

Vaginal Microbiota Composition according to real-time PCR in Patients with HPV-Associated Cervical Intraepithelial Neoplasias

E. Voroshilina^{1,2}, N. Vladimirova², K. Ignatova²

¹Ural State Medical University, Department of Microbiology, Virology and Immunology, Yekaterinburg, Russia.

²"Garmonia" Medical Center, Laboratory Department, Yekaterinburg, Russia.

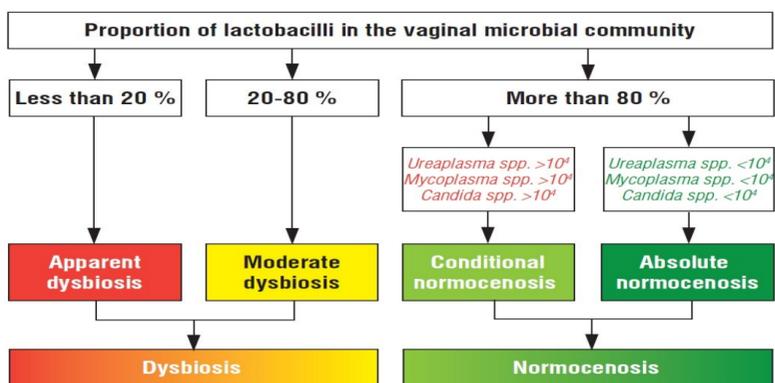
INTRODUCTION

It was shown that vaginal microbiota dominated by obligate anaerobes and therefore having a higher pH level increases the risk of HPV infection as well as makes the conditions for HPV persistence. The assessment of vaginal microbiota composition and HPV load is of great importance in prevention of cervical dysplasia and cervical cancer.

The introduction of the Femoflor®16 kit (DNA-Technology, LLC, Russia) made it possible to evaluate vaginal microbiota and its participants with high accuracy and specificity, identify the severity of dysbiotic processes.

THE AIM OF THIS STUDY WAS TO ANALYZE QUALITATIVE AND QUANTITATIVE COMPOSITION OF VAGINAL MICROBIOTA IN PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIAS (CIN) BY MEANS OF QUANTITATIVE REAL-TIME PCR WITH FEMOFLOR®16 KIT (DNA-TECHNOLOGY, RUSSIA).

ALGORITHM FOR LAB REPORT GENERATION FOR AN RT-PCR TEST OF VAGINAL MICROBIOTA



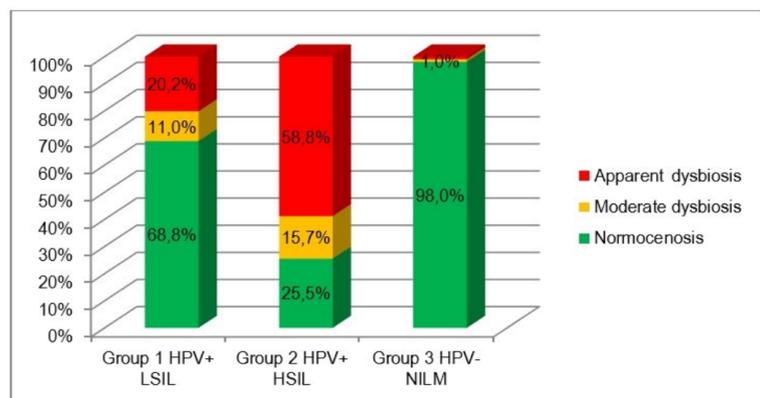
RESULTS

Depending on the cytology type and results of HPV-test, patients were divided into 3 groups:

Group 1 included 109 HPV-positive patients with LSIL

Group 2 - 102 HPV-positive patients with HSIL

Group 3 - 102 HPV-negative women with NILM (control group).



In the majority of cases vaginal microbiota of control group met the criteria of normocenosis: it was detected in 100 (98%) of 102 cases. Also normocenosis was detected in 75(68.8%) of 102 HPV-positive patients with LSIL (Group 1). While only 26(25.5%) of 102 HPV-positive patients with HSIL (Group 2) met the criteria of normocenosis ($p<0.01$). Moderate dysbiosis was detected in 12(11.1%) patients of Group 1, 16(15.7%) of Group 2 and 1(1.0%) of Group 3. Apparent dysbiosis in 22(20.1%), 60(58.8%) and 1(1.0%) of patients of Group 1, 2 and 3 respectively($p<0.01$). In the structure of apparent dysbiosis, anaerobic dysbiosis was prevalent in women of Group 1 and 2 (68.2% and 75.0% respectively).

HPV-associated CIN are accompanied by the development of apparent dysbiosis with the prevalence of obligate anaerobes.

STUDY DESIGN

Study population and sampling

312 women (aged 20-49, mean age 32.56±6.2) were recruited into the study upon presentation for cervical screening to "Garmonia" Medical Center, Yekaterinburg, Russia.

Cervical smears were collected with Rovers® Cervex-Brush® Combi with detachable head, which was placed into the BD SurePath collection vial, containing preservative fluid. Vaginal fluid was collected from the posterior vaginal fornix using urogenital swabs and placed in 1.5ml Eppendorf tubes with sterile saline solution and stored at -20°C prior to analysis.

