel criteria, vaginal pH).

Cluster 1 (n=171) included cases where lactobacilli were prevalent in the microbiota. Most of the patients in this cluster (94%) had normal microbiota according to the Nugent score. Cluster 2 (n=11) included cases where aerobic bacteria prevailed in the microbiota: *Enterobacteriaceae, Streptococcus, Staphylococcus*. 64% of patients from this cluster had intermediate microbiota according to the Nugent score. In clusters 3 (n=57) and 4 (n=31), facultative anaerobes (*Gardnerella vaginalis, Atopobium vaginae*) and obligate anaerobes (*Sneathia/Leptotrichia/Fusobacterium, Megasphaera/Veillonella/Dialister, Lachnobacterium/Clostridium*) were prevalent respectively. Representatives of these 2 clusters had BV according to the Nugent score.

The concentration of lactobacilli in cluster 1 was significantly higher compared to other clusters (where the concentrations of lactobacilli were similar). The concentration of *Gardnerella vaginalis/Prevotella bivia/Porphyromonas* was significantly

higher in clusters 1 and 2 than in clusters 3 and 4 where the levels of concentration were similar to each other. The same goes for the proportion of *Atopobium vaginae* in the TBL. Vaginal pH was different in all clusters, and it was highest in cluster 4.

Vaginal discharge was found significantly less often in women with lactobacilli prevalent in the microbiota (13%) compared to patients from clusters 2 (91%), 3 (82%), and 4 (97%). Amine test was negative, and no "clue" cells were detected in women from clusters 1 and 2.

The main goal of our work was to study bacterial communities in dysbiotic vaginal microbiota, therefore, we did not analyze species composition of lactobacilli. As a result of clustering vaginal bacterial communities were divided into groups characterized by lactobacilli prevalence (cluster 1), aerobic bacteria prevalence (cluster 2), facultative anaerobic bacteria prevalence (cluster 3), obligate anaerobic bacteria prevalence (cluster 4).

In the distribution of clusters according to the Nugent score, most patients in cluster 1 were put in the category of normal microbiota. Most patients in cluster 2 had intermediate microbiota. This coincides with data received by G. Donders (2002) that intermediate microbiota according to the Nugent score includes microbiota characteristic of aerobic vaginitis. Patients from clusters 3 and 4 mostly were in the BV category according to the Nugent score. It should be noted that in cluster 3 most samples scored 7–8 points, while in cluster 4 most samples scored 9–10. This might be due to the fact that both gardnerellas and other BV-associated bacteria were detected in samples from cluster 4, since it is only possible to receive maximum points according to the Nugent score if apart from the morphotypes of small gram-variable cocci and coccobacilli, a morphotype of gram-variable curved rods was detected, and there are no lactobacilli morphotypes.

In our study, both clusters of BV-associated bacterial communities (3 and 4) contained *G. vaginalis* and *A. vaginae*, and their quantities in the clusters were similar.

The results of this study support the hypothesis viewing BV as a polymicrobial biofilm infection where *G. vaginalis* triggers the pathological process leading to the development of BV.

It is also important to note the results of vaginal discharge test for *Eubacterium* (gram-positive bacteria of *Eubacteriaceae* family, *Clostridiales* order). This microorganism was detected almost in all women with BV with its proportions being rather high (up to 60% of TBL). In our previous work we showed that these bacteria have a significant association with BV. Our data do not support the results of studies conducted using deep DNA sequencing method, according to which *Eubacteria* are detected in a small number of samples, and their concentration is relatively low.

The proportions of other bacteria/bacteria groups, i.e. *Corynebacterium/Mobiluncus, Peptostreptococcus, M. hominis*, were relatively low, therefore, it difficult to determine their role in any type of microbiota. In terms of *Corynebacterium/Mobiluncus*, interpretation difficulties are also related to the fact that while both genera are in the *Actinomycetales* order (*Actinobacteria* phylum), *Mobilincus spp.* is associated with BV, and *Corynebacterium spp*. is not.

All clusters also significantly varied in vaginal pH. It is established that low pH is maintained by the production of lactic acid and hydrogen peroxide by lactobacilli in normocenosis. In our study, low concentrations of lactobacilli in "non-lactobacilli" bacterial communities (clusters 2, 3, 4) was associated with much higher pH values.

When analyzing other diagnostic criteria for BV (vaginal discharge, positive amine test, presence of "clue" cells), we did not find any statistically significant differences between the two clusters associated with BV. Further research could be focused on the connection between certain bacterial clusters with the clinically manifested BV and recurrent BV.

Conclusion

Thus, vaginal bacterial communities could be grouped into four main clusters or types of vaginal microbiota. Normal microbiota cluster comprises mainly of lactobacilli. Aerobic microbiota cluster is characterized by the prevalence of enterobacteria, streptococci or staphylococci. Clusters with the prevalence of facultative anaerobic or obligate anaerobic microbiota are associated with BV. The described clusters belong to different categories according to the Nugent system and vary in vaginal pH values.

Bacterial Vaginosis Diagnostic Criteria: Femoflor-16 Kit

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Bacterial vaginosis (BV) is the main cause of pathological vaginal discharge in women of the reproductive age. Recent studies show that BV is associated with a number of inflammatory disorders of the urogenital tract and pregnancy complications.

Amsel diagnostic criteria are considered the main method in clinical diagnostics. Nugent score is mainly used in laboratory diagnostics.

BV is manifested as a change in the balance between normal vaginal microbiota (lactobacilli) and opportunistic microorganisms (found in small quantities in the norm). Literature refers to such states as "vaginal dysbiosis". Another known form of vaginal dysbiosis is aerobic vaginitis (AV). During AV, the quantity of lactobacilli also decreases, vaginal pH increases, allowing for the increase in the quantity of opportunistic microorganisms (OM). However, BV is characterized by the lack of inflammatory processes and the presence of large quantities of anaerobic microbiota, while AV is characterized by an inflammatory reaction and/or pronounced vaginal epithelium atrophy, as well as a small amount of commensal gastrointestinal microbiota.

Lately, diagnostics of vaginal dysbiosis in Russia have been conducted by Femoflor-16 kit (DNA-Technology, LLC). The kit is based on multiplex quantitative real-time PCR (RT-PCR). It determines the total bacterial load (TBL) and the concentration (in absolute and relative values) of the following genera/species of microorganisms: *Lactobacillus, Enterobacteriaceae, Streptococcus, Staphylococcus, Gardnerella vaginalis/Prevotella bivia/Porphyromonas, Eubacterium, Sneathia/Leptotrichia/Fusobacterium, Megasphaera/ Veillonella/Dialister, Lachnobacterium/Clostridium, Corynebacterium/Mobiluncus, Peptostreptococcus, Atopobium vaginae*.

Algorithms for interpretation of Femoflor-16 results do not include BV. However, patient treatment based on the principles of evidence-based medicine requires a laboratory conclusion to provide information allowing to make an accurate diagnosis. The aim of this study was to develop diagnostic criteria for bacterial vaginosis using Femoflor-16 kit to analyze vaginal discharge.

Materials and Methods

The study included 280 women aged 20–45, presenting with discomofort and vaginal discharge. Exclusion criteria were pregnancy and treatment with antimicrobial drugs within the period of 4 weeks before the examination.

Vaginal discharge was tested by means of microscopy and Femoflor-16 kit.

Amsel diagnostic criteria (slightly modified so that "clue" cells were detected in a Gram-stained preparation) were used to diagnose BV. Laboratory analysis for BV was conducted, using Nugent score.

Femoflor-16 kit determines the TBL and the concentration of vaginal microbiota. According to the relationship between different representatives of the microbiota, the state of vaginal microbiota is determined — normocenosis (absolute or conditional) or dysbiosis. Depending on its severity, dysbiosis can be moderate or apparent, and depending on the prevalent bacteria, it can be aerobic, anaerobic, or mixed.

In the development of diagnostic criteria of BV, the quantity of all the microorganisms, excluding *M. hominis, Ureaplasma,* and *Candida*, was represented as the relationship between the concentration of their DNA and TBL. The quantities of *M. hominis, Ureaplasma,* and *Candida* were represented in absolute values (GE/ml). Correlation between the quantity of the detected microorganism groups/microorganisms and the clinical and microscopy characteristics of BV was analyzed to determine the association between these microorganisms and the BV. ROC-analysis was used to evaluate the capability of potential bacterial markers identified with Femoflor-16 to accurately categorize samples with normal microbiota and with BV. Statistical data processing was performed using Statistica (StatSoft) and SPSS (IBM).

Results and Discussion

According to Amsel diagnostic criteria, BV was detected in 86 women, 194 women did not have BV. According to Nugent score, 172 samples contained normal microbiota, 27 — intermediate microbiota, 81 — BV.

All samples were tested by Femoflor-16 kit. 10 samples were invalid; thus 270 samples were included in the analysis. Of them, 164 samples had normal microbiota, according to the Nugent score, 26 — intermediate microbiota, 80 — BV. Femoflor-16 results were interpreted as follows: 100 patients had absolute normocenosis, 24— conditional normocenosis, 45 — moderate dysbiosis (44 — anaerobic, 1 — aerobic), 101 — apparent dysbiosis (90 — anaerobic, 8 — aerobic, 3 — mixed).

Lactobacilli prevalence in the TBL showed negative correlation with BV. Anaerobes had a positive correlation with BV, with the highest coefficients observed for *Gardnerella vaginalis/Prevotella bivia/Porphyromonas, Sneathia/Leptotrichia/Fusobacterium,* and *Megasphaera/Veillonella/Dialister*. Positive correlation with BV was also observed for *M. hominis. Mobiluncus*, while it is traditionally associated with BV, did not show significant correlation with any of BV parameters. However, in Femoflor-16, these bacteria are detected together with *Corynebacterium* (which

is related to *Mobiluncus* phylogenetically but is not associated with BV). Aerobic bacteria, ureaplasmas, and yeast-like fungi had no correlation with BV characteristics.

The following bacterial markers showed the highest diagnostic accuracy according to ROC-analysis: *Lactobacilli* proportion in the TBL, *Gardnerella vaginalis/Prevotella bivia/Porphyromonas* proportion in the TBL, *Eubacterium* proportion in the TBL, *Sneathia/Leptotrichia/Fusobacterium* proportion in the TBL, *Megasphaera/Veillonella/Dialister* proportion in the TBL, Atopobium vaginae proportion in the TBL. *Lachnobacterium/Clostridium* proportion in the TBL and *Peptostreptococcus* proportion in the TBL had satisfactory diagnostic accuracy. TBL and *M. hominis* showed low diagnostic accuracy according to ROC-analysis.

Low concentration of lactobacilli (less than 10% of the TBL) is the most sensitive and specific criterion for BV. However, it should be noted, that cases of intermediate microbiota according to the Nugent score were excluded from the ROC-analysis. Most patients with apparent aerobic and mixed dysbiosis (also characterized by the small proportion of lactobacilli) have intermediate microbiota. Thus, lactobacilli proportion in the TBL cannot be the only criterion. Therefore, BV can be diagnosed if two conditions are met: low concentration of lactobacilli and high concentration of at least one of the bacterial markers of BV.

