Central Asian Journal of Pediatrics

Volume 2021 | Issue 1 Article 5

3-20-2021

CLINICAL PRACTICAL ASSESSMENT APPLICATION OF POLYMERASE CHAIN REACTION AS A TEST FOR ASSESSING MICROBIOCINOSIS IN PREGNANT WOMEN

D.T. Ruzmetova
Tashkent Medical Academy

S.A. Matyakubova
Urgen Branch of the Tashkent Medical Academy

UDC 618.514.8-07

CLINICAL PRACTICAL ASSESSMENT APPLICATION OF POLYMERASE CHAIN REACTION AS A TEST FOR ASSESSING MICROBIOCINOSIS IN PREGNANT WOMEN

[1]Ruzmetova D.T., [2]Matyakubova S.A.

[1]Tashkent Medical Academy,

[2]Urgen Branch of the Tashkent Medical Academy

Resume

This article is based on the assessment of a comparative analysis of vaginal microbiocenosis in 129 pregnant women with premature rupture of amniotic fluid. The control group consisted of 30 women with physiological pregnancy, without any complications. We also studied the effect of vaginal dysbiosis on the development of septic complications in the postpartum period. Smears from the posterolateral vaginal wall were examined by real-time PCR. We used the reagents included in the Femoflor 16 kit manufactured by NPO DNA-Technology, Russia.

As a result of the study, it was found that bacterial imbalance during premature rupture of amniotic fluid in pregnant women was established in 73.7% of cases, which is 7.3 times more often than during physiological pregnancy (p <0.001). Moreover, in women with septic complications in the postpartum period, dysbiosis was recorded more often. In the structure of anaerobic dysbiosis in women with premature rupture of amniotic fluid, the main role is played by Gardnerella vaginalis - Ureaplasma spp., Fungi of the genus Candida, Mobiluncus species, Atopobium vaginae. Aerobic dysbiosis was detected only in 9.3% of cases with the participation of Streptococcus spp., Mixed - in 7.0% of cases. Thus, the role of vaginal dysbiosis in premature rupture of amniotic fluid in pregnant women is confirmed. Based on

the data obtained, we consider it necessary to study vaginal dysbiosis during the period of pregravid preparation.

Key words: pregnant women with premature rupture of amniotic fluid, vaginal biocenosis, real-time PCR.

КЛИНИКО ПРАКТИЧЕСКАЯ ОЦЕНКА ПРИМЕНЕНИЕ ПОЛИМЕРАЗНОЙ ЦЕПНОЙ РЕАКЦИИ КАК ТЕСТ ОЦЕНКИ МИКРОБИОЦИНОЗА У БЕРЕМЕННЫХ

[1]Рузметова Д.Т. [2]Матьякубова С.А.

[1]Ташкентская медицинская академия,

[2]Ургенский филиал Ташкентской медицинской академии

Резюме

В основу данной статьи положена оценка сравнительного анализа микробиоценоза влагалища у 129 беременных женщин с преждевременным излитием околоплодных вод. Контрольную группу составили 30 женщин с физиологической беременностью, без каких-либо осложнений. Также проведено исследование влияния дисбиоза влагалища на развитие септических осложнений в послеродовом периоде. Мазки с заднебоковой стенки влагалища исследовали методом ПЦР в режиме реального времени. Использовали реактивы, входящие в набор «Фемофлор 16» производства фирмы «НПО ДНК-Технология, Россия».

В результате исследования было установлено, что бактериальный дисбаланс при преждевременном излитии околоплодных вод у беременных установлен в 73,7% случаев, что в 7,3 раза чаще, чем при физиологически протекающей беременности (p<0,001). Притом у женщин с септическими осложнениями в послеродовом периоде дисбиоз регистрировался чаще. В структуре анаэробного дисбиоза у женщин с преждевременным излитием

околоплодных вод основную роль играют Gardnerella vaginalis − Ureaplasma spp., грибы рода Candida, Mobiluncus species, Atopobium vaginae. Аэробный дисбиоз – определен только в 9,3% случаев с участием Streptococcus spp., смешанный – 7,0% случаях. Таким образом, подтверждается роль дисбиоза влагалища при преждевременном излитии околоплодных вод у беременных. На основании полученных данных считаем необходимым изучать дисбиоз влагалища в период прегравидарной подготовки.

