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The Results of Microbiological Investigations into Preterm Labor

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Abstract

The purpose of this study was to identify the relationship between the microbiological status of women and newborns, and the development of premature labor.

Materials and Methods: The study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus.

Results: Disturbances in the vaginal biotope were diagnosed in every second woman. We found that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope, and the risk of premature birth at an earlier time correlates with the presence of infection in the mother. The risk of giving birth to an infected child is 4.2 times higher at birth from a mother who has disturbances in the biotope, compared to a woman with a normal biotope.(**International Journal of Biomedicine. 2019;9(2):144-149.**)

Key Words: premature labor • vaginal biotope • vaginal infections • newborns

Abbreviations

PL, premature labor; **BG**, bacterial growth; **GA**, gestational age; **PB**, premature birth.

Introduction

The causes of spontaneous PL are heterogeneous. An important risk factor for PL is a high infectious index of the genital tract [1,2] during pregnancy and chronic placental insufficiency.[3] The achievements of clinical microbiology in recent decades have largely changed our understanding of the possible causative agents of vaginal infections.[4-9] In women with bacterial vaginosis, PB occurs 3–4 times more often [10] and the likelihood of postpartum endometritis is 5–7 times higher than in women without the infection.

Long-term clinical observations confirm the connection between vaginal infections and adverse pregnancy outcomes for the mother and fetus.[11-13] To date, convincing evidence of the linkage between vaginal infections and PL, fetal intrauterine infection, and inflammatory complications after childbirth has been published.[14-20] That study, of the reproductive health of the examined women, showed that inflammatory diseases of the body and uterine appendages occurred in 18.2% of cases. There is evidence of repeated, and often recurrent, inflammatory diseases of the external genital organs and the vagina in 36.9% of women.[17] Currently, among the infections of the vagina, bacterial vaginosis, aerobic vaginitis and candidal vulvovaginitis play a leading role.[21] Unfavorable predictors of PB are a combination of bacterial vaginosis and the persistence of Ureaplasma urealyticum, Mycoplasma hominis and Mycoplasma genitalium in the cervical canal of pregnant women.[22] Inflammation of the mucous membrane in these cases is due to the activation of various representatives of opportunistic flora. Usually, the pathogens are different

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types of staphylococci and streptococci, enterococci, E. coli, Klebsiella, Proteus and some other bacteria and their associations.[23]

However, the development of an imbalance in the vaginal microbiota is still poorly understood.[16,24] Therefore, etiotropic therapy is a difficult task, especially in the early stages of pregnancy. The use of antibacterial drugs during pregnancy is debatable, and treatment approaches do not have solid standards.[25] Numerous clinical studies and systematic reviews based on meta-analysis present conflicting views on antimicrobial therapy for opportunistic infections of the vagina during pregnancy.[26-29] The purpose of this study was to identify the relationship between the microbiological status of women and newborns, and the development of PL.

Materials and Methods

Our study included 227 pregnant women at gestational age between 28 and 36 weeks and 6 days. Depending on the gestational age, they were divided into 3 groups. Group 1 included 73 women at gestational age between 28 and 30 weeks and 6 days; Group 2 included 81 women at gestational age between 31 and 33 weeks and 6 days, Group 3 included 73 women at gestational age between 34 and 36 weeks and 6 days. All women underwent an assessment of vaginal microcenosis and the quantitative and qualitative composition of the biotope of the cervical discharge; the newborns underwent bacteriological examination of the auricle, pharynx and anus.

In order to study the state of vaginal microcenosis, as well as the diagnosis of trichomonas and gonococcal infections, we performed a microscopic examination of the vaginal discharges, which were taken with a sterile gynecological universal probe (Centrmed LLC, Russia) from the posterior and lateral vaginal vaults. The resulting material was applied to separate areas of a defatted slide, dried in air, and stained with methylene blue or Gram. We then evaluated the leukocyte reaction, the morphology of the microorganisms and their total number, the presence of "key" cells in the vaginal biotope.