Then the developed criteria for BV detection were compared with the kit manufacturer's criteria for apparent anaerobic dysbiosis (unofficially equated with BV). Of 92 patients with BV, 87 patients had apparent anaerobic dysbiosis, 2 — apparent aerobic dysbiosis, 3 — apparent mixed dysbiosis. At the same time, 3 out of 90 women with apparent anaerobic dysbiosis did not have BV. Thus, diagnostic criteria for BV developed in this study and the criteria developed by Femoflor-16 manufacturer's describe the same state of vaginal microbiota to a significant degree.

Femoflor-16 kit is based on quantitative PCR in real time. Compared to bacteriological method and microscopy, it has a number of advantages in identifying vaginal microbiota disorders. It detects non-culturable and difficult to culture microorganisms, as well as differentiates between the bacteria with similar morphotypes.

Femoflor-16 laboratory result includes the following main variants of vaginal microbiota: normocenosis (absolute and conditional), moderate dysbiosis (anaerobic and aerobic), apparent dysbiosis (anaerobic and aerobic). We believe that Femoflor-16 has the potential to accurately detect two main types of vaginal dysbiosis: BV and AV. After developing relevant diagnostic criteria, it could be possible, using this kit, to accurately identify the type of vaginal microbiota associated with aerobic vaginitis (it is also necessary to evaluate inflammatory reaction for the accurate AV diagnosis).

The developed criteria allow diagnosing BV with high sensitivity and specificity. It should be noted that when evaluating the developed criteria, the categories of normal and intermediate microbiota according to the Nugent score were both viewed as the lack of BV. Nugent intermediate microbiota composition is diverse, and according to literature, it is mostly made up of microorganisms associated with AV and of microbiota intermediate between norm and BV. The results of our study indirectly confirm it: 63% of cases of apparent aerobic dysbiosis had intermediate microbiota according to the Nugent score.

Another issue that fueled this study involves difficulties with interpretation of moderate dysbiosis which is an intermediate state between normocenosis and apparent dysbiosis. Moderate anaerobic dysbiosis is especially problematic since it is frequently detected. The comparison between Femoflor-16 and Nugent score results showed that most samples with moderate anaerobic dysbiosis were categorized as normal microbiota according to the Nugent score (84%). We believe that the intermediate state category identified by Femoflor-16 as moderate dysbiosis should be narrowed to include only borderline, difficult-to-interpret cases.

It should also be noted that Femoflor-16 kit's potential for analyzing vaginal microbiota has some limitations: *Gardnerella vaginalis*, facultative anaerobe, is detected together with *Prevotella bivia* and *Porphyromonas spp*., obligate anaerobes. A similar limitation is *Corynebacterium* being detected together with *Mobiluncus*.

Conclusion

Diagnostic criteria for BV using Femoflor-16 kit, intended to analyze vaginal microbiota. Small proportion of lactobacilli (less than 10% of the TBL) together with a large proportion of *Gardnerella vaginalis/Prevotella bivia/Porphyromonas*, and/or *Eubacterium*, and/or *Sneathia/Leptotrichia/Fusobacterium*, and/or *Megasphaera/Veillonella/Dialister*, and/or *Lachnobacterium/Clostridium*, and/or *Peptostreptococcus*, and/or *Atopobium vaginae* determine BV with high sensitivity and specificity.

Diagnostic criteria for BV developed in this study and the criteria developed by Femoflor-16 manufacturer's describe the same state of vaginal microbiota to a significant degree.

Vaginal Microbiota During Pregnancy in the Context of Quantitative PCR

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For pregnant women, dysbiotic diseases such as bacterial vaginosis and urogenital candidiasis are risk factors for threatened miscarriage, hydramnios, premature rupture of membranes, preterm labor. Bacterial vaginosis is also a risk factor for the development of pyoinflammatory puerperal complications. Vaginal microbiota also has an impact on the fetus and the newborn.

The introduction of the Femoflor-16 kit (DNA-Technology LLC) made it possible to comprehensively evaluate vaginal microbiota and its participants with high accuracy and specificity, identify the severity of dysbiotic processes.

The aim of this study was to analyze vaginal microbiota of pregnant women in the I, II, and III trimesters by means of quantitative PCR.

Materials and Methods

557 samples of vaginal content collected from pregnant women in the I (303 samples), II (144 samples), and III (110 samples) trimesters were examined. Women included in the study were aged 18–43. Samples positive for obligate pathogenic agents of urogenital diseases (*Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhea, Mycoplasma genitalium*) were excluded from the study, as well as HIV-positive patients and those who received systemic or topical antimicrobial or antimycotic treatment within 4 weeks before the check-up. The data is not representative of the whole population since most of the patients included in the study underwent pregnancy spacing.

Vaginal microbiota was evaluated by means of RT-PCR (Femoflor-16, DNA-Technology). Using the specialized software, we determined the amount of the total bacterial load (TBL), as well as quantity and the proportion of lactobacilli and different groups of opportunistic microorganisms (facultative and obligate anaerobic microorganisms, mycoplasmas and yeast-like fungi).

The following classification of microbiota variants was used:

1. Normocenosis (absolute normocenosis) is the variant where the proportion of lactobacilli is more than 80% of the TBL, and the quantity of *Ureaplasma spp., Mycoplasma spp., Candida spp*. are lower than 10^4 GE/ml.

2. Conditional normocenosis is the variant of microbiota where the proportion of lactobacilli is less than 80% of the TBL, and the quantity of *Ureaplasma spp., Mycoplasma spp., Candida spp.* are higher than 10^4 GE/ml.
3. Moderate (aerobic or anaerobic) dysbiosis is the variant where the proportion of lactobacilli is within the range of 20–80% of the TBL, and the quantity of aerobes or anaerobes are increased.
4. Apparent (aerobic, anaerobic or mixed) dysbiosis is the variant of microbiota where the proportion of aerobes or anaerobes is 80% of the TBL, and the proportion of lactobacilli is lower than 20% of the TBL.

   Statistical data processing was conducted using SPSS Statistics v.17.0.

Results and Discussion

All the women included in the study were divided into 3 groups according to trimesters. Absolute normocenosis was detected in 46.86% of women in the I trimester, in 45.83% of women in the II trimester, in 55.45% of women in the III trimester. Conditional normocenosis was detected in 32.34% of women in the I trimester, 34.72% of women in the II trimester, in 26.36% of women in the III trimester. microbiota variants where the proportion of lactobacilli was higher than 90% were more often detected in women in the II and III trimesters.

TBL was slightly lower in women in the II and III trimesters due to the decrease in the absolute quantity of all groups of facultative anaerobic and obligate anaerobic bacteria. Compared to the I trimester, in the III trimester of pregnancy the quantity of some anaerobes (*Eubacterium spp., Megasphaera spp., Peptostreptococcus spp.*) were statistically lower. Thus, by the end of the pregnancy, the proportion of lactobacilli increased.

The structure of conditional normocenoses changed throughout the pregnancy. In the I trimester it was associated with *Ureaplasma spp.* (64.29%) and *Candida spp.* (61.23%). Both microorganisms were detected in 25.52% of cases. In the II trimester, the amount of conditional normocenoses associated with mixed infection was lower (20%), while candidiasis was found more often (62%). *Mycoplasma spp*. was not detected in the majority of patients throughout the pregnancy.

In the III trimester, the amount of conditional normocenoses associated with *Candida spp*. was significantly lower (41.38%), and the amount of conditional normocenoses associated with *Ureaplasma spp*. was significantly higher (79.31%).

Both, TBL and the absolute quantity of lactobacilli, decreased in the II and III trimesters compared to the I trimester. Moreover, in the III trimester, the absolute quantity of a number of anaerobic microorganisms significantly decreased

(*Eubacterium spp., Megasphaera spp., Lachnobacterium spp., Mobilincus spp., Atopobium vaginae*).

The quantity of *Ureaplasma spp.* increased in the II and III trimesters in women with conditional normocenosis. The quantity of *Candida spp*. slightly increased in the II trimester and significantly decreased in the III trimester.

Apparent dysbiosis was detected in 13.73% of women in the I trimester, 7.80% of women in the II trimester and in 7.89% of women in the III trimester.

Anaerobic dysbiosis was found in the majority of women in the I trimester (75.61%), aerobic dysbiosis was detected 15 times less often (4.87%). Mixed aerobic-anaerobic apparent dysbiosis was found in 19.52% of women. This means that anaerobes prevailed in the majority of apparent dysbioses in the I trimester. Mixed dysbiosis was not detected in the II trimester. In the majority of cases apparent dysbiosis in the II trimester was associated with anaerobes. In the III trimester the proportion of facultative anaerobic bacteria in microbiota of patients with dysbioses increased (55.5%)

TBL in apparent dysbiosis was higher in the I trimester compared to other variants of microbiota, but in the II and III trimesters its quantity decreased. The quantity of lactobacilli was 10 times less than the TBL, and it decreased in the II and III trimesters.

Gardnerella vaginalis in association with *Eubacterium spp.* and *Atopobium vaginae* was detected in the highest quantity in apparent dysbiosis during the I trimester. *Atopobium vaginae* was detected in 41.46% of cases of apparent dysbiosis.

Peptostreptococcus spp., Lachnobacterium spp., Mobilincus spp. were detected in lower quantity. The quantity of all anaerobic microorganisms (except for *Gardnerella vaginalis*) decreased throughout the pregnancy and continued to do so in the III trimester.

In apparent dysbiosis, *Gardnerella vaginalis* prevailed in all the trimesters. By the end of the pregnancy due to the decrease in the quantity of all anaerobes, the proportion of facultative anaerobic microorganisms, especially streptococcus, increased.

Moderate dysbiosis was detected the least often in the I trimester (7.26%). In the II trimester it was detected in 11.11% of cases, and in 10% of cases in the III trimester.

In moderate dysbiosis, TBL was higher in the II and III trimesters compared to the I trimester, as well as the quantity of lactobacilli. *Gardnerella vaginalis* and *Eubacterium spp.* were detected in the majority of cases, moreover, in the II and III trimesters the quantity of the latter increased by 10 and exceeded the quantity of *Gardnerella vaginalis*.

Quantity of aerobes and facultative anaerobes varied throughout the pregnancy. The quantity of *Staphylococcus spp.* decreased by the end of the pregnancy, while the quantity of *Streptococcus spp.* and *Enterobacteriaceae spp*. increased. *Ureaplasma spp.* was higher in the II and III trimesters in women with moderate dysbiosis with the decrease in the quantity of anaerobes and the increase in the quantity of aerobes.

The quantity of *Candida spp*. increased by the III trimester, which was not typical of other variants of dysbiosis. Thus, different structure of moderate dysbiosis was found during different pregnancy stages, which was associated with multidirectional changes in the quantitative values of microorganisms in the vaginal microbiota.

Conclusions

Microbiota met the criteria of normocenosis in the majority of the examined women throughout the pregnancy. Absolute normocenosis was detected more often in the II and III trimesters.

In normocenosis in the II and III, TBL was slightly lower due to the decrease in quantity of all groups of facultative anaerobic and obligate anaerobic bacteria. Absolute quantity of lactobacilli were constant throughout the pregnancy. Thus, the proportion of normal flora in the microbiota increased by the end of the pregnancy.

In every fifth patient throughout the pregnancy the proportion of lactobacilli severely or moderately decreased due to the increase in the quantity of anaerobic bacteria.

