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Research Article

Study of the State of the Vaginal Microbiota in Women with Risk and Threat of Preterm Birth

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Abstract

In etiopathogenetic terms, premature birth is considered as a clinical syndrome characterized by polyetiological factors, the participation of the fetus in pathogenesis, a variety of clinical symptoms, and the involvement of genetic and environmental factors. At the same time, there is increasing evidence that the composition of a woman's vaginal microbiota significantly affects her sexual and reproductive health, including the risk of adverse pregnancy outcomes, including miscarriage and preterm birth. The purpose of the work was to assess the state of the vaginal microbiota in women with risk factors and the threat of spontaneous premature birth. 150 women of reproductive age took part in the study. The inclusion criteria for the study were the presence of risk factors for preterm birth or the threat of preterm birth. Determination of the pH of vaginal contents, molecular biological, and bacterioscopic methods were used to assess the state of the vaginal microbiota.

The conducted studies indicate a high risk of preterm birth in the presence of dysbiotic and inflammatory changes in the vaginal microbiota - odds ratio (OR) = 2.962 (95% CI 1.32-6.645). At the same time, for pregnant women with risk factors for preterm birth, OR makes up = 8.120 (95% CI 2.149-30.686), and for pregnant women with diagnosed threatened preterm birth - OR = 10.133 (95% CI 3.149-32.604). Thus, one of the risk factors for the development of spontaneous premature and threatened premature births is changes in the state of the vaginal microbiota, which requires the development of diagnostic and therapeutic measures to prevent premature termination of pregnancy and reduce the frequency of obstetric and perinatal complications.

Keywords: Preterm Labor; Vaginal Microbiota; Bacterial Vaginosis; Aerobic (Mixed) Vaginitis; Vaginal Dysbiosis

Introduction

Preterm birth affects approximately 11% of births worldwide [6,8,27]. In etiopathogenetic terms, premature birth is considered as a clinical syndrome characterized by polyetiological factors, the participation of the fetus in pathogenesis, a variety of clinical symptoms, and the involvement of genetic and environmental factors. Although many factors have been shown to increase the risk of spontaneous preterm birth, the majority of preterm births occur in women without a clear risk factor [6,27]. The conducted studies showed that among the risk factors associated with spontaneous PP, the previous history of preterm birth (OR 12.06; 95% CI 6.21-23.43) and cervical length less than 2.5 cm (OR 3.97; 95% CI 1.67-9.47), although the search continues for methods to predict preterm birth [5,6,21]. At the same time, there is increasing evidence that the composition of a woman's vaginal microbiota significantly affects her sexual and reproductive health, including the risk of adverse pregnancy outcomes, including miscarriage and preterm birth [2,3,24]. In some countries, low-cost screening programs involving the prevention and diagnosis of vaginal infection, especially asymptomatic ones, have been recognized

as an effective measure to reduce the risk of premature birth. To detect it, a pregnant woman uses an independent measurement of the pH level of vaginal discharge twice a week using an indicator strip that is administered intravaginally [14].

The vaginal ecosystem is characterized by polymorphic endogenous microflora, changes in which are caused by dysbiotic and inflammatory processes of the birth canal [7,15,21,26]. The vast majority of studies on the condition of the microbiota of the pregnant woman's vagina relate to preterm birth, which develops against the background of premature rupture of the fetal membranes, while studies on the relationship between vaginal dysbiosis and spontaneous preterm birth are few and the results are contradictory [4,16,23].

