



PECULIARITIES OF DIAGNOSTICS OF CERVICOVAGINAL INFECTIONS IN PREGNANT WOMEN WITH THREATTED MISSION

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Abstract

Cervicovaginal infections, which lead to a complicated course of pregnancy and childbirth, remain one of the urgent problems of modern obstetrics. Infections not diagnosed in a timely manner in pregnant women can cause a high frequency of threat and/or premature termination. The variety of methods for detecting cervical vaginal infection in pregnant women complicates rational diagnosis and remains an incomplete and controversial problem. In this regard, the analysis of the prognostic significance of various diagnostic criteria in assessing the course, outcome of pregnancy and childbirth, the prognosis of an unfavorable course of pregnancy in women with CVI seems relevant and justified.

The paper presents an analysis of the results of the study conducted from 2019 to 2021 at the Regional Perinatal Center of Samarkand on the basis of the Department of Obstetrics and Gynecology No. 2 of the Samarkand Medical Institute, at the Department of Physical and Colloidal Chemistry of the Samarkand State University, as well as the private medical firm "Bionur". The study included pregnant women at 12-24 weeks gestation with pathological discharge syndrome; all had cervical-vaginal infection. Pregnant women were divided into 2 groups: group 1 (main) - 115 pregnant women with CVI complicated by the threat of termination of pregnancy, group 2 (comparison) – 49 pregnant women with CVI without the threat of termination. The control group consisted of 50 healthy pregnant women with a physiological course of gestation and vaginal normocenosis.

The criteria for inclusion in the main group were: the consent of the pregnant woman to conduct the study, the established diagnosis of CVI and the threat of termination of pregnancy at 12-24 weeks of pregnancy.

The average age of pregnant women in the control group was 23.82 ± 3.98 years, the main and comparative groups – $25,31 \pm 2,8$ years. All pregnant women underwent a general examination, a standard obstetric examination, as well as ultrasound to assess the condition of the fetus. The microbiocenosis of the genital tract was also studied by microscopic, bacteriological, GLC studies and PCR diagnostics.





For microscopic examination, the discharge from the posterior arch of the vagina and the cervical canal with Gram staining. The contents of the vagina and the mucus of the cervical canal in pregnant women were subjected to bacteriological examination. Elective and differential diagnostic nutrient media were used. After the manifestation of growth, the number of grown colonies was calculated and recalculated per 1 ml of sample, expressing the degree of colonization of samples in CFU/ml.

The nature of the microflora in pregnant women with CVI was determined using markers characteristic of each type of microorganism, determined by the method of GC. The GC method was carried out on a chromatograph "Color 100, model 165" with a flame ionization detector at the Samarkand State University. Identification of markers of microorganisms was carried out on the gas chromatographic mass spectrometric system Agilent Technologies 5977A MSD in the "Center of High Technologies". Qualitative and quantitative chromato-mass spectrometric analysis of the biomaterial - the vaginal discharge – for the content of fatty acids and oxy-acids was carried out to isolate and identify a single microorganism by its (taxa) profile and marker characteristics, for which specific ions of markers of microorganisms were determined by mass-fragmentography, followed by the identification of a complete community of microorganisms using the internal standard method (template). [4]. Methyl esters of fatty acids were obtained by transesterification of glycerides with methanol in the presence of acetyl chloride according to the method [4]. Identification of fatty acids in the microorganism was carried out by the method of "witnesses" and on the basis of the method of structural group components (mcg/ml), and quantitative analysis by the method of absolute calibration (cl/mlx10⁵) [4, 6]. PCR study in patients with CVI was carried out on the Rotor Gene apparatus (Germany) in a private company "Bionur med service".

In all patients of the main group, pregnancy proceeded against the background of the threat of its termination, and in the first trimester of pregnancy, the threat of termination was observed in 88 (76.5%). The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package.

In the complex diagnosis of cervicovaginal infection, we identified the following nosological manifestations of infection: bacterial vaginosis in the main and comparison groups occurred with a frequency of 33 and 18.4%, respectively, aerobic vaginitis – in 12.2 and 14.3%, vulvovaginal candidiasis – in 27 and 49%; in 15.6 and 12.2% of pregnant women bacterial vaginosis was combined with vulvovaginal candidiasis, in Mixed infection was detected in 12.2 and 6.1% of patients. Thus,





bacterial vaginosis and its combinations in the main group were 2 times more common (48.6%).