Mycoplasma spp. was not detected in the majority of women in all the trimesters irrespective of the severity of dysbiotic states. *Ureaplasma spp*. was detected in women in all trimesters, moreover, in the II and III trimesters its quantity increased.

The quantity of *Candida spp*. in vaginal microbiota was stable throughout the pregnancy, except for the patients with moderate dysbiosis for whom the increase in the quantity of yeast-like fungi by the end of the pregnancy was typical.

Analysis of the qualitative and quantitative composition of vaginal microbiota in pregnant women showed the highest variability with intermediate states of microbiota, that is, with conditional normocenosis and moderate dysbiosis.

Vaginal Microbiota in the Context of Quantitative PCR: Changes and Correction During Pregnancy

E. S. Voroshilina, L. V. Tumbinskaya, A. E. Donnikov, E. E. Plotko, L. V. Khayutin

Currently, there are two different views on microbiota of pregnant women and the changes in the quantitative and qualitative composition of microbiota throughout the pregnancy. Some say that vaginal microbial community is very sensitive to various endogenous and exogenous factors and changes significantly during pregnancy. Others say that vaginal microbiota is stable and capable of restoring itself.

Nevertheless, most authors say that vaginal dysbiotic disorders in pregnant women affect not only the mother's health, but also the health of the newborn.

Real-time PCR will allow the physician to determine the etiology of a dysbiotic disorder, evaluate vaginal microbiota and choose the appropriate course of treatment.

The aim of this study was to identify the peculiarities of the vaginal microbiota dynamics during pregnancy by means of quantitative PCR, and to evaluate possibilities for correction of vaginal dysbiotic disorders in women in the I trimester.

Materials and Methods

119 pregnant women aged 20–43 were included in the study. Vaginal microbiota was examined once every trimester by means of quantitative RT-PCR (Femoflor 16, DNA-Technology LLC). 357 vaginal samples were examined in all.

Using the specialized software, we determined the amount of the total bacterial load (TBL), as well as quantity and the proportion of lactobacilli and different groups of opportunistic microorganisms (facultative and obligate anaerobic microorganisms). Microbiota variants were distinguished in accordance with the classification that was introduced in the previous study.

Statistical data processing was conducted using SPSS Statistics v.17.0.

Results and Discussion

By the end of the pregnancy the number of women with microbiota that met the criteria of absolute and conditional normocenosis (the proportion of lactobacilli higher than 80%) increased. Moreover, while the number of women with conditional normocenoses decreased, the number of women with absolute normocenosis increased. Conditional normocenosis was more often associated with *Ureaplasma spp.* (it was prevalent in all trimesters). The proportion of conditional

normocenoses with *Candida spp.* in the quantity of more than 10^4 GE/ml decreased almost by two by the III trimester.

In absolute and conditional normocenosis changes in the proportion of lactobacilli were insignificant (within 10%) throughout the pregnancy. Thus, these microbiota variants are relatively stable, and they are not significantly affected by exogenous factors, including drug treatment. Lactobacilli proportion changed the most throughout pregnancy (more than 80%) in women with apparent dysbiosis.

Women with moderate and apparent dysbioses hold an intermediate position with moderate changes in the proportion of lactobacilli (20–80%) throughout the pregnancy. The increase in the proportion of normal flora did not always lead to the restoration of normocenosis.

Normocenosis in the I trimester was detected in 56 women. In 85.71% of these women normocenosis remained until the end of the pregnancy. In 7.14% of the cases it changed to conditional normocenosis (due to the increase in the quantity of *Ureaplasma spp.*) during the II trimester. Conditional normocenosis associated with *Candida spp*. was detected in 3 women in the II trimester. Thus, normocenosis in the I trimester is a positive prognostic factor, suggesting high probability of this variant of microbiota being sustained throughout the pregnancy.

Conditional normocenosis associated with *Ureaplasma spp.* and *Candida spp.* was determined in 41 women in the I trimester. Positive dynamic was reached in 39.13% of cases. In 17.1% of cases microbiota dynamic was negative — after treatment the proportion of lactobacilli decreased, and moderate or apparent dysbiosis was formed.

Moderate dysbiosis was detected in 10 women in the I trimester. Positive dynamics by the end of the pregnancy was observed in half of the cases. In 4 cases microbiota state did not change, and in 1 case by the III trimester it became apparent dysbiosis.

Apparent dysbiosis was detected in 12 women in the I trimester. Positive dynamics in the II trimester was observed in 6 cases, and in the III trimester it was observed in 10 cases. In 2 cases the microbiota state did not change throughout the pregnancy. In women with apparent dysbiosis associated with *Ureaplasma spp.* and *Candida spp*. (more than 10^4 GE/ml), the quantity of these microorganisms remained the same, despite the conducted treatment and reaching conditional normocenosis. This might suggest that restoration of the normal balance between lactobacilli and opportunistic microorganisms in pregnant women is easier to achieve than suppression of *Ureaplasma spp.* and *Candida spp.*

Dysbiotic disorders in pregnant women increase the risk of various complications (including miscarriage) from the I trimester. The range of drugs that can be prescribed during the I trimester is rather limited, and the use of antibiotics for the treatment of urogenital infections is contraindicated. We evaluated the efficiency of vaginal microbiota correction by means of probiotics (Ecofemin) and drugs containing ascorbic acid (Vaginorm C) in 38 women in I trimester.If vaginal candidiasis was diagnosed, Natamycin was prescribed first.

After the conducted treatment, microbiota was restored to its normal state in 26.31% of cases, moreover, normocenosis in these patients remained in the III trimester. The number of apparent dysbioses decreased by a third. Positive dynamics was observed in 39.5% of the treated women, in 52.6% of cases there was no change, and in 7.9% of cases negative dynamics was registered.

Conclusion

Normocenosis in the I trimester is a positive prognostic factor, suggesting high probability of this variant of microbiota is being sustained throughout the pregnancy.

Absolute and conditional normocenosis during pregnancy is a stable system that is not significantly affected by exogenous factors, including drug treatment. The proportion of lactobacilli in the composition of these variants of biocenosis remains stable throughout the pregnancy.

The dynamics of vaginal dysbiotic disorders were controversial. The biggest positive shifts were observed in women who had moderate and apparent dysbiosis in the I trimester. Conditional normocenosis, associated with both *Candida spp.* and *Ureaplasma spp.* in the clinically significant quantity, remained stable throughout the pregnancy.

Received data show the lack of efficiency of treatment for vaginal microbiota correction in the I trimester of pregnancy. Positive dynamics was observed in 39.5% of patients.

Reproductive Behavior of Women and the State of Vaginal Microbiota

E. E. Plotko, E. S. Voroshilina, L. V. Hayutin, E. I. Abakumova, L. V. Tumbinskaya, A. E. Donnikov

Bacterial infections of the vagina are the leading cause of infectious inflammatory diseases in obstetrics and gynecology, and their incidence in population ranges from 30 to 80%. With the increase in the incidence of STIs, change in the nature of sexual and reproductive behavior, and irrational antibiotic treatment, there is an increase in the incidence of diseases associated with microorganisms from the normal vaginal microbiota. Vaginal dysbiosis can lead to the development of inflammatory urogenital diseases, bacterial vaginosis already in the I trimester of pregnancy, influencing the incidence of various complications, including miscarriage.

Methods, currently used to analyze vaginal microbiota (such as microscopy, bacteriological method, clinical method), are either not informative enough or too expensive. Only identifying the quantitative relationships between particular species of microorganisms can characterize the state of vaginal microbiota, determine the appropriate course of treatment.

The aim of this work is to conduct an in-depth analysis of vaginal microbiota by means of RT-PCR in pregnant women in their I trimester, to evaluate the impact of women's reproductive behavior on vaginal microbiota.

Materials and Methods

267 women (5 to 12 weeks pregnant) were included in the study. The women were divided into 2 groups: the 1st group comprised of 167 patients with wanted pregnancy, planning to carry it term; the 2nd group included 100 women with unwanted pregnancy, planning to terminate it either surgically or with medication.

All women underwent a standard examination, including microscopy of the vaginal discharge and the analysis of vaginal microbiota by means of RT-PCR (Femoflor 16, DNA-Technology LLC).

Statistical data processing was conducted using WINPEPI v.9.7 and Statistica v.7.0 for Windows.

Results and Discussion

The proportion of primigravidas in the 1st group was significantly higher than in the second, half of the women were uniparous. 24.6% women from the 1st group and 44%

women from the 2nd group had abortions (including miscarriages) before the current pregnancy. More than half of the women had a history of gynecological diseases.

Vaginitis was detected in 12.6% of women in the 1st group and 25% of women in the 2nd group; vaginosis was detected in 4.8% and 6% of women respectively. Disorders of the vagina and its microbiota were detected more often in women with unwanted pregnancy. At the same time 16.2% of women in the 1st group and 14% of women in 2nd group complained about their vaginal discharge, that is, woman's subjective evaluation of her condition differed from the results of the examination the most in women planning to terminate their pregnancy.

According to microscopy results, only 56.3% of women in the 1st group and 48% of women in the 2nd group had no signs of inflammation and normal state of vaginal microflora. This data did not correspond with the results of clinical examination (82.6% and 69% respectively), as well as with women's subjective evaluation of their state. Normal amount of WBC with the dysbiotic nature of vaginal microbiota was detected in every third patient.

Vaginal microbiota was then analyzed by means of RT-PCR (Femoflor 16, DNA-Technology LLC). Normocenosis (the proportion of lactobacilli is more than 80%) was detected only in 26.3% of women in the 1st group and 22% of women in the 2nd group. Compared to the 1st group, anaerobic dysbiosis was detected twice as often in pregnant women planning to terminate their pregnancy.

Conditional normocenosis (the proportion of lactobacilli is more than 80%; the quantity of opportunistic microorganisms such as *Ureaplsma spp., Mycoplasma spp., Candida spp*. is higher than 10^4 GE/ml) was detected in more than half of women in both groups. *Candida spp*. does not influence the quantity of lactobacilli even when found in significant quantities.

Anaerobic dysbiosis (the proportion of anaerobes is more than 10%) associated with both mycoplasmas and ureaplasmas were detected significantly more often in women with unwanted pregnancies. Normocenosis with low quantities of mycoplasmas and ureaplasmas was more typical for women planning to carry the pregnancy to full term.

3 variants of vaginal dysbiosis associated with *Ureaplasma spp., Mycoplasma spp.* were determined in pregnant women. *Mycoplasma spp*. was detected significantly less often than *Ureaplasma spp*.

Ureaplasma spp. may be present in the quantity meeting the diagnostic criteria while the quantity of lactobacilli remains the same (1st group — 2%, 2nd group — 37%). High quantities of *Ureaplasma spp*. (more than 10^4 GE/ml) are associated with the decrease in the proportion of lactobacilli to less than 20% of the total bacterial load.

The state of vaginal microbiota was analyzed in relation to reproductive behavior. Induced abortion in the medical history turned out to be an important factor influencing vaginal microbiota in both groups. Normocenosis with low quantities of *Ureaplasma spp*. was detected in 49.3% of women with a history of abortions compared to 62.8% of women without prior abortions. Moreover, the quantities of *Ureaplasma spp*. were higher (up to $10^{4.6}$) in the group of women who had abortions before.