Some studies have shown that women who had bacterial vaginosis in the first trimester have the increased risk of spontaneous premature birth before 35 weeks of pregnancy, approximately doubles the risk of preterm birth in asymptomatic patients, significantly increases the risk of late miscarriage and maternal infection [8,10,13]. A number of authors proved the relationship between premature birth and asymptomatic bacteriuria, bacterial vaginosis not treated before 20 weeks of pregnancy, revealed the relationship between the increased titer of opportunistic pathogens in the cervical canal and the frequency of premature birth [12,16,24]. Bacterial vaginosis is a common phenomenon and is caused by a violation of the microbiological environment in the lower parts of the genital tract [8,14,23]. In the United States, the prevalence of bacterial vaginosis among pregnant women ranges from 5.8% to 19.3% and is higher in some racial/ethnic groups [16]. Aerobic vaginitis, bacterial vaginosis during pregnancy is associated with adverse obstetric outcomes, including early miscarriage, premature birth, low birth weight, and postpartum endometritis [13,18]. However, the USPSTF recommends against screening for bacterial vaginosis in pregnant women who are not at increased risk of preterm birth and notes that the available data are insufficient to assess the balance of benefits and harms of screening for bacterial vaginosis in pregnant women at increased risk of preterm birth [16]. At the same time, the opinion is expressed that, the vaginal microbiome may be an effective prognostic indicator of preterm labor and of the effects of tocolytic treatment for preventing preterm birth [17,20].

Despite numerous studies, the use of new technologies and drugs, the prevention and prediction of spontaneous premature birth remains insufficiently effective. This requires the improvement and development of effective methods of primary and secondary prevention of this syndrome, which determines the relevance of the study of factors, regarding the participation of which in the pathogenesis of premature birth, a unanimous opinion has not been formed.

The purpose of the work was to assess the state of the vaginal microbiota in women with risk factors and the threat of spontaneous premature birth.

Materials and Methods

150 women of reproductive age took part in the study. The inclusion criteria for the study were the presence of risk factors for preterm birth or the threat of preterm birth. The first (I) group consisted of 80 women with risk factors for premature birth who applied for antenatal care at 10-12 weeks of pregnancy. The second (II) group was formed from 70 women hospitalized in an obstetric hospital with a threat of premature birth at 22-28 weeks of gestation. The control group consisted of 20 conditionally healthy pregnant women in the period of 10-12 weeks of pregnancy. Ultrasound (transvaginal) examination and evaluation of the condition of the vaginal microbiota were performed upon inclusion in the study, again at 17-21 weeks of gestation in pregnant women of the first group and control groups. The exclusion criterion from the study was the confirmation of premature rupture of the fetal membranes during the Actim Prom test.

The diagnosis of threatened preterm labor was made on the basis of preterm contractions and shortening of the cervix less than 2.5 cm. Determination of the pH of vaginal contents, molecular biological, and bacterioscopic methods were used to assess the state of the vaginal microbiota [18,22,25]. pH-metry of the vaginal environment was performed using CITOLAB-pH indicator test strips. The study of the state of the microbiota of the vagina was carried out by the PCR method with detection of the result in real time with quantitative assessment of the total bacterial mass, various groups of opportunistic pathogens and lactoflora (Florocenosis, 18 indicators). The study involved detection of DNA of *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma*

genitalium, *Trichomonas vaginalis* (qualitative), quantitative detection with DNA typing of *Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis/tropicalis*, quantitative detection of DNA of *Ureaplasma urealyticum, Ureaplasma parvum*, *Mycoplasma hominis* with parallel quantitative DNA typing of *Lactobacillus* spp., *Gardnerella vaginalis*, *Atopobium vaginae*, *Enterobacteriaceae, Staphylococcus* spp., *Streptococcus* spp.

Normobiocenosis of the vagina was interpreted according to the manufacturer's instructions: total bacterial mass - 10^6-10^8; Lactobacillus - 106-10^8, aerobic and anaerobic opportunistic microorganisms in an absolute amount less than 10^4 GE/ml (0.1%-1%), *M. genitalium, U. parvum, Candida* fungi are absent or less than 10^4 GE/ml. When the specified indicators are exceeded, the state of the microbiota of the vagina was considered as aerobic, anaerobic or mixed dysbiosis (in combination with yeast fungi of the genus Candida), depending on the predominant microorganisms [5,16,20,23,26].