Microscopic examination of the contents of the vaginal discharge in 77 (67%) pregnant women of the main group showed a microscopic picture of the inflammatory process. Vaginal trichomonas were found in 3 (2.6%) women of the main group.

Table 1 The state of the vaginal microflora during microscopic examination in pregnant women with cervicovaginal infection

Microscopy (Gram)	Control, n=50		Main group, n=115		Comparison group, n=49	
	absolute	%	absolute	%	absolute	%
Leukocytes <15 in sight	45	90,0	38	33***	9	18,4***
Leukocytes 20-40 in sight	-	-	20	17,4***	28	57,1***
Leukocytes >40 in sight	-	-	57	49,6***	12	24,5***
Squamous epithelium	47	94,0	65	61,9***	32	65,3***
Trichom. Vaginalis	-	-	3	2,61	1	2,0
"Key cells"	-	-	32	27,8**	3	6,1
Mushrooms candida	10	20,0	49	42,6**	17	34,7
Slime	-	-	103	89,6***	31	65,3***
Lactobacilli	50	100,0	4	3,5***	3	6,1***

Note: * - differences relative to the data of the control group are significant (* - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$)

Bacteriological examination mainly revealed conditionally pathogenic flora: Enterococcus faecalis, E. coli, Fungi of the genus Candida, Staph. epidermidis in high concentrations ($CFU/ml > 10^5$) (Table 2). A bacteriological study also revealed a general contamination of the cervical canal. As can be seen from Table 2, the level of contamination in the main group was higher. Violation of quantitative ratios in the bacterial community of the genital tract leads to the development of clinical manifestations of CERVICOVAGINAL INFECTION in the form of a threat of interruption.



Table 2 Total contamination of the cervical canal in pregnant women with cervicovaginal infection (CFU/ML)

Level of contamination	Main group, n=115		Comparison group, n=49		χ^2	P
	abc.	%	abc.	%		
Low ($0-10^3$ -)	-	-	-	-		
Medium ($10^3-9 \times 10^4$)	42	36,5	30	61,2	7,49	<0,01
High ($10^5-9 \times 10^5$) and above	73	63,5	19	38,8	8,51	<0,01

In order to increase the effectiveness of the diagnosis of cervicovaginal infection, PCR and GC studies were used. To detect "hidden" cervicovaginal infections, cervical smears were examined by PCR. We conducted a qualitative PCR study, which is the "gold standard" in the diagnosis of pathogens, pathogens of cervicovaginal infection, but at the same time is not quantitative, it is mainly used to detect obligate pathogens. The study of microbiocenosis by PCR for the presence of "hidden infections" showed that the most common microorganism in the main group were Ureaplasma urealyticum (20.7%) and cytomegalovirus infection (13.8%) ($p < 0.05$).

In the diagnosis of infection, the method of GLC was used, with which the composition of fatty acids, oxy-acids and aldehydes included in the the composition of microbial cell lipids and metabolites. GZHC allows to simultaneously determine markers of opportunistic flora, as well as "hidden" urogenital infection both qualitatively and quantitatively, and is an express method.

Table 3 The level of markers of microorganisms of the contents of the vagina (cl/ml) in pregnant women determined by the method of GLC

Type of microorganism	Marker (MIS MIDI "Sherlock", 1992)	Main group, n=75	Comparison group, n=23
Cytomegalovirus	Cholestadienon	$2,17 \cdot 10^5$	$1,92 \cdot 10^3$
Herpes simplex virus type 2	Cholestenediol	$3,24 \cdot 10^5$	$2,13 \cdot 10^3$
Chlamydia trachomatis	hydroxyeicosanoic acid (3h20)	$2,58 \cdot 10^7$	$1,88 \cdot 10^4$
E. coli	3-hydroxymyristic acid	$2,44 \cdot 10^8$	$1,98 \cdot 10^4$
Enterococcus faecalis	Cyclonodecanoic acid (19cyc)	$8,23 \cdot 10^7$	$6,23 \cdot 10^3$
Staphylococcus spp.	Anteisonodecanoic acid (a19)	$2,10 \cdot 10^8$	$1,70 \cdot 10^5$
Streptococcus agalactiae B	Decanoic acid (C10:0)	$7,07 \cdot 10^9$	$4,02 \cdot 10^5$
Mushrooms of the genus Candida	Heptadecenoic acid (C17:1)	$7,40 \cdot 10^7$	$5,63 \cdot 10^4$
Lactobacillus 1-	methylenodecanoic acid (C19cyc)	$5,29 \cdot 10^2$	$5,44 \cdot 10^3$