Urogenital tract dysbiotic diseases not associated with ureaplasmas are more typical for women with induced abortions in the medical history. At the same time, the incidence of vaginal dysbiosis associated with high quantities of *Ureaplasma spp.* does not depend on this medical history factor.

Conclusions

The use of RT-PCR allowed us to establish that 75% of pregnant women between 5 and 12 weeks had some form of vaginal dysbiosis. The question of clinical interpretation of conditional normocenosis in pregnant women needs to be further studied.

Anaerobes (including ureaplasmas and mycoplasmas) play the main role in the structure of vaginal dysbioses in pregnant women. Aerobic dysbiosis was found only in 1 case.

Anaerobic dysbiosis was detected more often in women with unwanted pregnancies, planning termination.

Induced abortion in the medical history is a risk factor for anaerobic dysbiosis.

The use of RT-PCR allows us to evaluate vaginal microbiota with more accuracy and optimize procedures for prevention of complications associated with pregnancy and its termination.

Vaginal Microbiota in the I Trimester of Pregnancy in Women with a History of Recurrent Pregnancy Loss

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Recurrent pregnancy loss (RPL) is the main cause of perinatal morbidity and mortality with persistently high incidence rate (15–20% of all pregnancies). It is caused by a variety of etiological factors, one of the significant ones being vaginal microbiota disorders in the I trimester of pregnancy.

Vaginal microbiota consists of more than 400 microorganism species, including opportunistic microorganisms. Lactobacilli are prevalent in the vaginal microbiota of a healthy woman, and they play an important role in maintaining normocenosis.

Undiagnosed infections caused by opportunistic microorganisms can lead to spontaneous abortions, premature labor, intrauterine growth restriction, in utero infections. A recent study showed that the risk of premature labor decreases by 75% in women with normocenosis, and vice versa, the risk more than doubles in women in the I trimester without lactobacilli and with bacterial vaginosis and aerobic vaginitis, while the risk of RPL increases more than by 6.

New microbiological technologies allowed us to establish the diversity of lactobacilli species and its biological characteristics. Recent studies showed that *Lactobacillus crispatus, L. jensenii, L. gasseri,* and *L. iners* prevail in the vaginal microbiota of pregnant women. Prevalence of *L. iners* is often seen in the intermediate stage between normocenosis and dysbiosis.

The aim of this study was to identify the characteristics of vaginal microbiota in the I trimester of pregnancy in women with a history of recurrent pregnancy loss.

Materials and Methods

The study included 60 pregnant women in the I trimester with a history of RPL aged 18 to 40 (main group) and 10 pregnant women in the I trimester without bad reproductive and obstetric history aged 25 to 31 (control group). Women from both groups underwent clinical and laboratory examination which included history taking, collection of complaint data, obstetric-gynecological examination, laboratory tests (microscopy, culture-based method, real-time PCR).

Vaginal microbiota was analyzed by means of real-time PCR (Femoflor-16, DNA-Technology, LLC).

Statistical data analysis was performed using NCSS 11 statistical package (NCSS, LLC).

Results and Discussion

Microscopy showed that the quantity of WBC in the vaginal discharge exceeded the quantity of epithelial cells in 21.7% of cases in the main group. Inflammatory reaction was not detected during microscopy in the control group. At the same time there were no statistically significant differences between the two groups.

According to the microscopy results, lactobacilli were prevalent in 85% of cases in the main group and in 100% of cases in the control group. The difference between the two groups was statistically insignificant.

According to the bacteriological test, *L. jensenii* and *L. crispatus* were prevalent in both groups. *L. iners* was detected only in the main group in 23.3% of cases. A significantly higher quantity of non-lactobacilli microorganisms was detected in women with a history of RPL compared to women without bad reproductive and obstetric history.

According to the results of quantitative real-time PCR, vaginal microbiota disorders were found only in women with a RPL history (15% of cases). The difference between groups was statistically insignificant. Both facultative (*Enterobacteriaceae spp.*, streptococci, staphylococci) and obligate anaerobes were detected in the two groups. Among obligate anaerobes, *Gardnerella vaginalis, Prevotella bivia, Porphyromonas spp., Sneathia spp., Leptotrichia spp., Fusobacterium spp*. were detected more often in the main group. *Candida spp*. was detected in women with a history of RPL and without bad reproductive and obstetric history.

The comparative analysis of vaginal microbiota in pregnant women showed that women a RPL history tended to have more apparent dysbiotic disorders compared to healthy women. *L. jensenii, L. crispatus,* and *L. iners* were the species of lactobacilli, detected the most often. *L. iners* was detected more often in women with a history of RPL. Most strains of *L. iners* do not synthesize hydrogen peroxide. Moreover, according to some studies the toxin produced by *L. iners* is similar to *Streptococcus intermedius* and *G. vaginalis* whose role in the pathogenesis of dysbiotic processes has been proven.

Our data coincides with the results of other studies. L. Petricevic et al. (2014) determined that *L. iners* is prevalent in 85% of women whose pregnancy ended with premature labor.

Vaginal dysbiosis in pregnant women from the main group was more often characterized by the increase in the quantities of non-lactobacilli microorganisms. D.B. Nelson et al. (2012) have shown that women with low quantity of lactobacilli are at a higher risk of pregnancy loss in the II trimester.

In our study vaginal dysbiosis and an inflammatory reaction was detected only in women with a history of RPL. G. Donders et al. (2000) noted the increased risk of miscarriage and premature labor in women with vaginal dysbiosis, bacterial vaginosis and aerobic vaginitis in the I trimester of pregnancy.

There was no significant difference in the quantities of *Ureaplasma spp.* and *Mycoplasma hominis* in both groups. Data from world literature show that the presence of *M. hominis, U. urealyticum* is associated with an increased risk of pregnancy loss after 20 weeks of pregnancy.

Conclusions

This study showed that vaginal dysbiosis is detected more often in pregnant women with a history of recurrent pregnancy loss. The problem needs to be further researched in order to determine the pathogenesis of the infection associated with RPL, premature labor, and the effectiveness of prophylactic treatment with antimicrobial drugs.

Correction of Vaginal Dysbiosis with Cavitated Solution of Chlorhexidine in the 1st Trimester of Pregnancy: Effectiveness and Safety

E. S. Voroshilina, D. L. Zornikov, E. E. Plotko

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Introduction

Today in Russia treatment and prevention of vaginal dysbiosis is performed using the method of irrigation of the vagina with cavitated solutions of antiseptics. The aim of this study was to evaluate the state of vaginal microbiota before and after irrigation of the vagina with cavitated 0.05% solution of chlorhexidine.

Materials and Methods

104 pregnant women were examined (gestational age — 6–12 weeks). All patients were planning to terminate the pregnancy by means of vacuum aspiration. In order to prevent inflammatory complications before the vacuum aspiration, vagina was treated with cavitated 0.05% solution of chlorhexidine using the cavitation ultrasonic unit AUZH-100 in accordance with the methodology guidelines (exposure time — 1–2 minutes, power — 6–8 units, solution amount — 150–200 ml).

Vaginal discharge was analyzed. The material for the study was collected before and after treatment of the vagina with cavitated 0.05% solution of chlorhexidine.

DNA was extracted using PREP-GS kit (DNA-Technology, LLC). Each specimen was analyzed by means of real-time PCR (RT-PCR) for 15 microorganism clusters (Femoflor-16, DNA-Technology, LLC).

After performing RT-PCR, total bacterial load (TBL) and the quantity and the proportions of the tested microorganism clusters from the TBL were determined in each sample.

The variant of vaginal microbiota (absolute normocenosis, conditional normocenosis, moderate dysbiosis, apparent dysbiosis) was determined in accordance with the earlier established criteria.

Results

Before treatment with cavitated 0.05% solution of chlorhexidine, vaginal microbiota met the criteria of normocenosis (proportion of *Lactobacillus spp.* is more than 80% of the TBL) in 73.1% of the examined patients. 37.5% of these patients had absolute normocenosis (*Ureaplasma spp.* and/or *Mycoplasma hominis* and/or *Candida spp.* — less than 10^4 GE/ml), and 35.6% of these patients had conditional normocenosis (*Ureaplasma spp.* and/or *Mycoplasma hominis* and/or *Candida spp.* — more than 10^4 GE/ml). After the irrigation normocenosis was detected in 79.8% of patients, including absolute normocenosis (67.3% of patients) and conditional normocenosis (12.5%). The increase in the number of patients with absolute normocenosis and the decrease in the number of patients with conditional normocenosis was due to the 10–100 times decrease in the concentration of *Ureaplasma spp.*

Vaginal dysbiosis before the irrigation was detected in 26.9% of patients. Moderate dysbiosis (*Lactobacillus spp.* — 20–80% of the TBL) was detected in 10.6% of the examined women, and apparent dysbiosis (*Lactobacillus spp.* — less than 20% of the TBL) was detected in 16.3% of patients. After the treatment, dysbiosis was detected in 20.2% of the examined women. Moderate dysbiosis was detected in 14.4% of cases, and apparent dysbiosis — only in 5.8% of cases. Three times decrease in the proportion of patients with apparent dysbiosis was caused on average by 100 times decrease in the quantity of opportunistic microbiota, while the quantity of lactobacilli decreased no more than 10 times.

Conclusion

Irrigation of the vagina with cavitated 0.05% solution of chlorhexidine led to the increase in the number of patients with absolute normocenosis (from 39 to 70) and to the decrease in the number of patients with conditional normocenosis (from 37 to 13), as well as to the decrease in the number of patients with apparent dysbiosis (from 17 to 6). These changes were caused by the decrease in the quantity of *Ureaplasma spp.* and in the quantity of anaerobic opportunistic microorganisms in relation to lactobacilli.

Vaginal Dysbiosis: the Species Composition of Lactobacilli and the Possibilities of Non-drug Correction

E. E. Plotko, D. L. Zornikov, L. V. Hayutin, E. S. Voroshilina

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Introduction

Effectiveness of the use of probiotics for vaginal dysbiosis correction is still a debated topic. At the same time, sequential irrigation of the vagina with cavitated antiseptic solutions and treatment with probiotics, containing lactobacilli species with high protective characteristics, seems to be a promising strategy for restoring vaginal microbiota. The aim of this study was to analyze vaginal microbiota composition before and after the use of the proposed algorithm.

Materials and Methods

88 women of the reproductive age with vaginal dysbiosis were examined. Vaginal discharge of the examined women was analyzed. The material for the study was collected three times: before and after irrigation of the vagina with cavitated 0.05% solution of chlorhexidine, and in a month after the end of probiotic treatment.

In order to correct vaginal dysbiosis, all patients underwent irrigation of the vagina with cavitated 0.05% solution of chlorhexidine using the cavitation ultrasonic unit AUZH-100 in accordance with the methodology guidelines (exposure time — 1–2 minutes, power — 6–8 units, solution amount — 150–200 ml). After the irrigation, all patients received treatment with Ecofemin-Floravag (EF): orally, 1 capsule twice a day for a period of 10 days.