Ключевые слова: беременные женщины с преждевременным излитием околоплодных вод, биоценоз влагалища, ПЦР в реальном времени.

HOMILADOR AYOLLARDA MIKROBIOTSINOZNI BAHOLASH TESTI SIFATIDA POLIMERAZA ZANJIRINING REAKSIYASINI KLINIK AMALIY BAHOLASH

[1]Ruzmetova D.T., [2]Matyakubova S.A.

[1]Toshkent tibbiyot akademiyasi,

[2]Toshkent Tibbiyot Akademiyasi Urgan filiali

Rezyume

Ushbu maqola amniotik suyuqlikning muddatidan oldin yorilishi bolgan 129 homilador ayolda vaginal mikrobiotsenozning qiyosiy tahlilini baholashga asoslangan. Nazorat guruhida hech qanday asoratlar bolmagan 30 ta hjmilador kiritilgan.Shuningdek biz vaginal disbiozning tugruqdan keyingi davrda septik asoratlarning rivojlanishiga tasirini organib chiqdik. Real vaqtda PSR yordamida posterolateral devordan tushgan izlar tekshirildi.Biz Rassiyaning NPO DNK-texnologiyasi tomonidan ishlab chiqarilgan Femoflor 16 toplamiga kiritilgan reaktivlardan foydalandik.Tadqiqot natijasida aniqlandiki homilador ayollarda amniotik suyuqlikning muddatidan oldin ketganida bakterial nomtanosiblik 73.7%

hollarda aniqlangan. bu fiziologik homiladorlik davriga qaraganda 7.3 barobar kop (p<0,001).

Bundan tashqari tugruqdan keyingi davrda septik asoratlari bolgan ayollarda disbioz tez-tez qayd etilgan. Amniotik suyuqlikning muddatidan oldin yorilishi bolgan ayollarda fnaerobik disbioz tuzilishida asosiy rolni Gardnerella vaginalis-Ureoplazma spp., Candida, Mobilunkus turlari, Atopobium vaginae oynaydi. Aerobik disbioz faqat 9.3% hollarda Streptococcus spp.,ishtirokida aniqlangan 7.0% hollarda aralashgan. Shunday qilib, homilador ayollarda amniotic suyuqlikning erta yorilishida vaginal disbiozning roli tasdiqlangan.Olingan malumotlarga asoslanib, biz buni zarur deb hisoblaymiz.

Kalit sozlar amniotik suyuqlikning erta yorilishi, vaginal biotsenoz, real vaqtda homiladorlarda PSR tekshiruvi.

Introduction

The vaginal microbiocenosis of women of reproductive age is a well-balanced and stable system. Normally, this biotope contains microorganisms that can survive, coexist and develop in a specific physical environment without causing disease of the macroorganism [3, 9].

The dominant agents in the microbiocenosis are lactobacilli, the metabolic product of which is a-hydroxypropionic lactic acid, which creates an acidic reaction of the vaginal contents. In addition to lactobacilli, more than a hundred species of various bacterial microorganisms, fungi, viruses and protozoa can be found in the urogenital tract of a healthy woman of reproductive age [1, 8].

The emergence of violations of the quantitative and qualitative relationships of resident microorganisms - saprophytic and conditionally pathogenic, inhabiting the genitourinary system in the norm, leads to an imbalance [4, 11]. The development of an imbalance in the microbiocenosis may be accompanied by immune disorders and, in some cases, clinical manifestations, the severity of which varies from asymptomatic carriage to severe clinical manifestation [6].

Conditionally pathogenic microorganisms can be present both in pathological conditions and in normal conditions (in limited quantities). Therefore, to assess the state of the biocenosis, both qualitative and quantitative characteristics are required, which became possible after the development of a real-time PCR (RT-PCR) technique [2, 5].

Femoflor is a unique technology based on the use of RT-PCR. This technology today allows you to give the most complete quantitative and qualitative characteristics of the normal and conditionally pathogenic flora of the urogenital tract in women [7, 10].

Thus, the urgency of the problem of objective laboratory diagnostics of microbiosis imbalance in case of premature rupture of membranes in pregnant women causes an urgent need for the development and implementation of new diagnostic methods in practical health care that allow timely diagnosis of this pathology.