To analyze the nature of the quantitative and qualitative composition of the biotope of the cervical discharge, a tampon-probe and test tubes with a transport medium ("Medical Wire & Equipment", England) were used. Seeding was performed on a series of nutrient media to isolate and cultivate various groups of microorganisms: 5% blood agar based on Brucella agar with the addition of vitamin growth factors to isolate and cultivate anaerobes, mannitol salt agar to isolate and cultivate gram-negative bacteria, and Saburo medium to isolate and cultivate fungi. Blood agar media were cultivated in a thermostat with a high content of carbon dioxide (5-10%). To cultivate anaerobes we used anaerostats (Becton Dickinson, USA). The isolated microorganisms were identified and their sensitivity to antibacterial drugs was determined using the Witek bacteriological analyzer.

The results obtained were recorded in accordance with the NCCLS standards (1999–2000). The number of isolated microorganisms was determined by the density of their growth on the sectors of the agar plate.

Bacteriological study of auricular secret and pharynx

The material obtained and transported to the laboratory was seeded on Petri dishes with 5% blood agar, chocolate agar, and yolk-salt agar, on Endo medium, Saburo medium, and in a tube with glucose broth. Seeding on dense nutrient media was carried out metered (according to Gould), which made it possible to quantify the number of grown colonies. Seedings were incubated at 37°C for 24–48 hours, and were examined daily. Plates with 5% blood agar were incubated under conditions with a high CO_2 content. With the appearance of growth on nutrient media, we counted colonies of various morphologies, taking into account their ratio and species identification of microorganisms, as well as determining their sensitivity to antibacterial drugs. A negative result of the study was issued in the absence of growth on all nutrient media for 72–96 hours.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the RUDN University Ethics Committee. Written informed consent was obtained from all participants.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA). Baseline characteristics were summarized as frequencies and percentages for categorical variables and as mean±SEM for continuous variables. The Mann-Whitney (U Test) was used to compare the differences between the two independent groups. The Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (r_s) were was used to determine the strength and direction of the relationship between two variables. The two-proportions z-test was used to compare two observed proportions. Group comparisons with respect to categorical variables are performed using chi-square tests. A value of $P<0.05$ was considered statistically significant.

Results

Bacterioscopy of the contents of the *vaginal and cervical canal contents*

According to the results of bacterioscopic examination (Table 1), normocenosis of the genital tract was diagnosed only in 84(37.0%) women. Disturbances in the vaginal biotope were diagnosed in every second woman. We found that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope (82.2%). We found a moderate inverse correlation between the term of labor and the presence of bacteria (r=-0.3657, $P<0.001$). Significant differences were found between Group 1 and Groups 2 and 3 (χ^2=23.036, P=0.0000), and between Groups 2 and 3 ($\chi^2$5.653, P= 0.017).

Table 1.
The results of bacterioscopic examination

Group	normocenosis		vaginal infections		Statistics
	n	%	n	%	
Group 1 (n=73)	13	17.8	60	82.2	χ^2=23.036 P=0.0000
Group 2 (n-81)	30	37.0	51	63.0	
Group 3 (n=73)	41	56.2	32	43.8	P_{1-2}=0.0079 P_{2-3}= 0.0174
Total (n=227)	84	37.0	143	63.0	P_{1-3}=0.0000

Table 2.