So, we found that each type of microorganisms has its own composition of fatty acids. In case of CERVICOVAGINAL INFECTION complicated by the threat of termination of pregnancy, the level of markers of microorganisms has higher values (in titer) concentrations of 10^{5-9} and higher, whereas in the comparison group their titer was in the range of up to 10^5 (Table 3).

In pregnant women with the threat of termination of pregnancy and CERVICOVAGINAL INFECTION of mixed genesis, peaks were detected on the chromatogram, that is, the absolute and relative retention time, confirmed by additional ions and the ratio of peak areas (templates were used to find the right ion) (Fig. 1, 2).

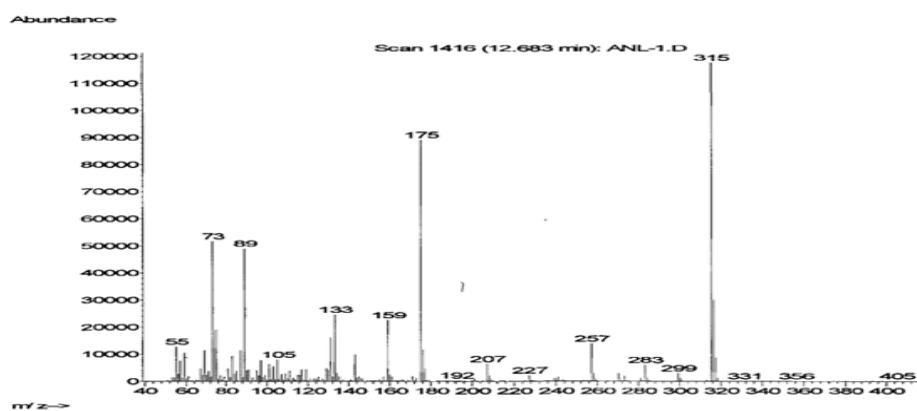


Fig. 1. Concentration of Enterobacteriaceae marker – β -hydroxymyristinic acid (h14) in a pregnant woman with a threat of termination + mixed infection

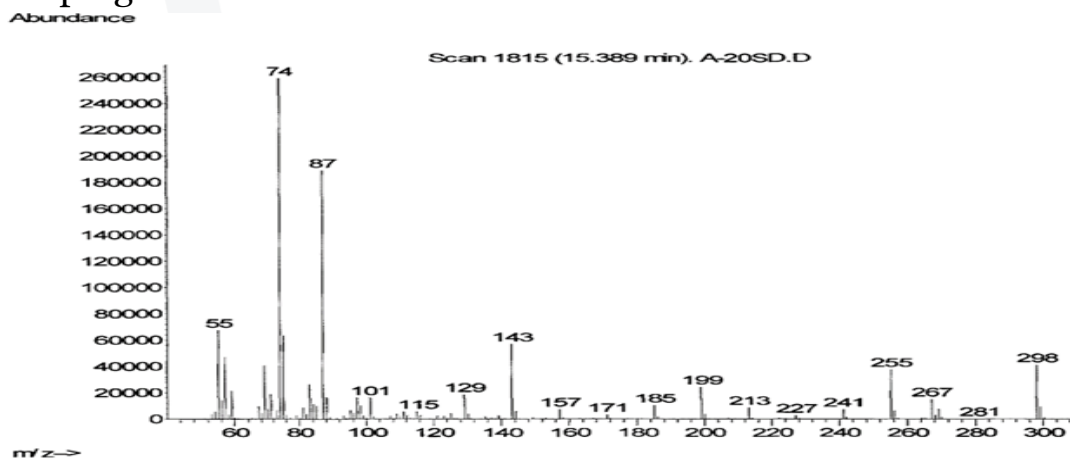


Fig. 2. Concentration of cyclonadecanoic acid (marker of *Enterococcus faecalis*) (19 cyc) in a pregnant woman with a threat of termination of pregnancy and cervicovaginal infection (BV+AB).