Objective: to study the biocenosis of the vagina in pregnant women with premature rupture of membranes using RT-PCR.

Materials and research methods: The study groups consisted of 129 pregnant women admitted to the "Khorezm Perinatal Center" at a gestational age of 24-36 weeks with premature rupture of amniotic fluid (main group) and 30 healthy women with pregnancy at 30-32 ± 2 days of weeks (group of clinical and laboratory control).

Among pregnant women with premature rupture of amniotic fluid, 57 were with a gestation period of 24-31 weeks and 72 - with a gestation period of 32-36 weeks.

Main part. Examined scrapings of the epithelium of the posterolateral fornix of the vagina. The state of the vaginal biocenosis was determined by RT-PCR using Femoflor-16 reagents in a DT-96 detecting amplifier (NPO DNA-Technology). This kit includes a set of reagents for detecting control of sampling, quantitative determination of total bacterial mass (TBM), quantitative assessment of normal flora (Lactobacillus spp.), And 23 opportunistic microorganisms with the identification of the relationship between them and TBM.

To obtain adequate results, only samples with a sufficient number of cervical canal cells that entered the test tube with the analyzed sample and a sufficient total bacterial

mass were used. Samples were taken into account in which the amount of DNA of human cells was more than 104 genome-equivalents (GE) in the sample, and the TBM value was from 106 to 109 GE in the sample.

The software was used to calculate the number of TBM, lactobacilli and various groups of opportunistic microorganisms by the quantitative method and relative to TBM. The results were assessed in accordance with the criteria proposed [5].

For a quantitative assessment of normal flora and opportunistic microflora (OCP), it was proposed to use relative indicators, which were calculated as the difference in logarithms to base 10, according to the formula: log10 (-) = log10x - log10y.

The classification of biocenosis species included:

1) absolute normocenosis - a variant of biocenosis, in which the proportion of normal flora is 80 to 100% relative to TBM, the amount of Ureaplasma spp., Mycoplasma spp. - less than 104 ge / ml; and for fungi of the genus Candida - less than 103 ge / ml;

2) conditional normocenosis - the proportion of normal flora is 80-100% relative to TBM, the amount of Ureaplasma spp., Mycoplasma spp. - more than 104 ge / ml, and Candida spp. - more than 103 ge / ml;

3) moderate imbalance (aerobic, anaerobic or mixed) - the proportion of lactobacilli is reduced to 20-80% relative to TBM due to an increase in the number of anaerobes and / or aerobes;

4) pronounced imbalance (aerobic, anaerobic or mixed) - the proportion of lactobacilli decreases to 20% or less, the proportion of opportunistic microorganisms reaches 80-100% relative to TBM.

The results were processed on a personal computer using Microsoft Office Excell 2010. Differences between the groups were considered significant at $p < 0.05$.

Results of the study: among all examined pregnant women (n = 129) with premature rupture of amniotic fluid, 2 subgroups were identified: 1st subgroup - 19 pregnant women with septic complications (11 pregnant women with a gestational age of 24-31 weeks, 8 pregnant women with a gestational age of 32 -36 weeks), the 2nd subgroup

consisted of 115 pregnant women with premature rupture of amniotic fluid without septic complications.

The study revealed significant changes in the structure of the vaginal biocenosis among the examined groups of pregnant women (Table 1).

The patients of the main group showed significant changes in the composition of the vaginal microflora in comparison with the control group. The total bacterial mass in the main group was 13.2% higher than Lg10 OBM in the control group (8.6 ± 0.13 versus 9.3 ± 1.5, respectively). The most pronounced increase in BMR was observed in women from the 1st subgroup, i.e. in cases where septic complications have developed. Lg10 content Lactobacittus spp. In pregnant women with premature rupture of amniotic fluid, it was reduced by 36.1% from the indicators of the control group (4.6 ± 0.1 versus 7.2 ± 0.2, respectively). The lowest indicators were found in the group of women with septic complications in relation to the control indicators, which was significant (P <0.01) and corresponded to the development of vaginal dysbiosis.