For bacterioscopic examination of discharge from the vagina, the material was obtained from the posterior and lateral vault with Gram staining. During the microscopic examination of the smear, the number of leukocytes, epithelial cells, and the nature of the microflora (lactobacteria, bacilli, cocci, "key cells", Trichomonas vaginalis, Neisseria gonorrhoeae, Candida spp.) in the field of view were determined. Bacterial vaginosis was also diagnosed according to Amsel's criteria (R. Amsel., *et al*. 1983): abundant homogeneous, white-gray vaginal discharge with an unpleasant odor; pH of discharge from the vagina is more than 4.5; positive amino test (whiff test); detection of "key" cells in a vaginal smear. The diagnosis was considered confirmed in the presence of three out of four signs. Observation of pregnant women of the studied cohort continued until the end of pregnancy.

Statistical processing of the results was carried out using the standard Microsoft Excel 7.0 and "Statistica 6.0" programs. Intergroup comparisons were made using the Mann-Whitney U test or one-way analysis of variance. Because of the exploratory nature of this study, a P value <0.05 was considered statistically significant.

Consent to the publication of data from the research units is provided by the methodology and design of the study.

The study was conducted in accordance with the principles of the Declaration of Helsinki, the Convention of the Council of Europe on Human Rights and Biomedicine, the relevant laws of Ukraine, modern bioethical norms regarding safety for the health of patients, obtaining informed consent and confidentiality of personal and medical data (Conclusion of the commission on the ethics of scientific research, experimental developments and scientific works of the Danylo Halytskyi Lviv National Medical University, protocol No5 dated 23.05.2022).

Research Results and their Discussion

The average age of the patients was 28.4 ± 3.5 years. There was no significant difference in age, life history, social status, somatic pathology between the formed groups (p > 0.05).

Bacterioscopic examination of vaginal swabs and diagnosis of bacterial vaginosis according to the Amsel criteria in women of the study cohort showed that the first (14; 70.0%) and second (3; 15.0%) degrees of purity prevailed in the pregnant women of the control group sheath. In 2 (10.0%) pregnant women of the control group, bacterial vaginosis was diagnosed in the absence of any complaints (Table 1).

Bacterioscopy results	**Studied cohort**		
	I (**n = 80**)	II (**n = 70**)	K (**n = 20**)
I-t degree of purity	46 (57,5) P > 0,05	20 (28,6) $P_{1,2}$ < 0,01	14 (70,0)
II-d degree of purity	10 (21,3) P > 0,05	16 (22,9) $P_{1,2}$ > 0,05	3 (15,0)
Bacterial vaginosis	15 (18,8) P > 0,05	22 (31,4) $P_{1,2}$ > 0,05	2 (10,0)
Mixed vaginitis	2 (2,5)	6 (8,6) P_2 > 0,05	–
Vulvovaginal candidiasis	7 (8,8) P > 0,05	8 (11,4) $P_{1,2}$ > 0,05	1 (5,0)

Table 1: Results of bacterioscopic examination of vaginal secretions and assessment of Amsel criteria in women of the studied cohort (n., %).

Note: P –reliability of the differences between the 1st group and the control group.

P1 – reliability of the differences between the II group and the control group.

P2 – reliability of differences between I and II groups.

A different picture took place during the bacterioscopic examination of vaginal swabs of pregnant women of groups I and II. In patients of group II, more often than in patients of group I and the control group, was diagnosed bacterial vaginosis (22; 31.4%), mixed vaginitis (6; 8.6%) and, accordingly, a significantly smaller proportion of pregnant women who had the first degree of vaginal cleanliness (20; 28.6%) ($P_{1,2} < 0.01$) (Table 1). Vaginal swabs, which were used to verify mixed vaginitis, showed signs of an inflammatory process - more than 50 leukocytes in the field of view, a large number of epithelial cells, a significant number of gram-positive and gram-negative cocci and rods with isolated lactobacilli.

We did not find any significant differences in the frequency of development of vulvovaginal candidiasis in pregnant women of the formed groups, however, it should be noted that its frequency was still higher in patients with a threat of premature birth (Table 1).

As further studies have shown, the use of molecular biological methods, in particular PCR with detection of results in real time, is more informative for assessing the state of the vaginal microbiota, and, accordingly, substantiating therapeutic and preventive measures.

Molecular biological analysis proved that the lowest frequency of vaginal normocenosis occurred in pregnant women with a threat of premature birth (Table 2).

