International Journal of Medical and Health Research
ISSN: 2454-9142
www.medicalsjournals.com
Volume 2; Issue 4; May 2016; Page No. 18-20

Evaluation of Microflora Features the Method of Gas-Liquid Chromatography in Pregnant Women with Infectious Risk

[1] Rabbimova GT, [2] Muhamadiev NK

[1] Samarkand State Medical Institute

[2] Samarkand State University

Abstract
The paper identified markers of microorganisms of the genital tract in pregnant women with infectious risk and provides a comparative assessment of various methods of identification of microorganisms. The possibilities of using of GLC when typing microorganisms.

Keywords: marker microorganism, PCR, of GLC, pregnancy, infectious risk

Introduction

Relevance. One of the urgent problems of modern obstetrics remains a problem of intrauterine infection and intrauterine fetal infection (IUI) as leading obstetrics and perinatology due to a high level of infection during pregnancy, childbirth and postpartum, the danger of fetal development disturbance and birth of a sick child. Contamination by pathogenic microflora of the vagina and cervix during pregnancy creates a risk of its premature interruption, intrauterine infection, and during delivery contributes to a purulent inflammatory infection in the form of chorioamnionitis, endometritis [1-3]. In this case, the timely determination of the type of microflora is essential to prevent the risk of infection in choice of treatment strategy aswell. It is known that the traditional methods of identification of causative agents of microorganisms, infectious diseases or purulent inflammatory processes - include several stages: sowing the biological material on nutrient media, production of pure (i.e, consisting of identical microbes) cultures their, growing in an enriched media, and only to identify them by the nature of the fermentation of certain substrates. Even for microorganisms that have the ability to rapid growth, these stages of study take at least two days, which is a drawback of this method. Currently the technique of assessing the condition of the body for infection by markers specific to a certain type of microorganism has been developed, which makes it possible to carry out accelerated (less than two hours) indication of microorganisms [4-6]. In this regard, the development of new and improvement of existing methodologies to evaluate the peculiarities of microflora in pregnant women with infectious risk by gas-liquid chromatography (GLC) is relevant. The aim of the research was identification of microorganisms species in pregnant and postpartum women from the group of infectious risk by gas-liquid chromatography GLC.

Material and methods.

The study was carried out on the basis of maternity complex №3 of Samarkand. The study included 45 pregnant women with high infectious risk. The criteria for inclusion in the group of pregnant women with the infection risk were: woman's consent for the research and clinical medical history and symptoms of vaginitis in conjunction with gestational pyelonephritis or exacerbation of chronic pyelonephritis during pregnancy, the threat of termination of pregnancy during the entire period of gestation, presence of this time non-specific inflammatory disease of female genitals in medical history, postpartum and post-abortion endometritis; singleton pregnancy at term of 30-41 weeks; signs of intrauterine infection according to echographic study: placentomegaly, the presence of particulate matter in the amniotic fluid, and ventriculomegaly and pyelectasy in the fetus.

The vaginal secretions and the contents of the cervical canal of uterus during pregnancy, the uterine cavity aspirate were used as research material. Taking of material was carried out under aseptic conditions. During the aspirate sterile conductors were used, precluding the possibility of contamination of the sample by the cervical and vaginal microflora. The complex analysis of 65 samples of vaginal content and cervical secretions was carried out by three methods: bacteriological, PCR method and GLC.

Microbiological testing was performed by standard methods [7-8].

Determination of the DNA of potential pathogens was performed by polymerase chain reaction (PCR) aspirates from the uterine cavity (reagents and equipment LLC "Liteh", LLC "InterLabService", "DNA Technology", Moscow).

The presence of microflora in pregnant women at risk of infection was determined by the markers specific to each kind of microorganism, determined by GLC. Methods of GLC determination of microbial markers - fatty acids in the form of methyl esters: stationary phase - 15% of lestosil on Chromaton NA-W with particles size of 0,150-0,250 mm, glass column with 0,04 x1,00m; consumption of gas flow - nitrogen - 32 ml / min; detector - flame ionization, the ratio of nitrogen: hydrogen: air = 1: 1: 10, volume of introduced test - 2.3 mkl of hexane extract of fatty acid methyl esters the lipid fraction is isolated from the test sample by the method of Folch, methyl esters of fatty acids were obtained by transetherification of

glycerides with methanol in the presence of acetyl chloride by the method of [9].

Identification of fatty acids in a microorganism was carried out by "witnesses" method and on the basis of structural-group components method [10], but a quantitative analysis by a method of absolute calibration [11]. Statistical processing of the data was performed using Statistica 6.0 package of applied programs.

Results

When examining the contents of bacterioscopic vaginal discharge in 98% of all pregnant women bacterioscopic picture of vaginal inflammatory process was observed (4 degree and pattern of evident inflammation). Of these, in 5 (7.2%) women Trichomonas vaginalis was found.