Having studied various methods of diagnosing the microflora of the vagina and cervix in pregnant women with cervicovaginal infection, we found a relationship between the methods of determining microorganisms by bacteriological, PCR and GLC.



Table 4 The value of the correlation coefficient of the content of microorganisms by various methods

Type of microorganism	Correlation coefficient	
	Bacteriological+GC	PCR+GC
Cytomegalovirus	-	0,87
Herpes simplex virus	-	0,83
Chlamydia trachomatis	-	0,81
E. coli	0,85	-
Staphylococcus	0,76	-
Streptococcus	0,84	-
Fungi of the genus Candida	0,71	-
Enterococcus faecalis	0,72	-
Lactobacillus	0,66	-

It can be seen from Table 4 that the correlation coefficient of the content of some microorganisms between the bacteriological method and GC; PCR and GC is high in all cases, especially high correlation is observed between GC and PCR, which exceeds 0.8 when detecting all the markers of microorganisms encountered, but qualitative PCR has disadvantages such as the inability to adequately quantify.

Thus, the method of GLC in combination with bacteriological and PCR studies allows to qualitatively and quantitatively identify the causative agents of cervicovaginal infections, to conduct a comprehensive assessment of the etiological role of infectious agents in the development of cervicovaginal pathology; diagnostic efficacy was 85.5% (sensitivity - 90%, specificity – 81%), while separately diagnostic efficacy in GLC was 82%, with the bacteriological method – 56%, PCR – 79%. Data on the detection of infection by various methods are presented in Table 5.

Table 5 Comparative evaluation of the effectiveness of detection of markers of microorganisms by various methods

The causative agent	Bacteriological examination	Polymerase chain reaction	Gas-liquid chromatography
Cytomegalovirus	-	+	+
Herpes simplex virus type 2	-	+	+
Chlamydia trachomatis	-	+	+
E. coli	+	-	+
Enterococcus faecalis	+	-	+
Staphylococcus spp.	-	-	+
Streptococcus spp.	+	-	+
Candida	+	-	+
Lactobacillus	+	-	+



Thus, with cervicovaginal infection complicated by the threat of interruption, bacterial vaginosis was 2 times more common than in the comparison group. The structure of cervicovaginal infections in pregnant women with the threat of termination is as follows: bacterial vaginosis in 33% of pregnant women, aerobic vaginitis – in 12.2%, VVC – in 27%. In 15.6% of the examined bacterial vaginosis was combined with vulvovaginal candidiasis, in 12.2% of cases there was a mixed infection with the presence of "hidden" cervicovaginal infection, which was observed significantly more often than in the comparison group ($p < 0.05$).

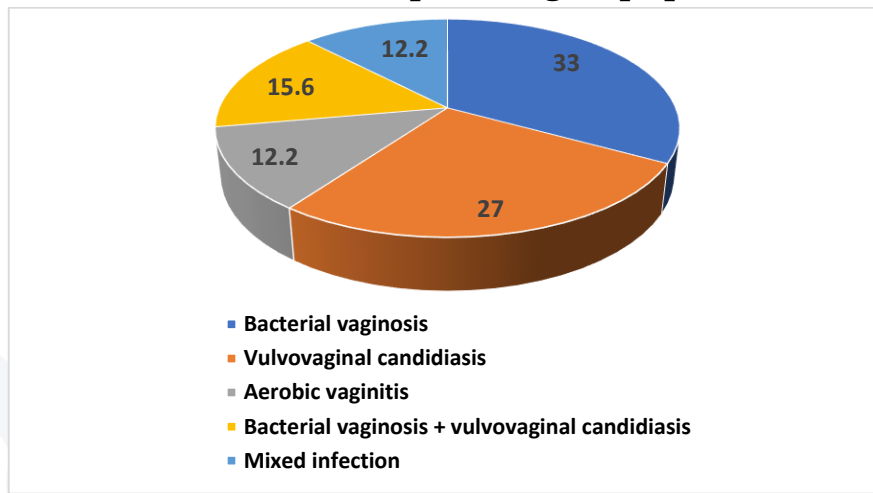


Fig. 3. The structure of cervicovaginal infections in pregnant women (%) in pregnant women of the main (a) group