Table 1

The structure of the vaginal biocenosis among the examined pregnant women in a comparative aspect (Lg10; M ± m)

Indicators	Main group (n=129)	1st subgroup (n=19)	2nd subgroup (n=115)	Control
ОБМ	8,6±0,13*	9,3±1,5*	8,3±0,17	7,6±0,12
Lactobacittus spp.	4,6±0,1*	2,9±0,15**^	3,4±0,14**	7,2±0,2
Aerobic microorganisms				
Enterbacteriactae	4,1±0,2**	4,7±0,22**^	3,4±0,2*	2,0±0,16
Streptococcus spp.	4,3±0,21***	4,8±0,23***^	3,2±0,14***	1,6±0,14
Staphylococcus spp.	3,6±0,1***	4,1±0,15***	3,4±0,14***	1,9±0,12
Anaerobic microorganisms				
Gardnerella vaginalis/Privotella bivia/Porphyromonas spp.	7,6±0,21***	8,6±0,23^***	5,6±0,25**	3,7±0,11
Eubacterium spp.	6,7±0,08**	7,7±0,21***^	5,4±0,18**	3,8±0,08

Sneathia spp./Leptotrihia spp./Fusobacterium spp.	7,0±0,07***	8,1±0,27**^	6,7±0,18***	2,8±0,07
Megasphaera spp./Vellonella spp./Dialister spp.	7,2±0,1	8,2±0,21	6,2±0,24	2,7±0,09
Lachnobacterium spp./Clostridium spp.	5,3±0,12**	6,3±0,22***^	4,2±0,18*	2,8±0,12
MobiLuncus spp./Corynebacterium spp.	4,6±0,12***	7,6±0,28***^	4,2±0,24**	1,3±0,11
Peptostreptococcus spp.	6,2±0,2**	8,2±0,29***^	5,2±0,18*	3,2±0,14
Atopobium vaginae	7,3±0,24***	8,3±0,25***^	6,3±0,27**	2,7±0,13
Mycoplasma				
Mycoplasma spp.	3,8±0,15***	4,2±0,21***^	3,4±0,20***	1,2±0,12
Ureaplasma spp.	4,2±0,18***	4,8±0,22***^	3,2±0,18***	1,6±0,11
Yeast-like fungi				
Candida spp.	3,2±0,3***	4,4±0,21***^	2,6±0,18***	1,6±0,08
Pathogenic microorganisms				
Mycoplasma genitalium	2,7±0,15	3,2±0,18^	2,1±0,11	0,0±0,0

*Note: * reliability of data in relation to the control group (P <0.05-0.001); ^ - reliability of data between 1st and 2nd subgroup (P <0.05-0.001)*

The vaginal microbiota in pregnant women of the main group was enriched with aerobic microorganisms, an increase in which indicates the development of a pyoinflammatory process in the postpartum period in the mother and child, which is confirmed by high rates among women with septic complications in the postpartum period.

The number of all obligate anaerobes was statistically higher in the main group (P <0.05-0.001) than in the control group, and the highest indicators were recorded in the 1st subgroup and were significantly high in relation to the data of the 2nd subgroup (P < 0.05-0.01).

Anaerobic opportunistic microorganisms prevailed in the main group and had statistically significant indicators in relation to the data of the control group (P <0.05-0.001). Content of Mycoplasma spp. and Ureaplasma spp. among pregnant women of

the main group had significantly high rates in relation to the control group (P <0.05-0.01), the severity of which was noted in the 1st group in relation to the second (P <0.05).

Pathogenic microorganisms in the form of Mycoplasma genitalium were significantly more common in the 1st subgroup and were significant in relation to the 2nd subgroup (P <0.05). In the control group, these microorganisms were not registered.

Comparative analysis of biocenosis indicators revealed that normocenosis in women of the main group occurs 3.1 times less frequently in relation to the data of the control group (2.8% versus 90%; p <0.01) (Table 2). In subgroup 1, 5 pregnant women (26.3%), who subsequently developed postpartum septic complications, had normocenosis, moreover, in all cases, only conditional, in the second group, absolute normocenosis was observed in 4.3%.