In bacteriological examination of vaginal contents in pregnant women of high infectious risk the following microflora was sown: Enterococcus (faecalis) - in 23,9%, E. coli - in 18,0%, Staphylococcus (epidermidis, aureus) - in 62.0%, yeast fungi of the Candida genus - at 62,0%, Ureaplasma urealyticum - at 10,7%, Gardnerella vaginalis at 9,6%, Streptococcus - 12,4%. Corynebacterium spp - 10%, Mycoplasma hominis - 6,7%. In monoculture microorganisms occurred in 18% in associations - in 84% of pregnant women. Sterile bacterial cultivation were in 3 patients (6%) of pregnant women (see table 1).

Normally, in the cervical secretions bacteria and viruses not included. Only in the lower third of the cervical canal representatives of vaginal biotope are found in minimal quantities. In bacteriological examination of the contents of the cervical canal the following microflora was determined in our study: Enterococcus (faecalis) - in 17,2%, E.coli - in 27,0%, Staphylococcus - at 70,0%, Ureaplasma urealyticum - at 9.3%, Mycoplasma hominis - from 5,8%, Gardnerella vaginalis - 2,3%, Corynebacterium spp - 5,9%.

Table 1: Microbiocenosis of the vagina and cervix contents during bacteriological examination, in%

The spectrum of pathogens	Vagina	Cervix
E. coli	18,0	27,0
Staphylococcus	62,0	70,0
Streptococcus	12,4	17,4
Corynebacteriumspp	10,0	5,9
Fungi of the genus Candida	62,0	41,0
Enterococcus (faecalis,	23,9	17,2
Ureaplasma urealyticum	10,7	9,3
Mycoplasma hominis	6,7	5,8
Gardnerella vaginalis	9,6	2,3

The specific interest is the study of the spectrum of pathogens discharge from the vagina and cervix in pregnant women of a high risk of infection, suffering from colpitis, verified by PCR-diagnostics, which is performed with the DNA of potential pathogens (table 2).

Table 2: Microbiocenosis of the vagina and cervix contents using PCR method, at %

The spectrum of pathogens	Vagina	Cervix
Cytomegalovirus	29,0	21,6
Mycoplasma hominis	8,9	7,8
Herpes simplex virus	12,4	26,4
Chlamydia trahomatis	2,3	5,1
Ureaplasma urealyticum	21,8	9,8

Thus, the study of microbiocenosis by PCR method revealed the following: cytomegalovirus - in 29.6% of examined female patients, Mycoplasma hominis - at 8.9%, the herpes simplex virus - at 26.4%; Chlamydia trahomatis - at 5,1%, Ureaplasma urealyticum - at 9.8% of patients. In a number of the examined patients the massive growth of the bacterial flora in bacteriological methods on the background of the identified viral infection was revealed by PCR method. This indicates the disturbance of secretory immunity under the influence of viruses and probably accession of secondary bacterial infection.

Based on the foregoing, it can be stated that by culture techniques we can determine all known organisms, but this technique takes 48-72 hours and comprises the stages of growing on enrichment medium, in character identification of fermentation of certain substrates - all this is a drawback of the method. PCR method in our study identified pathogens that are difficult to cultivate by bacteriological methods, PCR diagnostics makes of possible to diagnose the presence of viruses, chlamydia, mycoplasma, ureaplasma and most other bacterial infections. The reliability of the study reaches 100%. PCR diagnostics detects the presence of infectious agents, of diseases even in those cases when it is impossible to do it using the other methods (immunological, bacteriological, microscopic) and even makes it possible to reveal single cells of bacteria or viruses.

The presence of the microflora is also defined by an improved method of GLC. These qualitative and quantitative results are presented in Table 3.

Table 3: The results of determination of microorganisms markers from vagina and cervix contents using GLC method

N	Type of microorganism	Marker	Contents*
1	Cytomegalovirus	Holestadienon	Y=2,17*10 ^{5}*x
2	Herpes simplex virus	Holestendiol	Y=3,24*10 ^{5}*x
3	Chlamydia trahomatis	Hydroxyeicosanic acid (3h20)	Y=2,53*10 ^{5}*x
		3- Hydroxydocosanic acid	Y=2,27*10 ^{5}*x
4	E. coli	3-hydroxymyristinic acid	Y=3,25*10 ^{5}*x
		Oxytridekanic acid (h13)	Y=2,51*10 ^{5}*x
5	Staphylococcus	Anteisononadecanic acid (a19)	Y=1,38*10 ^{5}*x
		Anteisotridecanic acid	Y=1,52*10 ^{5}*x
6	Streptococcus	Decanoic acid (C10: 0)	Y=4,21*10 ^{5}*x
7	Fungi of the Candida genus	Heptadecenoic acid (C17: 1)	Y=0,37*10 ^{5}*x
8	Enterococcus faecalis	Cyclononadecanoic acid (19cyc)	Y=8,23*10 ^{5}*x
9	Lactobacillus	1 methylenoctadecanoic acid (S19cyc)	Y=4,73*10 ^{5}*x
		Heptadecanoic aldehyde (7a)	Y=12,16*10^{5}*x
		Cyclononadecanoic aldehyde	Y=7,16*10 ^{5}*x

Note: * Y - the content of the marker in the microorganism;
x - height of the peak in the chromatogram mm.