Bacterial cultures of the cervical canal of pregnant women

Bacterial cultures	Total	Group 1	Group 2	Group 3	P_{1-2}	P_{1-3}	P_{2-3}
E. coli	27 (11.9 %)	11 (15.1 %)	10 (12.3 %)	6 (8.2 %)	0.6249	0.1965	0.3979
Candida spp.	38 (16.7 %)	15 (20.5 %)	13 (16 %)	10 (13.7 %)	0.8389	0.5472	0.6773
Enterococcus faecalis	22 (9.7 %)	8 (11 %)	5 (6.2 %)	9 (12.3 %)	0.2924	0.7967	0.1909
Strep. agalactiae	8 (3.5 %)	7 (9.6 %)	0 (0 %)	1 (1.4 %)	0.1002	0.0281	0.7782
Strep. oralis	7 (3.1 %)	5 (6.8 %)	2 (2.5 %)	0 (0 %)	0.2025	0.2515	0.6507
Staphyl. epirmidis	17 (7.5 %)	7 (9.6 %)	9 (11.1 %)	1 (1.4 %)	0.7568	0.0281	0.0103
Staph.aureus	15 (6.6 %)	6 (8.2 %)	3 (3.7 %)	6 (8.2 %)	0.2413	1.0000	0.2413
Staph. haemolyticus	6 (2.6 %)	2 (2.7 %)	3 (3.7 %)	1 (1.4 %)	0.7346	0.5601	0.3522
Staph.saprophyticus	6 (2.6 %)	2 (2.7 %)	4 (4.9 %)	0 (0 %)	0.4755	0.6195	0.3874
Gemella morbillorum	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Enterobacter aerogenes	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Strep.viridans	3 (1.3 %)	1 (1.4 %)	1 (1.2 %)	1 (1.4 %)	0.9412	1.0000	0.9412
Staph. warneri	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Serratia odorifera	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Enterobacter cloacae	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Streptococcus mitis	3 (1.3 %)	0 (0 %)	2 (2.5 %)	1 (1.4 %)	0.6507	0.7979	0.6174
Corynebacterium spp.	4 (1.8 %)	1 (1.4 %)	2 (2.5 %)	1 (1.4 %)	0.6174	1.0000	0.6174
Acinetobacter	6 (2.6 %)	1 (1.4 %)	2 (2.5 %)	3 (4.1 %)	0.6174	0.3106	0.5716
Klebsiella pneumoniae	1 (0.4 %)	0 (0 %)	0 (0 %)	1 (1.4 %)	1.0000	0.7979	0.7782

Table 3.

Data of bacteriological results from the studied loci (the anus. ears and pharynx) in newborns

Bacterial cultures	Total	Group 1	Group 2	Group 3	P_{1-2}	P_{1-3}	P_{2-3}
E.coli	20 (8.8 %)	9 (12.3 %)	8 (9.9 %)	3 (4.1 %)	0.6299	0.0695	0.1563
Candida spp	9 (4 %)	5 (6.8 %)	3 (3.7 %)	1 (1.4 %)	0.3869	0.0944	0.3522
Enteroccocus faecalis	20 (8.8 %)	8 (11 %)	5 (6.2 %)	7 (9.6 %)	0.2924	0.7855	0.4347
Enterobacter cloacae	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Strep.agalactiae	9 (4 %)	4 (5.5 %)	4 (4.9 %)	1 (1.4 %)	0.8804	0.1716	0.1988
Strep.oralis	5 (2.2 %)	2 (2.7 %)	1 (1.2 %)	2 (2.7 %)	0.5084	1.0000	0.5084
Streptococcus mitis	2 (0.9 %)	0 (0 %)	1 (1.2 %)	1 (1.4 %)	0.8163	0.7979	0.9412
Staph. aureus	18 (7.9 %)	8 (11 %)	7 (8.6 %)	3 (4.1 %)	0.6306	0.1160	0.2460
Staph. saprophyticus	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Staph. epirmidis	15 (6.6 %)	8 (11 %)	5 (6.2 %)	2 (2.7 %)	0.2924	0.0482	0.2979
Staph. haemolyticus	4 (1.8 %)	3 (4.1 %)	0 (0 %)	1 (1.4 %)	0.4313	0.3106	0.7782
Staph. warneri	3 (1.3 %)	2 (2.7 %)	1 (1.2 %)	0 (0 %)	0.5084	0.6195	0.8163
Strep. viridans	1 (0.4 %)	0 (0 %)	0 (0 %)	1 (1.4 %)	1.0000	0.7979	0.7782
Neisseria spp.	10 (4.4 %)	3 (4.1 %)	5 (6.2 %)	2 (2.7 %)	0.5611	0.6495	0.2979
Genella morbillorum	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Serratia marcescens	4 (1.8 %)	3 (4.1 %)	1 (1.2 %)	0 (0 %)	0.2756	0.4691	0.8163
Serratia odorifera	1 (0.4 %)	0 (0 %)	1 (1.2 %)	0 (0 %)	0.8163	1.0000	0.8163
Corynebacterium spp.	3 (1.3 %)	0 (0 %)	1 (1.2 %)	2 (2.7 %)	0.8163	0.6195	0.5084
Acinebacter	1 (0.4 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0.7782	0.7979	1.0000
Klebsiella pneumoniae	2 (0.9 %)	2 (2.7 %)	0 (0 %)	0 (0 %)	0.5873	0.6195	1.0000