Table 2

Indicators of vaginal dysbiosis by type and severity among surveyed pregnant women in a comparative aspect

The state of the vaginal biocenosis	Main group (n=129)		1st subgroup (n=19)		2nd subgroup (n=115)		Control (n=30)	
	абс.	%	абс.	%	абс.	%	абс.	%
Normocenosis	37	28,7*	5	26,3*	32	27,8*	27	90
absolute	5	3,9*	0	0,0	5	4,3*	13	43,3
conditional	32	24,8*	5	26,3	27	23,5*	14	46,7
Dysbiosis	92	71,3*	14	73,7*	78	67,8*	3	10
moderate	52	40,3*	3	15,8^*	49	42,6*	2	6,7
- anaerobic	31	24,0*	1	5,3	30	26,1*	2	6,7
- aerobic	12	9,3	0	0,0	12	10,4	0	0
- mixed	9	7,0	2	10,5^	7	6,1	0	0
expressed	40	31,0*	11	57,9^*	29	25,2*	2	6,7
- anaerobic	40	31,0*	11	57,9^*	29	25,2*	2	6,7

*Note: * - reliability of data in relation to the control group (P <0.05); ^ - reliability of data between 1st and 2nd subgroup (P <0.05)*

Dysbiosis developed in 71.3% of pregnant women in the main group, of which moderate in 40.3%, expressed in 31.0%, which was significant in relation to the indicators of the control group. It should be noted that pronounced vaginal dysbiosis is 2.3 times more often observed among women in group 1, in relation to group 2 (57.9% versus 25.2%; P <0.05)

Ureaplasma (urealyticum + parvum) in a titer of more than 104 ge / ml and Candida spp were identified in 3 patients of the 1st group and in 8 women of the 2nd group with conditional normocenosis. in a titer of more than 103 ge / ml.

Moderate anaerobic dysbiosis in the main group as a whole was found 6.0 times more often in relation to the control group (24.0% versus 6.7%; P <0.001). It should be noted that in the 1st group, moderate anaerobic dysbiosis of the vagina was observed 5.0 times less frequently than in women of the 2nd group (5.3% versus 26.1%; p <0.01). At the same time, in the etiological structure of the imbalance of pregnant women in the main group, a combination of Gardnerella vaginalis / Prevotella bivia / Porphyromonas sppx was established with other anaerobic pathogens in 7 cases and in 5 cases - Ureaplasma and Candida spp. Were the cause of dysbiosis. In the control group, moderate dysbiosis was recorded only in 2 cases: 1 - Gardnerella in combination with other anaerobic bacteria and 2 - mycoplasma.

Moderate aerobic dysbiosis (3 cases of Streptococcus spp. In high titers) and mixed moderate dysbiosis (in one patient - Streptococcus spp. + Gardnerella vaginalis and in the second - Streptococcus spp. + Candida spp.) Were revealed only in the main group of pregnant women. the observation group was found in relation to the sum of aerobic and mixed imbalance as 3: 1.

A pronounced degree of anaerobic dysbiosis was identified in 40 (31%) women with premature rupture of amniotic fluid, which is 4.6 times higher than in the control group - in 2 patients (6.7%, p <0.05). Its etiological structure was made up of pathogens associated with bacterial vaginosis: Gardnerella vaginalis, Atopobium vaginalis and Ureaplasma in various combinations. The most frequently detected were Gardnerella vaginalis (26) and Ureaplasma (urealyticum + parvum) (10).

In the control group, a pronounced anaerobic imbalance was determined in 2 cases: in the 1st - a combination of Eubacterium spp. + Atopobium vaginae, in the other - Ure-aplasma (urealyticum + parvum) + Candida spp.

Indicators of bacterial vaginosis in women with preterm labor were determined in the following quantities: Gardnerella vaginalis - in 34.9% (45/129) of cases, Ureaplasma spp. and fungi of the genus Candida in diagnostic titers - in 27.9% (36/129) and 10.1% of cases (13/129), Mobiluncus species - in 13.9% of cases (18/129), Atopobium vaginae - 7, 8% (10/129).

In the control group: Gardnerella v. - 3.3%, Ureaplasma spp. - 3.3%, Atopobium v. - 3.3%.

A number of anaerobes are difficult to cultivate and, upon microscopy, are indistinguishable in morphological properties. PCR - RT makes it possible to identify them. This is especially important when defining Atopobium v. - a specific marker of chronic bacterial vaginosis [6]. In 22 cases (17.1%), facultative anaerobic microorganisms Streptococcus spp., Staphylococcus spp. And Entero-bacteriaceae were identified, causing pyoinflammatory processes in the postpartum period in mother and child, and I would like to note that in most cases in women 1- th subgroup.