DNA was extracted using PREP-GS kit (DNA-Technology, LLC). Each specimen was analyzed by means of real-time PCR (RT-PCR) for 15 microorganism clusters (Femoflor-16, DNA-Technology, LLC). The quantity of 6 types of lactobacilli (*L. crispatus, L. iners, L. jensenii, L. gasseri, L. johnsonii, L. vaginalis*) was determined in the first and third sample from each patient by means of RT-PCR (DNA-Technology, LLC).

After performing RT-PCR, total bacterial load (TBL) and the quantity and the proportions of the tested microorganism clusters from the TBL were determined in each sample. The proportion of each lactobacilli species from the total detected *Lactobacillus spp*. was calculated separately.

The variant of vaginal microbiota (absolute normocenosis, conditional normocenosis, moderate dysbiosis, apparent dysbiosis) was determined in accordance with the earlier established criteria.

Results

During the initial examination, 70.4% of women had complaints indicating infectious inflammatory vaginal disorder. Clinical signs of vaginitis or vaginosis were present in 54.5% of patients. 18.8% of women had no complaints or clinical manifestations at the time of the examination.

After the irrigation was performed, only 35.2% of women presented with complaints; clinical signs of vaginal infectious disorder were detected in 28.4% of the examined women.

According to the analysis of the first sample of vaginal discharge, vaginal microbiota state met the criteria of moderate dysbiosis in 37.5% of cases and the criteria of apparent dysbiosis in 62.5% of cases. After the treatment of the vagina with cavitated 0.05% solution of chlorhexidine, microbiota was restored to normocenosis in 46% of women, the proportions of patients with moderate and apparent dysbiosis were 24.1% and 29.9% respectively.

After the treatment with EF, the share of patients with normocenosis did not increase compared to the similar parameter after the treatment of the vagina with cavitated 0.05% solution of chlorhexidine. However, the decrease in the proportion of patients with apparent dysbiosis from 29.9% to 20.5% was observed (statistically insignificant).

1–4 species of lactobacilli were detected in each sample simultaneously, and the quantitatively prevalent species was determined. Before the treatment, the following lactobacilli species were identified as prevalent: *L. iners* (48 (54,5%)), *L. gasseri* (22 (25%)), *L. crispatus* (6 (6,8%), *L. vaginalis* (5 (5,7%)), *L. jensenii* (4 (4,5%)). After the probiotic course, there were no statistically significant changes in the incidence rate of the prevalent lactobacilli species. Prevalence of *L. crispatus* (species contained in EF) was observed 2 times more often after the course of probiotic treatment, however, the differences were not significant. Moreover, this species was present in the microbiota composition before the treatment in lower quantities in all 12 patients. In 3.4% of samples, lactobacilli were not detected during the initial examination or after the course of EF.

Conclusion

Irrigation of the vagina with cavitated 0.05% solution of chlorhexidine and subsequent course of oral probiotic EF turned out to be effective in half of the cases. In a month after the end of the EF course, one in two patients had no symptoms of infectious inflammatory vaginal disorder. Moreover, vaginal microbiota met the criteria of normocenosis in every second patient. After the course of EF, there were no statistically significant changes in the incidence rate of the prevalent lactobacilli species.

Vaginal Microbiota in women with trichomoniasis

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Modern literature shows prevalence of opportunistic microorganisms (OM), both aerobic and anaerobic, in women's genital tract during trichomoniasis. At the same time, the quantity of lactobacilli is significantly decreased. Dysbiotic processes in the vagina during trichomoniasis have a negative influence on women's reproductive health, the course of pregnancy, fetal development, and the condition of a newborn. Symptoms and prognosis during urogenital trichomoniasis are dependent on a number of factors: antagonistic and symbiotic relationship between Trichominas vaginalis and the microorganisms in the vaginal microbiota, influence of the protective factors of the microorganism.

Qualitative and quantitative composition of the microbiota could change, as well as their bacterial virulence. There is also a horizontal gene transfer phenomenon. At present, specialists debate the question of detection of various microorganisms together with trichomonas in microbiota. It is also important to take into account the risk of vaginal dysbiosis associated with other infections including those sexually transmitted. Trichomoniasis treatment strategy should account not only for the elimination of the pathogen, but also for the restoration of normal microbiota. All the aspects mentioned above require accurate diagnostics.

Microscopy is traditionally used for trichomoniasis diagnostics and for the evaluation of vaginal microbiota. Currently, in microscopy, the Nugent method is considered a gold standard. However, microscopic examination, while it is highly specific, is less sensitive than nucleic acid amplification test (NAAT).

Modern methods of evaluating nucleic acids, including real-time PCR, allow for an accurate detection of species diversity of both aerobic and anaerobic components of vaginal microbiota.

The use of culture-based method for diagnosing trichomoniasis and detecting anaerobic microorganisms currently has serious limitations compared to microscopy and NAAT.

The aim of this study is to evaluate vaginal microbiota in patients with trichomoniasis using microscopy and nucleic acid amplification test.

Materials and Methods

Vaginal microbiota of 37 sexually active women of the reproductive age with trichomoniasis was examined.

Vaginal microbiota was studied by means of microscopic examination. The lack or the presence, as well as the quantity of various microorganisms (*Lactobacillus, Gardnerella, Mobiluncus*) were determined, and the results were interpreted in accordance with the Nugent scale: 1–3 points — no bacterial vaginosis (BV), 4–6 points — intermediate type of vaginal microbiota, 7–10 points — bacterial vaginosis.

DNA was extracted using the kit for DNA extraction. In order to check the viability of nucleic acids in the archive materials all the samples were tested to confirm the presence of human DNA.

Real-time PCR was used for detection of sexually transmitted pathogens (*Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae* and *Mycoplasma genitalium*) and evaluation of vaginal microbiota. The composition of vaginal microbiota was studied by means of real-time PCR (Femoflor-16, DNA-Technology, LLC).

Results and Discussion

An inflammatory reaction of the vaginal mucous was detected in 83.8% of patients, according to the microscopy. Yeast-like fungi were detected in 8.1% of patients.

According to the Nugent method, bacterial vaginosis was detected in 10.8% patients, intermediate type of vaginal microbiota was detected in 43.2% of cases and bacterial vaginosis — in 46% of cases.

Human DNA was found to be present in all the samples according to NAAT.

Trichomonas vaginalis were detected in all the samples, and *Chlamydia trachomatis* — in 8.1% of the samples. *Neisseria gonorrhoeae* and *Mycoplasma genitalium* were not detected.

According to the Femoflor test, *Eubacterium spp.* were detected in all the patients. In 8.1% of the cases they were detected together with *Atopobium vaginae*. In 89.2% of cases, obligate anaerobic microorganisms made up 8–100% of the total bacterial load.

The analysis of archive biomaterial was conducted using NAAT. The possibility of using this method was confirmed by the fact that human DNA and *Trichomonas vaginalis* were preserved in all the samples.

Received data showed that trichomoniasis can be accompanied by various states of vaginal microbiota with dysbiosis occurring the most frequently. Chlamydia, mycoplasmas, ureaplasmas and yeast-like fungi were detected most often in patients with an intermediate type of vaginal microbiota or in patients with BV.

According to the Nugent method, almost in half of examined patients with BV, prevalent microorganisms in the microbiota were anaerobic. Dysbiotic disorders were found almost in 90% of all patients.

In patients with trichomoniasis, lactobacilli were detected less often, and OMs were detected more often. Such changes reflect a decrease in the biotope resistance to colonization and are associated with the state of lactobacilli. Optimal conditions for the growth of anaerobic microorganisms, changes in the microbiota, and a decrease in the quantity of lactobacilli occur during trichomoniasis. A hypothesis has been drawn on the role of *Trichomonas vaginalis* in the development of microbiota states that contribute to the survival of the pathogen and its transmission to a sexual partner.

The quantity of mycoplasmas was significantly increased in a third of patients with trichomoniasis. Recent studies show a correlation between the detection of mycoplasmas and *Trichomonas vaginalis*.

Thus, significant dysbiotic disorders were identified in vaginal microbiota in patients with trichomoniasis. As the proportion of normal microbiota decreases, the proportion of pathogenic and opportunistic microorganisms increases, which may contribute to the development of inflammatory diseases.

It is necessary to conduct further studies to evaluate vaginal microbiota in women with trichomoniasis. Further development and use of NAAT is necessary. Next step of the research should be determining the exact roles of microbiota participants in its maintenance and development. Studies on the significance of lactobacilli species should give us valuable data. This knowledge will broaden our understanding of the issue and allow us to develop more effective patient treatment strategies.

Conclusion

Trichomoniasis detected in sexually active women of the reproductive age correlates with vaginal dysbiosis. This was confirmed by microscopic and molecular biological analyses of vaginal discharge. However, the results as determined by different methods could significantly vary, which has to be taken into account when choosing the appropriate method of examination.

Thus, the results of this study confirmed the necessity of a comprehensive laboratory examination of patients with trichomoniasis in order to receive comprehensive results and determine the appropriate course of treatment. The use of NAAT for the analysis of archive clinical material is promising in this respect.

HIV-Infection and Vaginal Microbiota in Women of Reproductive Age

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The spread of HIV-infection in Sverdlovsk Region (Russian Federation) has reached epidemic proportions. Moreover, the highest incidence is observed in people of the reproductive age. The proportion of women among the HIV-positive increases yearly, with sexual transmission becoming more and more significant.

Vaginal dysbiosis and sexually transmitted infections are considered to be risk factors for contracting HIV-infection through sexual intercourse. At the same time, HIV concentration in vaginal secretions increases during vaginal inflammatory disorders and bacterial vaginosis. Thus, HIV-positive women put their partners at a higher risk of contracting the infection.

In many cases dysbiotic disorders of vaginal microbiota in HIV-positive women are asymptomatic, and a physician can only rely on laboratory results.

Quantitative real-time PCR (RT-PCR) allows the specialists to conduct an in-depth analysis of the quantitative and qualitative composition of vaginal microbiota in women of the reproductive age.

The aim of this study was to describe vaginal microbiota in HIV-positive women of reproductive age using real-time PCR (RT-PCR).

Materials and Methods

The study included 90 HIV-positive women between the ages of 18 and 45 (the first group). 90 HIV-negative women were selected for the control group using the case-control method. Patients from the control group matched the HIV-positive women according to the following parameters: age, being not pregnant at the time of the examination, the nature of complaints and clinical manifestations, the state of microbiota according to the microscopy.

Exclusion criteria for the control group: the presence of sexually transmitted obligate anaerobes (*Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhea, Mycoplasma genitalium*), syphilis, systemic or topical antimicrobial or antimycotic treatment within 4 weeks before the examination.

Vaginal microbiota was analyzed by means of RT-PCR (Femoflor-16, DNA-Technology, LLC). Microbiota variants were classified according to the algorithm suggested earlier. Statistical data processing was performed using SPSS Statistics v.17.0 software package.

Results and Discussion

At the time of the examination, the 3rd stage of HIV was determined in 57.8% of the HIV-positive women, the 4th stage of HIV was determined in 42.2% of the HIV-positive women, including stage 4a — 33.3%, stage 4b — 5.6%, and stage 4c — 3.3%. 37.1% of women received highly active anti-retroviral therapy (HAART).

At the time of the examination 18 (37.8%) of 90 patients complained of itching and increased vaginal discharge (10 patients), foul smelling vaginal discharge (4 patients), frequent urination (1 patient), lower abdominal pain (1 patient). 62.2% of women did not have any complaints.