PCR data	Studied cohort		
	I (n = 80)	II (n = 70)	K (n = 20)
Normocenosis	43 (53,8) P<0,05	17 (24,3) $P_{1,2} < 0,01$	15 (75,0)
Intermediate state of microbiota	11 (13,7) P > 0,05	13 (18,6) $P_{1,2} > 0,05$	1 (5,0)
Anaerobic dysbiosis (BV)	17 (21,3) P > 0,05	24 (34,3) $P_{1,2} < 0,05$	2 (10,0)
Aerobic dysbiosis	–	8 (11,4)	–
Vulvovaginal candidiasis	9 (11,3) P > 0,05	8 (11,4) $P_{1,2} > 0,05$	2 (10,0)

Table 2: The condition of the vaginal microbiota in women of the study cohort according to PCR data (n., %).

Note: P – reliability of the differences between the 1st group and the control group.

P1 – reliability of the differences between the II group and the control group.

P2 – reliability of differences between I and II groups.

It should be noted that the intermediate state of the vaginal microbiota was quite often detected both in patients from the risk group of premature birth and those with a threat of premature birth (13.8% and 18.6%, respectively), which, in the absence of preventive measures, could turn into a state of dysbiosis.

The frequency of detection of anaerobic dysbiosis was the highest among pregnant women with a threat of premature birth (34.3%) compared to pregnant women of the 1st group (21.3%) ($P < 0.05$) (Table 2).

It is extremely important that aerobic dysbacteriosis (aerobic vaginitis) was diagnosed only in pregnant women with the threat of preterm labor (group II) (8; 11.4%), which indicates the presence of an inflammatory process that can initiate the development of labor activity.

Vulvovaginal candidiasis was detected with almost the same frequency both in the control group and in the pregnant groups at risk of premature birth and the threat of premature birth (Table 2). Typing of Candida fungi showed that Candida nonalbicans fungi were found in both groups. A comparison of the obtained microbiological data with the anamnesis of pathology of the lower parts of the genital tract in pregnant women of the studied cohort proved that Candida nonalbicans is mainly found in patients with recurrent episodes of vulvovaginal candidiasis before pregnancy.

It was noteworthy that in 2 (2.9%) cases, Chlamydia trachomatis DNA was detected in pregnant women with a threat of premature birth and a burdened obstetric and gynecological history (infertility, early spontaneous miscarriages), and in 1 (1.4%) case - Mycoplasma genitalium (Table 3), while this examination was not carried out in these patients before. This highlights the importance of testing women at risk of preterm birth for sexually transmitted infections [22,25] for appropriate treatment prior to pregnancy. In all cases of diagnosis of STD, bacterial vaginosis and anaerobic vaginitis, appropriate etiotropic therapy was carried out [22,25].

The next stage of the study was to determine the correlation of the detected changes in the vaginal microbiota with the consequences of pregnancy, which were traced from the moment the patients were included in the study until delivery. Thus, the threat of premature birth developed in 23 (28.8%) pregnant women of the 1st group, premature rupture of the fetal membranes - in 5 (6.3%) cases. Premature births in group I took place in 12 (15.0%) cases, of which 7 (8.75%) cases were spontaneous preterm births, 5 (6.3%) cases were preterm births for obstetric indications (Table 3).

All (100%) pregnant women of group II, when included in the study, were diagnosed with the threat of preterm labor, in connection with which the patients received therapy aimed at stopping labor and prolonging pregnancy (tocolytic and gestagenotherapy). However, in 16 (22.9%) women, pregnancy was complicated by premature rupture of the fetal membranes, in 22 (31.4%) cases it ended in premature birth, of which in 6 (8.6%) cases - for medical reasons (Table 3).

Pregnancy results	Studied cohort		
	I (n = 80)	II (n = 70)	K (n = 20)
The threat of premature birth	23 (28,8)	70 (100,0)	–
Premature rupture of membranes	5 (6,3)	16 (22,9) $P_{1,2} < 0,05$	1 (5,0)
Premature birth for medical reasons	5 (6,3)	6 (8,6)	–
Premature (spontaneous) birth 22-36: of them	12 (15,0)	16 (22,9) $P_2 > 0,05$	–
Before 28 weeks of gestation	2 (2,5)	9 (12,9) $P_2 < 0,05$	–
From 29 to 36 weeks of gestation	5 (6,3)	7 (10,0)	1 (5,0)
Term delivery	68 (85,0)	48 (68,6) $P_{1,2} < 0,05$	19 (95,0)

Table 3: The results of pregnancy in women of the study cohort (n., %).

