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Research Article

SIGNIFICANCE OF THE FEMOFLOR TEST IN ASSESSING THE STATE OF VAGINAL MICROBIOCENOSIS IN PRETERM VAGINAL DISCHARGE

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ABSTRACT

The frequency of premature rupture of the membranes before the onset of labor, according to various authors, varies widely from 1 to 19.8% of cases. pathways, impaired uteroplacental circulation and the development of fetal hypoxia.

KEYWORDS

Pregnant women with premature rupture of membranes, genital tract biocenosis, polymerase chain reaction, preterm birth, vaginal microbiocenosis.

INTRODUCTION

The microflora of the urogenital tract of women is a collection of microorganisms that causes resistance to colonization, pH stability of the vaginal mucosa, participates in the maintenance of local immunity and, as a result, ensures the body's general resistance to infections [1,2,3]. The normal microflora of the vagina of healthy women of reproductive age includes gram-positive and gram-negative aerobic, facultative aerobic and obligate anaerobic microorganisms, 95-98% of all microorganisms are lactobacilli. Normal microflora also includes genital mycoplasmas with a titer of less than 104 KOE/ml, fungi of the genus Candida with a titer of

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less than 103 KOE/ml [4,5,8]. Bacterial vaginosis occurs as a result of qualitative and quantitative changes in the microflora of the urogenital tract. Microorganisms associated with bacterial vaginosis include Gardnerella, Mobiluncus, Prevotella, Bacteroides, Fusobacterium, Peptostreptococcus, Leptotrichia, Atopobium vaginae, and others [6,7,9]. The cause of vaginosis can be infectious-inflammatory diseases, such changes often lead to the development of complications in the organs of the reproductive system. [12,14]. Diseases caused by conditionally pathogenic microflora in the mother can lead to spontaneous abortions, premature discharge of amniotic fluid, intra- and postnatal infection of the fetus, postpartum vaginal inflammation, and even the development of premature labor. can come [13,15]. The most common methods of diagnosing infectious and inflammatory diseases of the genitourinary tract are microscopic examination of the condition of the vaginal epithelium and leukocyte reaction, assessment of the composition and number of microorganisms by microscopic and bacteriological examination of smear, qualitative polymerase chain reaction (PCR). microorganisms can be present both in pathological conditions (significant amounts) and in normal conditions (limited amounts). Therefore, both qualitative and quantitative characteristics are necessary to assess the state of biocenosis [10,11].

The purpose of the study is to determine the qualitative and quantitative composition of microorganisms that make up the microbiocenosis of the genital tract in pregnant women with premature discharge using PCR.

RESEARCH MATERIALS AND METHODS

Vaginal swabs of 28 pregnant women who came to the perinatal center with discharge before the period of 26-34 weeks and 24-36 years of age were studied. The control group consisted of 11 pregnant women aged 24-36 with a physiologically developing pregnancy of 20-25 weeks. We used PZR (Femoflor-16) and the DT-96 detection amplification reagent kit manufactured by OOO "NPO DNK-Technology" (Russia) to study the biocenosis of the urogenital tract in women. Samples with sufficient number of cells and sufficient total bacterial mass from the vagina and partial cervix were used to obtain appropriate results. Samples were considered in which the DNA content of human cells was greater than 104 genome-equivalents (GE) in the sample and the total bacterial mass value was 106 to 109 GE/sample. After amplification, the total bacterial mass value of Lactobacillus spp. and each of the conditional-pathogens was detected automatically. The state of vaginal microbiocenosis was determined by their ratio calculated using the software. Quantitative evaluation of Qin microflora was carried out both in absolute and relative terms. The absolute indicator is the amount of DNA of the desired microorganism in the sample, expressed in GE, expressed as a decimal logarithm - lg. The relative quantitative indicator of the microorganism was calculated as the ratio of the amount of the desired microorganism to the amount of the total bacterial mass. It is presented in two formats: the decimal log difference of the number of the respective group of microorganisms and the total bacterial mass and as a percentage of the total bacterial mass.

The state of biocenosis was assessed according to the criteria developed by G.T. Sukhish et al. The classification of types of biocenosis includes:

- absolute normocenosis - a variant of biocenosis, in which 80-100% of the total bacterial mass of normoflora is: Ureaplasma spp., Mycoplasma spp. – less than 104 ge/ml, and fungi of the genus Candida spp. - less than 103 g / ml;

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- conditional normocenosis - a variant of biocenosis, in which 80-100% of the total bacterial mass of normoflora is: the number of Ureaplasma spp and or Mycoplasma spp. – more than 104 ge/ml and Candida spp. - more than 103 g / ml;
- moderate (aerobic or anaerobic) imbalance - due to an increase in the percentage of aerobes or anaerobes, the percentage of lactobacilli decreases by 20-80% compared to the total bacterial mass;
- clearly expressed (aerobic, anaerobic or mixed) imbalance (makes 30-50%) - a variant of biocenosis, in which the share of aerobes or anaerobes reaches 80-100% of the total bacterial mass, and the share of lactobacilli decreases to 20% or less. Research results Processed using Statistica 6.0 software. Differences were considered significant at $p<0.05$.

Research results. Comparison of indicators of biocenosis in the compared groups showed that the proportion of normocenosis in women with premature amniotic fluid was 2.5 times lower than in the control group (p=0.0019). Dysbiosis was detected in 18 out of 28 cases (64.3%), and in the control group in 1 out of 11 women (9.1%; p=0.0019). Average anaerobic, pronounced aerobic and mixed dysbiosis was found only in the main group of women (table 1). Anaerobic bacteria were detected faster than aerobes, which corresponds to the results obtained earlier.