The study of cervical canal cultures

In all pregnant women (n=227), immediately upon admission to the hospital, a discharge content from the cervical canal was taken for bacteriological examination with the determination of sensitivity to antibacterial drugs. The data of the first seeding are the most important, since after the course of antibiotic therapy, the subsequent results of bacteriological seeding are uninformative for the diagnosis of vaginal microflora. The growth of microorganisms was observed in 143(63.0%) pregnant women; in 29(12.8%) women who gave birth, the growth of the bacterial flora in the newborns was not observed.

The leading positions among the sowed microorganisms were occupied by *Candida spp.* (38/16.7%), *E. coli* (27/11.9%), and *Enterococcus faecalis* (22/9.7%), and the growth of 50% of the detected bacteria was observed in Group 1 (Table 2). In bacterial vaginosis, microorganisms of intestinal origin were predominant against the background of a significant decrease in the frequency of lactobacilli. In vaginitis, associations of microorganisms represented by 2–5 species with an anaerobic or aerobic component were found in all cases. At the same time, 84 women with normal flora gave birth to 16(28.6%) children, who showed bacterial growth in diagnostically significant titers. Thus, respectively, only 68(30%) of the women gave birth to children who did not show an increase in bacterial opportunistic flora.

All newborns were bacteriologically examined (the anus, ears and pharynx). The growth of microorganisms was observed in 131(57.7%) newborns (Table 3). The leading positions among the sowed microorganisms in children were occupied by *E. coli* (20/8.8%), *Enteroccocus faecalis* (20/8.8%), *Candida spp.* (9/4%), and *Streptococcus agalactiae* (9/4%). At the same time, the growth of microflora was found significantly more often in newborns from mothers of Group 1. According to the study, in 127(55.9%) of 227 children, the growth of bacteria from the studied loci was detected in diagnostically significant titers (>10^5cfu/ml). At the same time, in 114(89.8%) of these children, the growth of microflora was associated with the identified infection in mothers (Table 4, Fig.1). The birth of a child with microorganisms correlated with the presence of infection in the mother (r_s=0.706, P<0.0001).

Table 4.

Comparative analysis of bacterial growth in women and newborns

	Total (n/%)	Group 1 (n/%)	Group 2 (n/%)	Group 3 (n/%)	P_{1-2}	P_{1-3}	P_{2-3}
BG in women	143/63	60/82.2	51/63	32/43.8	0.007	0.000	0.017
BG in newborns	127/55.9	54/74	43/53.1	30/41.1	0.006	0.000	0.136
BG in women and newborns	114/50.2	50/68.5	38/46.9	26/35.6	0.006	0.000	0.154
BG in women, 100%	143	60	51	32			
The proportion of newborns with BG in % of mothers with BG	114/79.7	50/83.3	38/74.5	26/81.3	0.259	0.805	0.466