Note: P – reliability of the differences between the 1st group and the control group.

P1 – reliability of the differences between the II group and the control group.

P2 – reliability of differences between I and II groups.

The conducted studies indicate a high risk of preterm birth in the presence of dysbiotic and inflammatory changes in the vaginal microbiota with an OR = 2.962 (95% CI 1.32-6.645), while the risk is even higher for pregnant women with other risk factors for preterm birth - OR = 8.120 (95% CI 2149-30686) and for pregnant women at risk of preterm labor OR = 10.133 (95% CI 3.149-32.604).

Discussion

We analyzed the relationship between the state of the vaginal microbiota and preterm birth in 150 women, among which the first group consisted of 80 women with risk factors for preterm birth at 10-12 weeks of gestation, the second - 70 pregnant women hospitalized with a confirmed threat of preterm birth at 22-28 weeks' gestation. Among the patients of the studied cohort, the highest frequency of bacterial vaginosis (34.3%) and aerobic vaginitis (11.4%) was in pregnant women with the threat of preterm labor. Despite the etiotropic therapy of vaginal microbiota disorders, 2 (2.5%) pregnancies in group 1 and 9 (12.9%) pregnancies in group 2 ($p < 0.05$) ended in preterm birth before 36 weeks of gestation. The results obtained are consistent with the data on the association of the vaginal microbiome with preterm birth [17], however, many authors focus on differences depending on race/ethnicity, socioeconomic status [2,3,7]. All patients included in our study were Caucasian, which makes the results congruent. In addition, the results vary due to the study of the vaginal microbiota at different times of antenatal observation [21,23,26].

Brown RG., *et al.* [4] indicate that the composition of the vaginal microbiota is a risk factor for PPROM and consider the vaginal microbiota as a potentially modifiable antenatal risk factor for PPROM. Regarding the association of preterm birth with changes in the vaginal microbiota and the advisability of treating BV to prevent preterm birth, there are different data in the literature [16,21,24].

Faruqui A., *et al.* [8] suggest that in women at risk of preterm birth, an adverse outcome is more likely if bacterial vaginosis is detected in the first trimester, but treatment reduces the rate of preterm birth, which is consistent with our data. Shimaoka M., *et al.* [23] in a retrospective study of the relationship between vaginal bacterial status and preterm birth rates showed that untreated preterm birth rates were higher. Based on the results obtained, and given the limitations of our study (small sample size), we consider the feasibility of studying the vaginal microbiota at the beginning of the second trimester of pregnancy, which is recommended by the CDC and other specialized organizations [22,25].

Conclusion

A comprehensive study of the vaginal microbiota of women in the study cohort showed that in patients with the threat of preterm

birth, more often than in patients with risk factors for preterm birth, and the control group, was diagnosed with bacterial vaginosis (22; 31.4%), mixed vaginitis (6; 8.6%) and, accordingly, a significantly smaller proportion of pregnant women who had the first degree of vaginal cleanliness (20; 28.6%).

One of the risk factors for spontaneous preterm birth is bacterial vaginosis, aerobic vaginitis, and etiotropic therapy at the beginning of the second trimester of pregnancy made it possible to significantly reduce the frequency of preterm birth before 28 weeks of gestation from 12.9% to 2.5% ($p < 0.05$) with an insignificant decrease in the overall incidence of preterm birth from 22.9% to 15.0% ($p > 0.05$).

Author's Contribution

OL, VP contributed to the concept and design of this study. OL performed the research and wrote the first draft of the manuscript. VP is essential edited and reorganized sections of the manuscript. All authors participated in revising, read and approved the manuscript the given version.

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Conflict of Interests

The authors declare no conflict of interest.

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