Speculum examination showed no clinical signs of vaginal disorders in 82.2% of women, and abnormal vaginal discharge and/or vaginal mucosa hyperemia.

Thus, 54.4% of women did not present with subjective or objective signs of vaginal disorders and could be considered as clinically healthy, however, microscopic examination of vaginal discharge showed that they had pathological variants of vaginal microbiota. Vaginitis was detected in 60% of HIV-positive women, every fourth patient had candida vaginitis, and every third patient — nonspecific vaginitis. Normal and intermediate variants of vaginal microbiota were detected only in 20% of women, intermediate variant was not often detected.

Absolute normocenosis, according to RT-PCR was detected only in 14.6% of women. Conditional normocenosis was detected in 29% of women. Thus, more than half of all women had dysbiosis (moderate dysbiosis — 17.8% of cases; apparent dysbiosis — 38% of cases).

Vaginal microbiota in HIV-positive women, according to RT-PCR, was compared to vaginal microbiota in HIV-seronegative women.

In the group of HIV-positive women microbiota variants with normal microbiota were detected significantly less often than in the control group. Absolute and conditional normocenosis was detected in 43.3% of HIV-positive women and in 55.5% of women in the control group, moreover, the proportion of absolute normocenosis in HIV-positive women was almost 1.5 times smaller than the control group.

The proportion of dysbiosis in the group of HIV-positive women was statistically significantly higher than in the group of HIV-negative women. Apparent dysbiosis was detected 1.5 times more often in the group of HIV-positive women. Thus, HIV-infection was associated with high incidence of dysbiotic disorders of vaginal microbiota, according to real-time PCR.

Notwithstanding their HIV status, conditional normocenosis in women of the reproductive age in most cases was associated genital mycoplasmas, with *Ureaplasma spp.* detected in the quantity of more than 10^4 GE/ml. *Candida spp.* in the quantity of

more than 10^4 GE/ml was present in every third patient with conditional normocenosis. *Candida spp.* was detected slightly more often as single-agent infection in HIV-positive women. Mixed mycoplasma-yeast infection was detected in 11.5% of HIV-positive women and 18.8% HIV-negative women.

Moderate dysbiosis in HIV-positive women was associated with aerobic microorganisms in 75.1% of cases, with only 43.8% of these cases associated with mixed aerobic-anaerobic dysbiosis. Apparent dysbiosis was mostly associated with anaerobic microorganisms. This suggests that in the early stages of vaginal dysbiosis in HIV-positive women, facultative anaerobes have a larger role, and with further development of dysbiotic disorders they are being displaced by obligate anaerobes.

In the control group moderate dysbiosis is associated with obligate anaerobes in 92.9% of cases. Aerobic microorganisms are mostly limited to mixed dysbiosis. At the same time, apparent dysbiosis in 31.8% of cases was associated with facultative anaerobes: in 18.2% of cases in aerobic dysbiosis, in 13.6% — in mixed dysbiosis.

The quantity of lactobacilli in HIV-positive women is statistically significantly lower compared to HIV-negative women, while TBL is the same in both groups. These data support the notion that the proportion of microbiota with normal microbiota decreases in HIV-positive women.

While the quantity of *Enterobacteriaceae spp.* was significantly higher in HIV-positive women, *Streptococcus spp.* and *Staphylococcus spp*. were detected in higher quantities in healthy women. At the same time, the quantities of the *Enterobacteriaceae spp., Streptococcus spp., Staphylococcus spp.* were practically the same in HIV-negative women. In HIV-positive women the quantity of *Enterobacteriaceae spp.* was 1000 higher than the quantities of *Streptococcus spp., Staphylococcus spp*.

To determine the role of particular representatives of opportunistic microbiota in the development of different microbiota variants, their incidence in clinically significant amounts was evaluated: if the proportion of a microorganism in the microbiota was more than 1% of the sum of all the detected microorganisms.

Enterobacteriaceae spp. was detected in the amount of more than 1% in 54.5% of HIV-positive women in absolute normocenosis, while in healthy women the presence of enterobacteria and other facultative and obligate anaerobes was rare. *Gardnerella vaginalis, Eubacterium spp., Peptostreptococcus spp.* was detected in HIV-positive women in 9.1% of cases. In healthy women in absolute normocenosis *Eubacterium spp*. was detected most often (19% of cases). Similar distribution was typical for conditional normocenosis.

In moderate dysbiosis in HIV-positive women enterobacteria were detected in 76.5% of cases, and together with *Gardnerella vaginalis* they are detected the most often in vaginal dysbiosis in HIV-positive women.

In apparent dysbiosis and given the decrease in the quantity of lactobacilli, enterobacteria are detected less often, however, the incidence of obligate anaerobes increases, particularly *Megasphaera spp., Gardnerella vaginalis, Eubacterium spp., Atopobium vaginae. A. vaginae* was detected in 76.5% of HIV+ women with apparent dysbiosis, which is 1.5 times more often than in HIV-negative patients. Given the resistance of *Atopobium vaginae* to metronidazole (used for treatment of bacterial vaginosis), it is possible that recurrent bacterial vaginosis in HIV-positive women is associated with high incidence of *A. vaginae* persistence in vaginal microbiota together with the decreased quantity of normal microbiota.

The results of this study suggest that absolute and conditional normocenosis in HIV-positive women are not very stable microbiota variants due to the presence of opportunistic microorganisms in clinically significant quantities which given the increase in their proportion cause the development of dysbiotic disorders.

The development of infectious inflammatory disorders in HIV-positive women is associated with the increase of opportunistic microorganisms, including genital mycoplasmas and yeast-like fungi. *Candida spp*. in the quantity of more than 10^4 GE/ml was detected in HIV-positive patients 1.5 times more often than in the control group. *Mycoplasma spp.* also was detected significantly more often in HIV-positive women. The results of this study suggest that presence of *Mycoplasma spp*. and *Candida spp.* in the quantity of more than 10^4 GE/ml in the vagina of HIV-positive women facilitates the development of inflammatory disorders associated with these pathogens. Vaginal dysbiosis is also followed by the change in the vaginal pH, which creates favorable conditions for *Mycoplasma spp.*

It is necessary to solve a number of questions concerning treatment of HIV-positive women with vaginal dysbiosis. To what degree is the increase in the quantity of genital mycoplasmas associated with high incidence and severity of vaginal dysbiotic disorders in HIV-positive women? Is it possible to decrease the quantity of genital mycoplasmas by correcting vaginal dysbiosis in this category of women? Given that most variants of detected variants of vaginal dysbiosis are asymptomatic, it is necessary to follow a more active tactic of detection and treatment of vaginal disorders in HIV-positive women.

Conclusions

Qualitative and quantitative composition of vaginal microbiota in HIV-positive women of the reproductive age significantly differs from that in HIV-seronegative women. According to RT-PCR, absolute normocenosis was detected only in 14.6% of HIV-positive patients. Dysbiosis was detected more than in half of the women, 38% of whom had apparent dysbiosis.

Enterobacteriaceae spp. and *Atopobium vaginae* have a significant role in the development of dysbiotic disorders in HIV-positive women.

Mycoplasma spp. and *Candida spp.* were detected in every third HIV-positive patient in the quantities higher than 10^4 GE/ml. Such incidence of mollicutes and yeast-like fungi in significant amounts can create conditions the development of inflammatory genital disorders associated with these opportunistic infections.

Recurrent Vaginal Dysbiosis Associated With Bacterial Vaginosis In Pregnant Women With HPV Infection

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The rate of human papillomavirus (HPV) infection among pregnant women makes up from 5.5 to 65% and higher compared to nonpregnant women — 25% against 13% respectively.

The rate of other infections concurrent with HPV is more than 70%. A positive association was established between bacterial vaginosis (BV) and HPV. Vulvovaginal candidiasis (VVC) and aerobic vaginitis (AV) occur more often in women diagnosed with HPV infection, however, their interrelation has not been identified.

The aim of this study is to determine the rate of recurrent vaginal dysbiosis associated with BV, their patterns, clinical features, and laboratory parameters in HPV-positive pregnant women.

Materials and methods

A total of 682 HPV-positive pregnant women aged 21–45 were examined. The patients were in their II–III trimesters (18–36 weeks), and they were diagnosed with HPV either before the current pregnancy (while cervical screening or preconception care) or in their I trimester (while cervical screening). Their ICD-10 diagnoses included N88 — other noninflammatory disorders of cervix uteri (bacterial vaginosis — BV), B37.3 — candidiasis of vulva and vagina (vulvovaginal candidiasis — VVC), N76.0 — acute vaginitis (nonspecific or aerobic vaginitis — AV); and they had a recurrent episode of the disease during the current pregnancy. All patients who participated in the study signed a consent form.

In order to identify, genotype and evaluate the quantity of HPV,real-time PCR test was performed using HPV QUANT-21 kit (DNA-Technology).Clinical presentation was evaluated based on patients' complaints and on the speculum examination of the vulva, vagina and the cervix.Vaginal content pH was measured using pH strips.

Microscopy was performed to analyze vaginal microbiota and vaginal inflammatory response; 4 vaginal smear types were distinguished. Total bacterial load (TBL) and presence of microorganisms in the significant amounts were analyzed using Femoflor-16 real - time PCR kit (DNA-Technology LLC). The sensitivity of the method is 99%, the specificity is 93%.The *Lactobacillus spp.* proportion >80% of the TBL with $<10^4$ genome equivalents (GE) / ml of *Ureaplasma spp., M. hominis* was interpreted as absolute normocenosis; *Lactobacillus spp.* proportion >80% of in the TBL with

Ureaplasma spp., M. hominis >10^4 GE / ml of— conditional normocenosis; 10–20% of one or more opportunistic microorganisms in the TBL with <80% of *Lactobacillus spp.* — anaerobic and aerobic dysbiosis; both obligate and facultative anaerobes, *Candida spp*., often a decrease in the proportion of *Lactobacillus spp*. — mixed dysbiosis. *Candida spp*. may be present both in normocenosis and dysbiosis.

The classification of cervical cytology (Papanicolaou test) was performed according to the Bethesda System.

Statistical data analysis was performed using SAS 9.4, Statistica 12 and IBM-SPSS-24 statistical packages.

Results

High-oncogenic HPV types were detected in the majority of the patients included in the study (30.8% had HPV 16; 57.5% had other high-oncogenic HPV types). 38.7% (264/682) of the examined women had vaginal dysbiosis; 48.1% (127/264) of these women had a recurrent episode during the current pregnancy, these patients had 1–2 episodes of dysbiosis during the current pregnancy,before the start of the study, and were treated with antimicrobial agents in accordance with Russian clinical guidelines.

The majority of patients with recurrent dysbiosis had STDs in the past — 59.1% (75/127) and / or nonspecific infections — 79.5% (101/127). Recurrences of the latter (before the current pregnancy) occurred more often in patients with BV in combination with AB — 23.8% (24/101), less often–in patients with isolated BV — 14.9% (15/101) and in combination with VVC— 11.9% (12/101).

This study found that clinical manifestations do not always accompany recurrent dysbiosis in pregnant women with HPV. Complaints on moderate or heavy vaginal discharge were presented by 65.4% (83/127) patients, including the complaints about an unpleasant odor — 41.7% (53/127), itching and burning — 51.2% (65/127). For the rest of the patients, dysbiosis was asymptomatic (34.6%). An objective examination showed that a third of the patients had inflammation.