After examining various methods of diagnosis of vaginal flora and cervix in pregnant women at risk of infection we found a relationship between the methods of determining of microorganisms (microbiological, GLC and PCR) the results of which are shown in table 4.

Table 4: The value of the correlation coefficient of microorganisms contens by different methods

№	Type of microorganism	Correlation coefficient		
		M+ PCR	M + GLC	PCR + GLC
1	Cytomegalovirus	0,907	0,947	0,779
2	Herpes simplex virus	0,781	0,949	0,783
3	Chlamydia trahomatis	0,872	0,955	0,783
4	E. coli	-	0,957	-
5	Staphylococcus	-	0,965	-
6	Streptococcus	-	0,942	-
7	Fungi of Candida genus	-	0,913	-
8	Enterococcus faecalis	-	0,929	-
9	Lactobacillus	-	0,932	-

Table 4 shows that the correlation coefficient of microorganisms content between microbiological and PCR; microbiological and GC; PCR and GLC in all cases is high, particularly high correlation is observed between microbiological and GLC and makes more than 0.9 in detection of all found markers of microorganisms. Thus, it can be argued that GLC method of determination of microorganisms in the genital tract in pregnancy is informative and at the same time in rapidity it exceeds microbiological, and in terms of economic efficiency - PCR diagnostics.

Conclusions

1. It was found that the microbiological, PCR and GC methods for determining the type of the microorganism from the vagina and cervix in pregnant women give identical results with an error of 3-5%.
2. Methods of GLC determination of microbial markers with a choice of stationary phase have been improved and can be recommended for screening of microbiocenosis in pregnant women. Technique by its rapidity, economic efficiency exceeds microbiological and PCR methods.
3. Typing of microorganisms by GLC method can be used in evaluation of quality and quantity of microorganisms, as well as in monitoring of therapeutic measures.

References

1. Kubanova AA. Urogenital infections caused by genital mycoplasmas. Clinical guidelines. Journal of Dermatology and Venereology. 2009; 3:78-72.
2. Agayeva MI. Comprehensive assessment of the functional state of the system mother-placenta-fetus in pregnant women with "high" index of infectious. The intellectual potential of the XXI century: level of cognition. 2010; 4(1):87-89.
3. Podzolkova NM. The possibility of early diagnosis of postpartum purulent - septic complications with the use of chromatography and mass spectrometry analysis // Mother and Child: Proceedings of the 8th All Russia Sciens Forum, Moscow, 3-6 October. 2006; Moscow, 2006, 203-204.
4. Krymtseva TA, Osipov GA, Boiko NB. Minor fatty acid in biological fluids of urogenital organs and their significance in the diagnosis of inflammatory processes. Journal. microbe. epidem. Immune. 2003; 2:92-101.
5. Osipov GA, Rodionov GG. The use of mass spectrometry microbial markers in clinical practice. Laboratory Diagnostics. Special Issue. Laboratory. 2013; 2:68-73.
6. Aripovsky AV, Kolesnik PO, Vezhdel MI, Titov VN. Method of preparation of samples for gas chromatographic determination of fatty acids without first extracting of lipids. Clinical Laboratory Diagnostics. 2012; 1:3-6.
7. Kanashkova TA. Medical Microbiology, Virology, Immunology: Workshop (8th ed.). Belarusian State Medical University. Minsk, 2015, 120.
8. Emma Tait, John D Perry, Stephen P Stanforth, John R. Dean Use of volatile compounds as a diagnostic tool for the detection of pathogenic bacteria. Trends in Analytical Chemistry. 2014; 53:117-125.
9. Muhamadiev NK, Ibatova Sh M. Gas chromatographic study of fatty acids in the blood serum of children with rickets. Proceedings of the 2nd West Ukrainian Symposium on Adsorption and chromatography. Lvov, 2000, 211-214.
10. Muhamadiev NK, Husainov Kh Sh, Rozhenko IN, Sakodynsky KI. Structurally-group methods in gas chromatographic identification of organic compounds. DAN UzSSR. 1989; 4:42-44.
11. Sakodynsky KI, Brajnikov VV, Volkov SA. Analytical chromatography. Chemistry, M, 1993, 464.