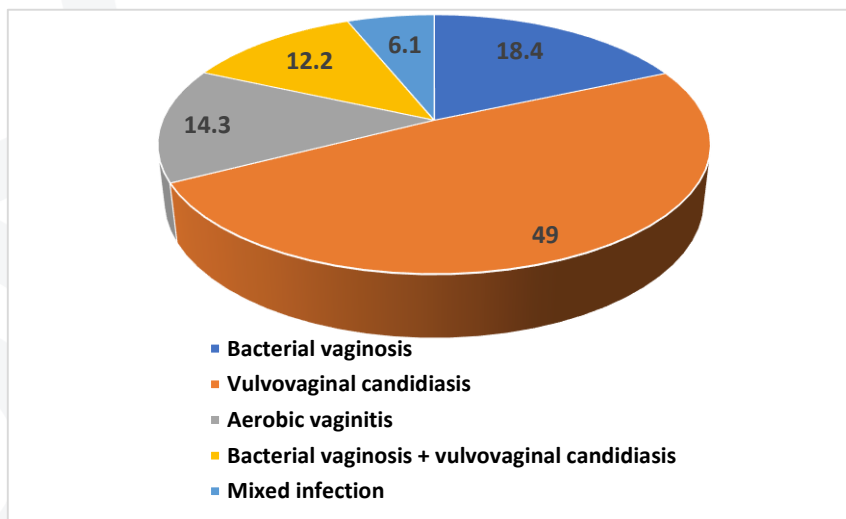


Fig. 3. The structure of cervicovaginal infections in pregnant women (%) in pregnant women of the comparison group (b)



Thus, in pregnant women with cervicovaginal infection complicated by the threat of termination of pregnancy leads to an increased level of contamination of the vagina and cervical canal and microbial load (CFU during bacteriological examination and titer of microorganisms by GC).

In order to determine whether a cervicovaginal infection during pregnancy will lead to a complicated course of its threat of termination and premature birth, prognostic markers of the risk of the threat of termination of pregnancy with cervicovaginal infection were studied. Using the heterogeneous recognition procedure of A.A. Genkin and E.V. Gubler (1978), they selected informative signs and ranked them. Diagnostic coefficients – scores were calculated for the selected features using the Kullback informative measure. Summing up the diagnostic criteria by points, we obtained the risk of developing the threat of termination of pregnancy with cervicovaginal infection, which allows us to predict the outcome of pregnancy; apply a differentiated algorithm of actions and tactics for managing such pregnant women (Table 6). The table shows that each feature corresponds to a certain score calculated by mathematical methods. Thus, the analysis of the significance of the above criteria for cervicovaginal infection makes it possible to determine the priority directions of therapeutic and preventive measures for the management of pregnant women with cervicovaginal infection complicated by the threat of termination of pregnancy and to prevent the transition of the threat of termination of pregnancy into premature birth.

Table 6 Prognostic criteria for the risk of the threat of interruption in cervicovaginal infection

Index	Low risk	Informative ness index	Medium risk	Informative ness index	High risk	Informative ness index
The content of leukocytes in a vaginal smear	<15 in sight	1	20-25 in sight	2	>25 in sight	3
Bacteriological.research microflora of the cervix and vagina	<10 ³	2	10 ³ -10 ⁴	2,5-4,5	>10 ⁵	5
Microorganism markers by GLC	<10 ³	2	10 ³ -10 ⁵	2,5-4,5	>10 ⁵⁻⁹	5
Total points	<15 в II/3p	5		6-11		12

Thus, the threat of termination of pregnancy with cervicovaginal infection occurs in the event of an increase in the titer of microorganisms more than 10⁵. Prognostic criteria for assessing the risk of the threat of interruption in cervicovaginal infection are also a high level of titer markers of microorganisms of 10⁵ or more. The sum of points over 12 is a high risk, the sum of points 6-10 is a moderate risk, 5 or less points



is a low risk of interruption in cervicovaginal infection; a positive prognosis was confirmed in 92%, negative in 8% of cases. Diagnostic efficiency of detection of microorganisms by GC methods in combination with bacteriological and PCR studies was 85.5% (sensitivity – 90%, specificity – 81%), while separately diagnostic efficiency in GC was 82%, with bacteriological method – 56%, PCR – 79%.