The use of real-time PCR made it possible to establish that in 71.3% of women with premature rupture of amniotic fluid, bacterial vaginosis of the vagina is detected (anaerobic dysbiosis - 24.0%, aerobic - 40.3%, mixed - 7.0%). In the group of women with physiologically developing pregnancy, only anaerobic dysbiosis was established (6.7%).

Conclusion

Our data indicate the high diagnostic capabilities of the real-time PCR method for assessing vaginal microbiocenosis in women, the relationship of bacterial vaginosis with premature rupture of amniotic fluid, the need to identify bacterial vaginosis during pregravid preparation and early pregnancy, followed by restoration of normal

microflora and selective decontamination of microorganisms associated with vaginosis.

List of literature:

1. Kira Ye.F. Sovremennyye podkhody k vyboru preparata lokal'nogo deystviya v terapii bakterial'nogo vaginoza //Akusherstvo i ginekologiya. - 2012. - .№ 7. -S. 59-66.
2. Nazarova V.V., Shipitsyna Ye.V., Gerasimova Ye.N., i dr. Kriterii diagnostiki bakterial'nogo vaginoza s ispol'zovaniyem testa Femoflor-16 //Zhurnal akusherstva i zhenskikh bolezney. – 2017. – T. 66. – № 4. – S. 57–67.
3. Plakhova K.I., Gomberg M.A., Atroshkina M.Ye., Il'ina Ye.N., Govorun V.M. Rol' Atopobium vaginae pri retsidivirovanii bakterial'nogo vaginoza //Vestnik dermatologii i venerologii. - 2007. - № 5. - S. 10-13.
4. Rakhmatulina M.R., Plakhova K.I. Bakterial'nyy vaginoz, assotsiirovannyy s Atopobium vaginae: sovremennyye printsipy diagnostiki i terapii //Akusherstvo i ginekologiya. 2012. №3. S.88–92.
5. Sukhikh G.T., Prilepskaya V.N., Trofimov D.YU., Donnikov A.Ye., Aylamazyan E.K., Savicheva A.M., Shipitsyna Ye.V. Primeneniye metoda polimeraznoy tsepnoy reaktsii v real'nom vremeni dlya otsenki mikrobiotsenoza urogenital'nogo trakta u zhenshchin (test FemoflorR): meditsinskaya tekhnologiya. M., 2011. 36 s.
6. Uvarova Ye.V., Kazakova A.V. Sravnitel'nyy analiz sostava mikrobioty vlagalishcha u devochek v pubertatnom periode //Sovremennyye problemy nauki i obrazovaniya. – 2016. – № 6.
7. Shipitsyna Ye. V., Martikaynen Z. M., Vorob'yeva N. Ye., Yermoshkin M. S. Primeneniye testa Femoflor dlya otsenki mikrobiotsenoza //Zhurnal akusherstva i zhenskikh bolezney, T. LVIII, vypusk 3/2009
8. Donati L, Di Vico A, Nucci M, et al. Vaginal microbial flora and outcome of pregnancy. Arch Gynecol Obstet. 2010;281(4):589-600.

9. Haggerty CL, Totten PA, Tang G, et al. Identification of novel microbes associated with pelvic inflammatory disease and infertility. //Sex Transm Infect. 2016.92(6):441-6.
10. Kenyon C., Colebunders R., Crucitti T. The global epidemiology of bacterial vaginosis: a systematic review //Am J Obstet Gynecol. 2013;209(6):505-23.
11. Tansarli GS, Kostaras EK, Athanasiou S, Falagas ME. Prevalence and treatment of aerobic vaginitis among non-pregnant women: evaluation of the evidence for an underestimated clinical entity. //Eur J Clin Microbiol Infect Dis. 2013;32(8);977-84.
12. Ravel J., Gajer P., Abdo Z., et al. Vaginal microbiome of reproductive-age women. //Proc Natl Acad Sci USA. 2011;108:S4680-7.
13. Taylor B.D., Darville T., Haggerty C.L. Does bacterialvaginosis cause pelvic inflammatory disease. //Sex Transm Dis. 2013;40(2):117-122.

Entered 09.08.2021