According to microscopy and Femoflor-16 results BV was detected in 34.7% (44/127) of HPV-positive patients with recurrent vaginal dysbiosis, BV in combination with AB — in 44.1% (56 / 127), BV with VVC - in 18.1% (23/127), BV, AV and VVC in 3.2% (4/127). The total frequency of mixed infections was 65.4% (83/127).

AV (according to microscopy) in pregnant women was characterized by the absence of basal and parabasal cells.

Femoflor-16 detected the prevalence of *Gardnerella vaginalis* (*G. vaginalis*) and/or *Atopobium vaginae* (*A. vaginae*) over the concentration of *Lactobacillus spp.* in all patients.

In women with BV the most frequent combinations of bacteria were *G. vaginalis* with *A. vaginae* and *Ureaplasma spp., G. vaginalis* with *Mobiluncus spp*. and *Corinebacterium spp*. At the same time, in patients with BV and AV, *G. vaginalis, A. vaginae, Mobiluncus spp.* prevailed in combination with *Streptococcus spp.* and *Staphylococcus saprophyticus*; in patients with BV and VVC, *G. vaginalis* and *Candida spp*. prevailed; in patients with BV, AB, and VVC, *G. vaginalis, Enterobacterium spp., Candida spp.* were prevalent.

Cervical intraepithelial neoplasia (CIN) was detected in every fourth patient, while squamous epithelial cells with atypical squamous cells of undertermined significance (ASC-US) were determined in 12.6%, low grade squamous intraepithelial lesions (LSIL) — 11.8%, high grade squamous intraepithelial lesions (HSIL) — 0.8%.

Discussion

Currently dysbiosis is considered as qualitative and quantitative changes of the local microbiota, including BV and other nonspecific infections which often recur.

Given the polymicrobial nature of the disease in the majority of the women in our study, evaluation of vaginal microbiota and detection of any opportunistic microorganisms were especially important.

Firstly, vaginal microbiota and vaginal inflammatory response were analyzed using microscopy. However, this method is known to be of low sensitivity, and its results are considered subjective, since it does not allow us to identify most of the morphotypes of bacteria and quantify them.

Vaginal microbiota was also assessed using real-time PCR (Femoflor-16 kit), one of the most sensitive and specific microbiological methods; it is especially informative in the detection of non-culturable, difficult to culture and persistent bacteria.

Anaerobic dysbiosis was characterized by the prevalence of obligate anaerobic bacteria, including the metronidazole-resistant *A. vaginae* (considered to be a marker of recurrent processes), clinically significant amounts of *Ureaplasma spp*., and a very small amount of lactobacilli.

In the presence of HPV, the proportion of *Lactobacillus spp.* is lower and the content of anaerobes in the vaginal microbiota is higher. According to the literature, the abundance of *Lactobacillus spp.* in vaginal microbiota reduces the risk of HPV infection compared to microbiota, predominated with obligate anaerobes, including *G. vaginalis, A. vaginae* and *Prevotella spp*. Literature also indicates a positive relationship between BV and HPV infection. Although the mechanisms of these associations are not fully understood, it is assumed that the vaginal communities of bacterial commensals and adaptive/innate host immune systems are involved.

The absence of basal and parabasal cells in vaginal smears of pregnant patients with BV in combination with AV Donders et al. explain with high estrogen levels in pregnant women.

Vaginal microbial community might be involved in the development of cervical intraepithelial neoplasia (LSIL and HSIL) and cervical cancer. In the present study, cervical intraepithelial changes were detected in every fourth patient, and the frequency of LSIL was 6 times higher compared to the study of P. Mongelos et al., who found that patients with benign changes in the cervical epithelium are characterized by initially high incidence of concomitant disorders of vaginal microbiota, including BV (44.6%) and vaginitis (26.8%). This indicates the possible influence of opportunistic vaginal microorganisms on the persistence of HPV in cervical epithelium cells and the development of low-grade intraepithelial neoplasias.

Earlier we have shown that correction of vaginal dysbiosis and the restoration of normal vaginal microbiota contributes to the recovery from the cervix inflammation and to the reduction of ASC-US in 100% of the patients, however the rate of intraepithelial damage to the cervix caused by the persistent HPV (LSIL and HSIL) does not change under the influence of dysbiosis treatment.

Conclusion

There is a high incidence (38.7%) of vaginal dysbiosis in HPV-positive pregnant women, and it is characterized by recurrent course (48.1%) in most of them.

Asymptomatic dysbiosis was determined in 34.6% of HPV–positive pregnant women . When dysbiosis recurs in II and III trimesters, the average pH values are 4.53 ± 1.62; mainly mixed infection with opportunistic microorganisms are observed in various combinations (65.4%), with the prevalence of anaerobic-aerobic infection (44.1%). With the presence of aerobic microorganisms, the absence of basal and parabasal cells is typical. Cervical intraepithelial changes occur in every fourth patient of predominantly low risk.

Characteristics of Vaginal Microbiota in HIV-Positive Pregnant Women

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Inflammatory vaginal disorders, such as bacterial vaginosis (BV), vulvovaginal candidiasis, are often detected in HIV-positive women and are characterized by a recurrent course. In pregnant women BV is a risk factor for a number of complications — pregnancy loss, polyhydramnios, premature rupture of membranes, preterm labor. BV could also be a cause of postpartum inflammatory complications. The state of vaginal microbiota has an impact not only on the woman but also on the fetus and the newborn.

Real-time PCR (RT-PCR) allows specialists to perform a comprehensive (quantitative and qualitative) evaluation of complex relationships between the microorganisms of the urogenital tract of HIV+ pregnant women.

The aim of this study was to describe the structure of vaginal microbiota, according to real-time PCR, in HIV-positive pregnant women.

Materials and Methods

The study included 106 HIV-positive pregnant women aged 18–39. 27.4% of these women were planning to terminate the pregnancy, and 72.6% of the women were planning to carry to term. 75.5% of the pregnant women were examined in the I trimester, 19.8% — in the II trimester, 4.7% — in the III trimester.

The control group included 106 HIV-negative pregnant women, selected using case-control method. Patients from the control group matched the HIV-positive women according to the following parameters: age, gestational age at the time of examination, the nature of complaints and clinical manifestations, the state of vaginal microbiota according to microscopy.

Exclusion criteria for the control group were: presence of obligate pathogenic sexually transmitted infections (*Chlamydia trachomatis, Trichomonas vaginalis, Neisseria gonorrhea, Mycoplasma genitalium*), syphilis, systemic or topical antimicrobial or antimycotic treatment within the period of 4 weeks before the examination.

Vaginal microbiota was evaluated by means of RT-PCR using Femoflor-16 kit (DNA-Technology, LLC). Vaginal microbiota variants were classified according to the earlier established algorithm. Statistical data processing was performed using SPSS Statistics v.17.0 software package.

Results and Discussion

At the time of examination 81.2% of women had the 3rd stage of HIV-infection, 18.8% — the 4th stage, including stage 4a in 17.9% of patients, stage 4c in 1 patient (0.9%). 19.8% of women received highly active anti-retroviral therapy (HAART), including 10.4% women with the 3rd stage of HIV-infection, 8.5% of women with stage 4a HIV, 0.9% of women with stage 4c HIV.

Complaints at the time of the examination were presented by 19.8% of women, including increased vaginal discharge (9.4%), itching and vaginal discharge (1.9%), vaginal discharge with odor (1.9%). 80.2% of the pregnant women did not have any complaints. Objective signs of vaginal disorder (pathological discharge and/or vaginal mucosa hyperemia) were found in 29.2% of women, 70.8% of patients were clinically healthy. Thus, at the time of the examination 61.3% of women did not have subjective or objective signs of vaginal pathology.

According to the results of RT-PCR, absolute normocenosis was detected only in 22 (20.8%) pregnant women. Conditional normocenosis was detected in 31 (29.2%) women. Thus, variants of microbiota with normal microbiota were found in half of the patients. According to RT-PCR results, 50% of pregnant women had dysbiotic disorders of the vaginal microbiota, including moderate dysbiosis in 18 (17%) women, apparent dysbiosis in 35 (33%) women.

Microbiota variants with normal microbiota were detected significantly less often in HIV-positive women, with absolute normocenosis detected almost twice less often compared to the control group. At the same time, the incidence of conditional normocenosis was similar in both groups.

HIV-infection in pregnant women was associated with higher incidence of vaginal dysbiosis according to RT-PCR.

Conditional normocenosis in HIV-positive women was associated with genital mycoplasmas in the quantity of more than 104 GE/ml in 80.7% of cases. In 48.4% of cases this was mycoplasma monoinfection, and in 32.3% of cases it was mixed infection associated with mycoplasmas and *Candida spp.* in the quantity of more than 10^4 GE/ml. *Ureaplasma spp*. (24 out of 25 cases) and *Mycoplasma spp*. (5 cases) were detected in conditional normocenosis; both microorganisms were detected in 4 cases. Conditional normocenosis associated with *Candida spp*. in the quantity of more than 10^4 GE/ml was detected in 51.6% of women; in 19.3% of patients it was present as a monoinfection.

The structure of conditional normocenosis in pregnant women did not vary depending on their HIV status. In half of the cases conditional normocenosis in patients from the control group was associated with *Candida spp*., in every third patient —

with mixed mycoplasma-yeast infection. Thus, it is possible that high incidence of *Candida spp.* is caused by the reproductive status of a woman rather than by the presence of HIV-infection.

Aerobic microorganisms (in particular, *Enterobacteriaceae spp*.) were present in moderate dysbiosis in 38.9% of HIV-positive women, with aerobic dysbiosis in 11.1% of cases and mixed aerobic-anaerobic dysbiosis in 27.8% of cases. In apparent dysbiotic disorders and with the significant decrease in the quantity of lactobacilli, enterobacteria are detected less often, while the incidence of obligate anaerobes (*Gardnerella vaginalis, Megasphaera spp., Eubacterium spp., Atopobium vaginae*) increases. Apparent anaerobic dysbiosis was detected in 78.6% of cases, and apparent aerobic-anaerobic dysbiosis was detected in 12.8% of cases. Similar changes were observed in the control group.

The quantity of lactobacilli in HIV-positive women is statistically significantly lower compared to HIV-negative women.

Facultative anaerobes have different roles in vaginal microbiota in HIV-positive and HIV-negative women. While the quantity of *Enterobacteriaceae spp*. was significantly higher in HIV-positive women, *Streptococcus spp.* and *Staphylococcus spp.* were detected in higher quantities in healthy women. At the same time, the quantities of *Enterobacteriaceae spp., Streptococcus spp., Staphylococcus spp*. were practically the same in HIV- women. In HIV+ women the quantity of *Enterobacteriaceae spp.* was 1000 higher than the quantities of *Streptococcus spp., Staphylococcus spp*.

In the group of obligate anaerobes, statistically significant differences were found in the quantities of *Gardnerella vaginalis, Eubacterium spp., Megasphaera spp., Peptostreptococcus spp., Atopobium vaginae. Mycoplasma spp*. was detected significantly more often in HIV-positive women compared to the control group. The quantities of *Ureaplasma spp., Candida spp.* did not differ between the two groups.