Literature

1. Azbukina L. N. risk factors, forecasting and tactics of pregnancy management with a threat of premature birth. Mezhdunar. Journal. adj. and the foundation. research. 2015, no. 11-5, pp. 633-635.
2. Ankirskaya A. S., Muravyeva V. V., Karapetyan T. E. Aerobic vaginitis in the structure of opportunistic vaginal infections. Debatable question of nosological terminology // Akush. and gin. 2013, No. 1, pp. 107-110.
3. Aripovsky A.V. et al. Method of sample preparation for gas chromatographic determination of fatty acids without preliminary extraction of lipids // Klin. lab. diagnostics. 2012, No. 1, pp. 3-6.
4. Bondarenko K. R. et al. Features of the vaginal microecosystem in the gestation period (review of literature) // Vestn. Russian state. med. UN-Ta. 2014, No. 4, pp. 6-12.
5. Glukhovets B. I., Khodareva A. G. Aanalysis of the microbiocenosis of the reproductive system by chromato-mass spectrometry (practical manual) St. Petersburg, 2016, 52 p.
6. Kravchenko E. N. et al. Modern approaches to diagnosis and therapy of vaginal infections // mother and child in Kuzbass. - 2017. - №3. - pp. 10-12.
7. Lashkevich E. L. premature birth as a consequence of urocervicaginal infection. Moscow, 2016, 22 p. (in Russian).
8. Osipov G. A., Rodionov G. G. application of the method of mass spectrometry of microbial markers in clinical practice. 2013, no. 1-3, pp. 68-73.
9. Rabbimova G. T., Negmadzhanov B. B. evaluation of the effectiveness of treatment and Prevention of complications in pregnant women with vaginal infection //the Journal of scientific articles "Health & Education Millennium". Moscow, 2017. Vol. 19. № 1. - pp. 48-50.
10. Rebrova O. Yu. statistical analysis of medical data. Using the Statistica application software package. Moscow: Media Sphere Publ., 2002, Vol. 305.
11. Rumyantseva T. A. Optimization of clinical and laboratory approaches to the diagnosis of vulvovaginal and cervical infections: Dis. or cand. med. Nauk, Moscow, 2014, 197 p. (in Russian).





12. Sinyakova A. A. et al. Microbiocenosis of the vagina in the first trimester of pregnancy in women with a history of miscarriage // Journal. akush. and wives. Bol. - 2016. - VOL. 65, N^o4. – PP. 44-49.
13. Yankovsky D. S. microbiome and women's health // reproductive endocrinology. 2015, No. 4, p. 24.
14. Baqui A. H. et al. Prevalence of and risk factors for abnormal vaginal flora and its association with adverse pregnancy outcomes in a rural district in north-east Bangladesh //Acta obstetricia et gynecologica Scandinavica. – 2019. – V. 98. – N^o. 3. – P. 309-319.
15. Leeper C., Lutzkanin A. Infections during pregnancy // Primary Care: Clin. Office Pract. – 2018. – Vol. 45, N^o3. – P. 567-586.
16. Rabbimova G.T., Muhamadiev N.K. Evaluation of microflora features the method of gas-liquid chromatography in pregnant women with infectious risk // Int. J. Med. Health Res. – 2016. – Vol. 2, N^o4. – P. 18-20.
17. Sentilhes L. et al. Prevention of spontaneous preterm birth: Guidelines for clinical practice from the French College of Gynaecologists and Obstetricians (CNGOF) // Europ. J. Obstet. Gynecol. Reprod. Biol. – 2017. – Vol. 210. – P. 217-224.
18. Stout M.J. et al. Early pregnancy vaginal microbiome trends and preterm birth // Amer. J. Obstet. Gynecol. – 2017. – Vol. 217, N^o3. – P. 356. e1-356. e18.