Table 1

Determination of the type and level of vaginal dysbiosis in the examined groups, abs (%)

Groups	Normostenosis	Dysbie			
		Average Anaerobic	Clearly expressed		Mixed
			Anaerob	Aerob	
Portion of the water out of order (N = 28)	10 (35,7)	7 (25,0)	7 (25,0)	2 (7,1)	2 (7,1)
Physiological pregnancy (N = 11)	10 (90,9)	0	7 (25,0)	0	0
	0,0019	0,0376	0,1362	0,1869	0,1869

Normocenosis was found in 10 cases of women with premature amniocentesis. Among them, only 1 patient was diagnosed with absolute normocytosis, and in the rest, 8 out of 9 cases had conditional normocytosis with fungi of the genus Ureaplasma (urealyticum + parvum) and Candida spp. In 1 case, the titer was more than 104 and 103, respectively. Anaerobic dysbiosis was detected on average in 7 (25%) women with premature amniotic fluid. 24-70% of the total bacterial mass in cervical smears of patients was Lactobacillus spp. organizes. Eubacterium spp. It was detected in high titers in 2 pregnant women.

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A combination of anaerobic bacteria and microorganisms associated with bacterial vaginosis was found in 5 cases:

- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp.+ Candida fungi;
- Megasphaera spp./Veillonella spp./Dialister spp. +Sneathia spp./Leptotrihia spp./Fusobacterium spp.;
- Mobiluncus spp./Corynebacterium spp. +ureaplasma (urealyticum+parvum);
- Megasphaera spp./Veillonella spp./Dialister spp.+ Ureaplasma (urealyticum+parvum) + Candida fungi;
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Atopobium vaginae + Candida fungi.

Of the main group, 9 (32.1%) pregnant women, including 7 (25.0%) had anaerobic dysbiosis, 2 (7.1%) had aerobic dysbiosis. In severe dysbiosis, Lactobacillus spp. amount is from 0 to 18% of the total bacterial mass.

The etiological structure of clearly expressed anaerobic imbalance consists of the following combinations of pathogens of anaerobic infections and microorganisms associated with bacterial vaginosis:

- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp.;
- Megasphaera spp./Veillonella spp./Dialister spp. +Atopobium vaginae + Ureaplasma (urealyticum + parvum);
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Ureaplasma (urealyticum + parvum);
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Eubacterium spp. + Sneathia spp./Leptotrihia spp./Fusobacterium spp. + Ureaplasma (urealyticum + parvum);
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Eubacterium spp. + Mobiluncus spp/|Corynebacterium spp. + Megasphaera spp./Veillonella spp./Dialister spp.;
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Eubacterium spp.;
- Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp. + Sneathia spp./Leptotrihia spp./ Fusobacterium spp.

Streptococcus spp. observed in high titers. In one of the patients, along with streptococci, we detected fungi of the genus Candida in the diagnostic titer. In both cases Lactobacillus spp. not defined. Moderate aerobic-anaerobic (mixed) dysbiosis was also detected in 2 patients. In 1 patient with mixed dysbiosis, the following were detected: Streptococcus spp./Gardnerella vaginalis/Prevolla bivia/Porphyromonas spp., in another case Megasphaera spp./Veillonella spp./Dialister spp./Ureaplasma (urealyticum+parvum).

Normocenosis was found in 10 out of 11 cases (90.9%) in physiologically developing women of pregnancy. Of them, absolute normocenosis was observed in 4 women, conditional normocenosis in 6 women. Among women with conditional normocenosis, Ureaplasma (urealyticum + parvum) was detected in 4 cases, fungi belonging to the Candida genus were detected in 1 patient, and both pathogens were detected in diagnostic titers in 1 pregnant woman. Severe anaerobic dysbiosis with a physiologically developing pregnancy from 11 pregnant women It was found in 1. The etiological structure is represented by the following pathogens: Gardnerella

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vaginalis/Prevotella bivia/Porp Streptococcus spp., Gardnerella hyromonas spp., Eubacterium spp., Atopobium vaginae, Ureaplasma (urealyticum+parvum).

CONCLUSION

Thus, as a result of the study, the effectiveness of using a new modern PCR method for the diagnosis of bacterial vaginosis was shown. The method allows to determine the number of lactobacilli and bacteria associated with bacterial vaginosis, anaerobic and aerobic flora, including difficult-to-cultivate flora, and to assess their ratio and the state of the biocenosis of the female genital tract in a short period of time. For the first time, using the Femoflor test, the microbial landscape of the genital tract of women with premature ejaculation was compared with the microbiocenosis of the genital tract of women with physiological pregnancy. In 64.3% of cases Lactobacillus spp. a decrease in the percentage, bacterial imbalance was detected in pregnant women with premature amniocentesis. At the same time, in women with physiological pregnancy, the decrease of lactobacilli was detected only by 9.1% (p=0.002), in most cases, normocytosis was detected. Anaerobic microorganisms (14 out of 28 or 50%) played the main role in the structure of dysbiosis in pregnant women with premature amniocentesis: Gardnerella vaginalis (17.8%), Candida spp. genus fungi (10.7%), Ureaplasma (urealyticum + parvum) - 17.8%, Atopobium vaginae (7.1%). Aerobic dysbiosis was noted only in 2 women (7.1%), Streptococcus spp. and Candida spp. fungi of the genus, mixed - observed in 2 (7.1%) cases. The identified facts confirm the point of view about the important role of bacterial vaginosis in the genesis of premature birth, emphasize the need to diagnose dysbiosis in the restoration of normal microflora.

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