To determine factors, influencing the state of vaginal microbiota in HIV-positive women, the structure of vaginal microbiota was analyzed in relation to the gestational age, the stage of HIV-infection, viral load, quantity of CD4 cells, and HAART.

The proportion of microbiota with normal microbiota (absolute and conditional normocenosis) in HIV-positive women increased during the course of pregnancy, and was 43.9%, 54.8%, and 71.4% in the I, II, and III trimesters respectively. Similar pattern was established before in the observation of HIV-seronegative pregnant women.

No relationship was established between the state of vaginal microbiota and viral load or the quantity of CD4 cells. This could be explained by the fact that pregnant women with high viral load and the quantity of CD4 cells of less than 200 per ml

received HAART, which turned out to be the factor that had a significant impact on the state of vaginal microbiota. Vaginal dysbiosis persisted in women who did not receive HAART. Normocenosis was detected statistically significantly more often in women who received HAART.

Moreover, a significant decrease in the quantity of genital mycoplasmas was observed in HIV-positive women who received HAART. Genital mycoplasmas in the quantity of more than 10^4 GE/ml were detected significantly less often in patients who received HAART (47.6%) compared to women who did not receive HAART (74.1%).

Conclusion

HIV-infection is a factor, significantly worsening the state of vaginal microbiota in pregnant women. Vaginal dysbiosis was detected in half of the examined women, while absolute normocenosis was diagnosed only in every fifth HIV-positive pregnant woman.

Vaginal microbiota state in HIV-positive women does not undergo significant changes with the decrease of CD4 cells and the increase of the viral load.

The decrease in the proportion of dysbiosis and the increase in the proportion of normocenoses, as well as the decrease in the quantity of genital mycoplasmas, were detected in HIV-positive pregnant women receiving HAART.

Characteristics of Endometrial Microbiota in Patients with Chronic Endometritis and a History of Unsuccessful In Vitro Fertilization

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Pelvic inflammatory diseases (PID), including chronic endometritis (CE), have a negative impact on endometrium receptivity which is one of the main factors of successful implantation. The role of endometrial microbiota in the development of chronic inflammatory processes, receptivity disorders and unsuccessful in vitro fertilization (IVF) is unclear and needs to be further researched.

The aim of this study was to evaluate the characteristics of endometrial microbiota in patients with chronic endometritis of varying severity and a history of ineffective IVF protocols.

Materials and Methods

Inclusion criteria: women aged 20–40; one or more unsuccessful IVFs with good quality embryo transfer; morphologic features of chronic endometritis. Chronic endometritis diagnosis was based on the results of histological and immunohistochemical examination of endometrium.

Patients were divided into 3 groups depending on the severity of morphologic changes in the endometrium. The 1st group included the patients with mild CE (n=34), the 2nd group — moderate CE (n=64), the 3rd group — severe CE (n=9).

Endometrial microbiota was evaluated by means of RT-PCR (Femoflor 16, DNA-Technology).

Results

Staphylococcus spp. (75.5%), *Enterobacteriaceae spp.* (41.1%), *Eubacterium spp.* (41.1%), *Streptococcus spp.* (35.2%), *Ureaplasma* (23.5%), *Lachnobacterium spp.* + *Clostridium spp.* (20.5%) were most often detected in patients with mild CE.

Compared to the 1st group, *Enterobacteriaceae* (67.1%), *Streptococcus spp.* (59.3%), *Atopobium vaginae* (28.1%) were detected significantly more often in patients with moderate CE. Moreover, *Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.* (4.6%), *Megasphaera spp. + Veillonella spp. + Dialister spp.* (4.6%) were detected only in the 2nd group.

Microbiota identified in patients with severe CE did not have statistically significant differences compared to the 1st and 2nd groups. The following microorgan-

isms were often present in the endometrial microbiota of women from the 3rd group: *Staphylococcus spp.* (55.5 %), *Enterobacteriaceae spp*. (55.5%), *Eubacterium spp.* (55.5%), *Streptococcus spp.* (33.3%), *Ureaplasma* (33.3%), *Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.* (22.2%), *Mobilincus spp. + Corynebacterium spp.* (22.2%), *Atopobium vaginae* (22.2%).

Conclusions

The study showed a correlation between morphologic features of CE and the presence of specific microbiota in the endometrium. In particular these are facultative anaerobic microorganisms: *Staphylococcus spp., Enterobacteriaceae spp., Streptococcus spp.* Moreover, *Sneathia spp. + Leptotrichia spp. + Fusobacterium spp.* (4.6%), *Megasphaera spp. + Veillonella spp. + Dialister spp.* (4.6%) were detected only in the 2nd group.

Negative impact of gram-negative microorganisms could be caused by bacterial endotoxin which is an essential element of the cell surface of all gram-negative microorganisms (bacteria and cocci), and it is released in the environment only after their destruction.

Some studies show that endotoxin has an influence on the endometrial and trophoblast receptors (TLR-2, TLR-4), as well as on the activation of immunocytes. This, in turn, leads to the activation of trophoblast cells apoptosis, implantation disorders, miscarriage, preterm labor.

Vaginal Microbiota in Patients with HPV-Associated and HPV-Negative Cervical Intraepithelial Neoplasias

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Chronic inflammation is one of the etiological factors for the development of precancer and neoplastic diseases of cervical epithelium. Previous studies established a correlation between bacterial vaginosis (BV) and the persistence of human papilloma virus (HPV) in the cervical canal. According to some studies, BV is one of cofactors for the development of cervical neoplasias. According to J.M. Klomp et al., out of all anaerobes *Gardnerella vaginalis* is found most often in cervical neoplasias.

Since opportunistic bacteria in high concentrations in the vagina and the cervix potentially can cause and maintain dysplasia, it is important to study vaginal microbiota during cervical intraepithelial neoplasias and HPV persistence in order to understand the mechanisms of cervical epithelium neoplastic transformation.

Quantitative and qualitative evaluation of vaginal microbiota composition will allow us to develop a differentiated approach to the treatment of dysbiotic diseases in patients with cervical neoplasias before ablation.

The aim of this study was to analyze qualitative and quantitative composition of vaginal microbiota in patients with cervical intraepithelial neoplasias (CIN).

Materials and Methods

413 patients (311 with CIN and 102 without CIN) were examined in accordance with the diagnostic standard: speculum and bimanual examination, ecto- and endocervical cytological screening, PCR test for HPV, extended colposcopy, histological examination of the cervix. Vaginal microbiota was evaluated by means of RT-PCR (Femoflor-16, DNA-Technology).

Vaginal microbiota was evaluated according to the following classification:

1. Absolute normocenosis — the proportion of lactobacilli is more than 80% of the total bacterial load (TBL), and the quantity of *Ureaplsma spp., Mycoplasma spp., Candida spp*. is less than 10^4 GE/ml;
2. Conditional normocenosis — the proportion of lactobacilli is more than 80% of total bacterial load, and the quantity of *Ureaplsma spp., Mycoplasma spp., Candida spp*. is more than 10^4 GE/ml;
3. Moderate (aerobic or anaerobic) dysbiosis — the proportion of lactobacilli is 20–80% of the total bacterial load, and the proportion of opportunistic microorganisms is more than 20%;

4. Apparent (aerobic or anaerobic) dysbiosis — the proportion of lactobacilli is less than 20% of the total bacterial load, and the proportion of opportunistic microorganisms is more than 20%.

   HPV was detected by means of RT-PCR (HPV Quant 21 kit, DNA-Technology).

Patients were divided into 3 groups: the 1st group included 100 HPV-negative patients with LSIL, the 2nd group included 109 HPV-positive patients with LSIL, the 3rd group comprised of 102 HPV-positive patients with histologically confirmed HSIL. Control group included 102 HPV-negative women with visually unchanged cervix.

Statistical data processing was performed using SPSS Statistics v. 20.0.

Results and Discussion

The analysis of vaginal microbiota composition showed significant differences between the groups of examined patients, depending on the state of the cervical epithelium and the presence of HPV infection. Normocenosis was detected in 98% of clinically healthy women (control group).

Normocenosis was detected significantly less often in patients from the 1st group (LSIL, HPV-negative): absolute normocenosis — 41%, conditional normocenosis — 33%. Dysbiosis, both moderate and apparent, was detected in 26% of the patients. Vaginal microbiota composition of women in the 2nd group (LSIL, HPV-positive) was similar. Thus, dysbiotic diseases were detected significantly more often in women with LSIL, regardless of their HPV status. These points to the significance of vaginal dysbioses in the development of dysplasia in the cervix initially even without HPV infection. However, patients, HPV-negative at the time of the examination, might have been infected in the past. In case of a past spontaneous elimination, HPV cannot be detected at present, and the concomitant dysbiosis contributes to maintaining the changes in the epithelium typical of LSIL.

Dysbiosis, both moderate and apparent, was detected in 58.8% of women from the 3rd group (HSIL), which is significantly more often compared to women in the control group and in patients with LSIL. This could support the theory on the role of viral-bacterial associations in the progression of cervical dysplasia.

In the structure of apparent dysbioses, anaerobic dysbiosis was prevalent in women from the 1st, 2nd, and 3rd groups (52.9%, 68.2%, 75% respectively). Aerobic and mixed aerobic-anaerobic dysbiosis was detected significantly less often. This corresponds with the results of previous studies, suggesting that there is a correlation between CIN and BV.

TBL was the highest in patients from the 3rd group, while the number of lactobacilli was significantly lower compared to women in the control group and groups 1 and 2. Absolute quantity of obligate and facultative anaerobes was significantly higher in women with HSIL compared to patients in other groups (especially *Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp., Atopobium vaginae, Eubacterium spp.,* and *Megasphaera spp./Veillonella spp./Dialister spp.* the quantity of which was 1000–10000 higher).

The results of this study suggest that there is a correlation between the grade of CIN and the severity of dysbiosis in patients with cervical lesions. The question is whether it is vaginal dysbiosis that contributes to the development of more pronounced changes in the cervical epithelium infected with HPV, or is it the persistence of HPV that creates a favorable background for the proliferation of opportunistic obligate anaerobes in the vagina, leading to apparent dysbiosis. Given the high rates of dysbiotic diseases in patients with cervical lesions, especially with HSIL, it is necessary to conduct a comprehensive analysis of vaginal microbiota, as well as individual treatment of dysbiosis for this group of patients.

Conclusions

The development of cervical intraepithelial neoplasias in HPV-negative women is accompanied by vaginal dysbiosis, both anaerobic and mixed aerobic-anaerobic dysbiosis. In the majority of cases, *Gardnerella vaginalis* was detected in association with *Eubacterium spp., Megasphaera spp., Mobiluncus spp*.

HPV-associated cervical neoplasias are accompanied by the development of apparent dysbiosis with the prevalence of obligate anaerobes (*Gardnerella vaginalis* in association with *Atopobium vaginae, Megasphaera spp./Veillonella spp./Dialister spp., Eubacterium spp.*).

Diversity of the prevalent pathogens shows the need for a comprehensive analysis of vaginal microbiota in patients with cervical precancerous processes, allowing to give a quantitative and qualitative evaluation of the vaginal microbial community and determine the appropriate course of treatment.

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