DNA extraction, HPV test and quantitative analysis of vaginal microbiota by real-time PCR

Total nucleic acid (NA) was extracted from swabs using the kit for NA isolation PREP-NA-PLUS (DNA-Technology, LLC).

Quantitative real-time PCR (RT-PCR) was performed using Femoflor®16 kit (DNA-Technology, Russia).

The kit allows detecting the quantity (expressed in genome equivalents per 1 ml (GE/ml)) of lactobacilli and 15 groups of opportunistic microorganisms (OM). The special software was used to automatically calculate the total bacterial load (TBL) and the proportion of OM and lactobacilli in relation to the TBL.

HPV test was performed using real-time PCR HPV-Quant-21 kit (DNA-Technology, Russia), which allows genotyping of HPV for 21 types and evaluation of the absolute viral load (DNA copies/sample) and relative viral load (RVL, DNA copies/105 epitheliocytes).

Liquid based cytology (LBC) was performed according to the protocol for the BDSurePath® equipment. Cervical samples were evaluated according to the Bethesda system (2014).

Statistical analysis was carried out using the IBM SPSS Statistics version 21.0 software package.

VAGINAL MICROBIOTA VARIANTS ANALYZED USING RT-PCR

Depending on the proportion of lactobacilli and opportunistic microorganisms (OM) in the TBL, three basic variants of vaginal microbiota were identified:

1. "Normocenosis". This variant of vaginal microbiota is predominated by lactobacilli. The proportion of lactobacilli is more than 80 % of the TBL, and the proportion of opportunistic microorganisms (specifically obligate anaerobes) is less 20 % of the TBL.
2. "Moderate dysbiosis" (MD) is an intermediate state of vaginal microbial community when the proportion of lactobacilli decreases and constitutes less than 80 % but more than 20 % of the TBL. Thus, the proportion of opportunistic microorganisms is more than 20 % but less than 80 % of the TBL.
3. "Apparent dysbiosis" (AD) – this variant of vaginal microbiota is predominated with various opportunistic bacteria: the proportion of lactobacilli is less than 20 % of the TBL, and the diverse microbial community (specifically strictly anaerobic bacteria) constitutes more than 80 % of the TBL.

No	Test title	Result	Quantitative	Relative Lg (V/TBL)	% of TBL
1	Sample intake control	not detected	10 ¹⁰	0.1	100
2	Total bacterial mass	not detected	10 ¹⁰	0.1	100
NORMAL MICROBIOTA					
3	Lactobacillus spp.	not detected	10 ¹⁰	0.0 (0-100 %)	0
FACULTATIVE ANAEROBIC MICROORGANISMS					
4	Enterobacteriaceae	not detected	10 ¹⁰	0	0
5	Streptococcus spp.	not detected	10 ¹⁰	0	0
6	Diphtheriobacterium spp.	not detected	10 ¹⁰	0	0
OBLIGATE ANAEROBIC MICROORGANISMS					
7	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	10 ¹⁰	0.7 (10-25 %)	0
8	Lactobacillus spp.	not detected	10 ¹⁰	0.0 (0-28 %)	0
9	Streptococcus spp. + Lactobacillus spp. + Fusobacterium spp.	not detected	10 ¹⁰	0	0
10	Mycoplasma spp. + Ureaplasma spp. + Chlamydia spp.	not detected	10 ¹⁰	0	0
11	Mollicoccus spp. + Corynebacterium spp.	not detected	10 ¹⁰	0	0
12	Propionibacterium spp.	not detected	10 ¹⁰	0	0
13	Absolutus vaginae	not detected	10 ¹⁰	0	0
YEAST-LIKE FUNGI					
14	Candida spp.*	not detected	10 ¹⁰	0	0
MYCOPLASMAS					
15	Mycoplasma hominis**	not detected	10 ¹⁰	0	0
16	Ureaplasma urealyticum + parvum**	not detected	10 ¹⁰	0	0
PROTEINOLYTIC MICROORGANISMS					
17	Mycoplasma genitalium**	not detected	10 ¹⁰	0	0
* - Quantitative analysis ** - Qualitative analysis					
Conclusion: absolute normocenosis.					