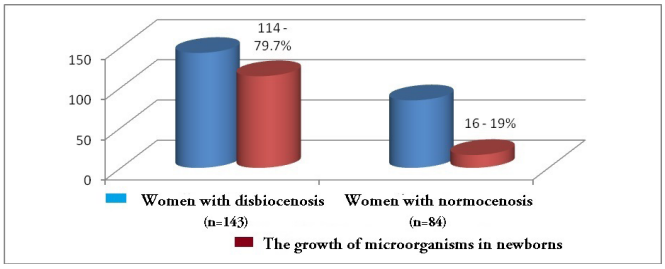

Fig. 1. *Bacterial growth in women and newborns.*

Discussion

High contamination of the genital tract of pregnant women with opportunistic and pathogenic bacterial and viral microflora is a high risk factor for PB. It is noted that the shorter the gestation period, the higher the frequency of disturbances in the vaginal biotope (82.2%), and the risk of PB at an earlier time correlates with the presence of infection in the mother (r=-0,327, P<0.001; r_s=-0.331, P=0.004) (Fig.2). The study by D. Nelson[16] showed that the prevalence of bacterial vaginosis among pregnant women was 3.88%, and the presence of G. vaginalis was 67.48%; but the presence of G. vaginalis was not enough to cause bacterial vaginosis. However, the presence of G. vaginalis may be considered a significant risk factor for PB.

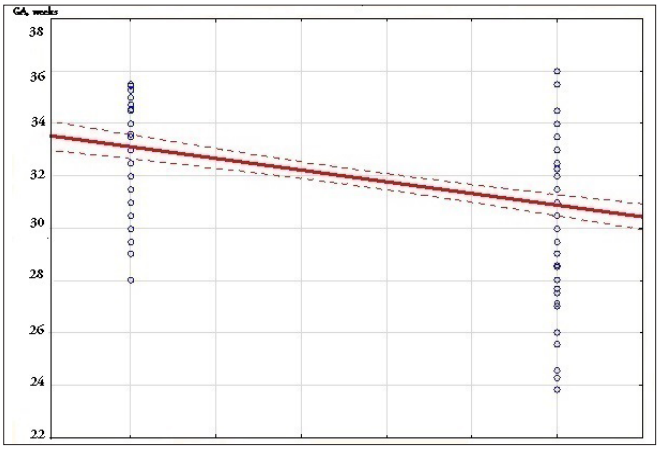

Fig.2. *The growth of microorganisms in the cervical canal of pregnant women (r=-0.327, P<0.001; OR=4.19, 95% CI: 2.67 – 6.55)*

In the previous study,[17] we showed the main risk factors leading to PL: early sexual debut, inflammatory diseases of the urinary organs, sexually transmitted infections, reproductive losses in history, anemia, etc. The present study shows the association of PL with an infectious factor. In our opinion, and according to a number of authors, a high bacterial contamination of the genital tract, already at the stage prior to the onset of pregnancy, contributes to the infection of the fetal membranes, with impaired amniotic fluid production, starting from the early stages of gestation.

Comparing the data obtained with the results of neonatal outcomes, according to a previous study,[30] we can note the

high risk of having children with congenital pneumonia in women of Group 1. With an increase in the period of gestation, the frequency of this complication is significantly reduced. In the future, we plan to perform a placental histology study and identify the presence of possible relationships among the data of microbiological studies.

Conclusion

The risk of giving birth to an infected child is 4.2 times higher at birth from a mother who has disturbances in the biotope, compared to a woman with a normal biotope. The result is a long-term presence of the newborn in the intensive care unit, antibiotic therapy, and a long rehabilitation period. Despite the ongoing preventive antibiotic therapy in pregnant women and mothers, the frequency of detectable pathogenic microorganisms in newborns has not decreased. The data obtained are explainable from several positions:

- First, microorganisms are resistant to antibacterial drugs used.
- Second, and probably the most important: A contamination of the newborn with pathogenic microorganisms occurs in-utero (prenatally) at earlier gestational periods.

In our study, 7(3.4%) women showed resistance to antibacterial drugs during bacteriological examination of discharge content from the cervical canal: E. coli was found in 5 women and Staphylococcus epidermidis in 2 women.

Thus, the results of the study allow us to:

- identify the group of pregnant women at risk for the infection process
- carry out therapeutic measures for the prevention and treatment of microflora biocenosis disorders in the early stages of gestation, and
- carry out measures to improve the reproductive potential of the population.

Competing Interests

The authors declare that they have no competing interests.

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