No	Test title	Result	Quantitative	Relative Lg (V/TBL)	% of TBL
1	Sample intake control	not detected	10 ¹⁰	0.1	100
2	Total bacterial mass	not detected	10 ¹⁰	0.1	100
NORMAL MICROBIOTA					
3	Lactobacillus spp.	not detected	10 ¹⁰	0.3 (40-55 %)	0
FACULTATIVE ANAEROBIC MICROORGANISMS					
4	Enterobacteriaceae	not detected	10 ¹⁰	0	0
5	Streptococcus spp.	not detected	10 ¹⁰	0	0
6	Diphtheriobacterium spp.	not detected	10 ¹⁰	0	0
OBLIGATE ANAEROBIC MICROORGANISMS					
7	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	10 ¹⁰	0.7 (10-25 %)	0
8	Lactobacillus spp.	not detected	10 ¹⁰	0.0 (0-28 %)	0
9	Streptococcus spp. + Lactobacillus spp. + Fusobacterium spp.	not detected	10 ¹⁰	0	0
10	Mycoplasma spp. + Ureaplasma spp. + Chlamydia spp.	not detected	10 ¹⁰	0	0
11	Mollicoccus spp. + Corynebacterium spp.	not detected	10 ¹⁰	0	0
12	Propionibacterium spp.	not detected	10 ¹⁰	0	0
13	Absolutus vaginae	not detected	10 ¹⁰	0	0
YEAST-LIKE FUNGI					
14	Candida spp.*	not detected	10 ¹⁰	0	0
MYCOPLASMAS					
15	Mycoplasma hominis**	not detected	10 ¹⁰	0	0
16	Ureaplasma urealyticum + parvum**	not detected	10 ¹⁰	0	0
PROTEINOLYTIC MICROORGANISMS					
17	Mycoplasma genitalium**	not detected	10 ¹⁰	0	0
* - Quantitative analysis ** - Qualitative analysis					
Conclusion: moderate anaerobic dysbiosis with significant amounts of Candida spp.					

No	Test title	Result	Quantitative	Relative Lg (V/TBL)	% of TBL
1	Sample intake control	not detected	10 ¹⁰	0.1	100
2	Total bacterial mass	not detected	10 ¹⁰	0.1	100
NORMAL MICROBIOTA					
3	Lactobacillus spp.	not detected	10 ¹⁰	0	0
FACULTATIVE ANAEROBIC MICROORGANISMS					
4	Enterobacteriaceae	not detected	10 ¹⁰	0	0
5	Streptococcus spp.	not detected	10 ¹⁰	0	0
6	Diphtheriobacterium spp.	not detected	10 ¹⁰	0	0
OBLIGATE ANAEROBIC MICROORGANISMS					
7	Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	not detected	10 ¹⁰	0.1 (0-14 %)	0
8	Lactobacillus spp.	not detected	10 ¹⁰	0.1 (0-27 %)	0
9	Streptococcus spp. + Lactobacillus spp. + Fusobacterium spp.	not detected	10 ¹⁰	0.1 (4-13 %)	0
10	Mycoplasma spp. + Ureaplasma spp. + Chlamydia spp.	not detected	10 ¹⁰	0.1 (0-25 %)	0
11	Mollicoccus spp. + Corynebacterium spp.	not detected	10 ¹⁰	2.0 (0-12 %)	0
12	Propionibacterium spp.	not detected	10 ¹⁰	1.8 (1.5-2.3 %)	0
13	Absolutus vaginae	not detected	10 ¹⁰	2.4 (0.3-6.4 %)	0
YEAST-LIKE FUNGI					
14	Candida spp.*	not detected	10 ¹⁰	0	0
MYCOPLASMAS					
15	Mycoplasma hominis**	not detected	10 ¹⁰	0	0
16	Ureaplasma urealyticum + parvum**	not detected	10 ¹⁰	0	0
PROTEINOLYTIC MICROORGANISMS					
17	Mycoplasma genitalium**	not detected	10 ¹⁰	0	0
* - Quantitative analysis ** - Qualitative analysis					
Conclusion: apparent anaerobic dysbiosis.					

Example of lab results of HPV Quant test for detecting 21 genotypes of HPV by means of quantitative real-time PCR

No	Name of research	Relative, (X/SIC)	Quantitative, Lg (copies/sample)	Qualitative
1	HPV 31	not discovered	not discovered	
2	HPV 35	not discovered	not discovered	
3	HPV 16	not discovered	not discovered	
4	HPV 52	not discovered	not discovered	
5	HPV 33	not discovered	not discovered	
6	HPV 68	not discovered	not discovered	
7	HPV 45	not discovered	not discovered	
8	HPV 82	not discovered	not discovered	
9	HPV 51	not discovered	not discovered	
10	HPV 6	not discovered	not discovered	
11	HPV 44	not discovered	not discovered	
12	HPV 11	not discovered	not discovered	
13	HPV 18	not discovered	not discovered	
14	HPV 39	not discovered	not discovered	
15	HPV 58	not discovered	not discovered	
16	HPV 66	not discovered	not discovered	
17	HPV 26	not discovered	not discovered	
18	HPV 53	not discovered	not discovered	
19	HPV 59	not discovered	not discovered	
20	HPV 56	not discovered	not discovered	
21	HPV 72	not discovered	not discovered	
22	SIC		5.1	
23	Total HPV load	5.0	5.1	

Patient 32 years old, cytology – LSIL

CONCLUSION

We used the commercial Femoflor®16 kit for evaluation of vaginal microbiota in patients with cervical intraepithelial neoplasias (CIN). Vaginal microbial community was predominated with Lactobacilli spp. in the majority of HPV-negative women with NILM and therefore met the criteria of normocenosis. These findings are consistent with previous studies in the literature. HPV-associated CIN are accompanied by the development of apparent dysbiosis with the prevalence of obligate anaerobes.