

Vaginal biocoenosis examining comparing to exfoliative cervical cytology

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ABSTRACT

Introduction: At present, the gynaecologists have been increasingly frequently switching from vaginal biocoenosis assessment towards cervical cytology results to obtain information on the type of infection. Exfoliative cervical cytology is a screening test for dysplastic intraepithelial lesions and ectocervical cancers. One should emphasize however that one of the four parts of the new Bethesda classification specifies such inflammatory lesions as: *Trichomonas vaginalis*, *Candida*, *Actinomyces*, *Chlamydia*, cellular changes consistent with HSV infection and changes of bacterial flora. The gynaecologists however may perform vaginal biocoenosis assessment individually and diagnose its abnormalities in a relatively short timeframe.

Purpose: To analyse the association between lesions revealed during vaginal biocoenosis assessment in correlation to lesions described in the studies dedicated to cytological assessment of ectocervical smear.

Material and methods: The study group included 1991 female patients scheduled for the follow-up

cytological screening in a gynaecological office. Patients underwent gynaecological examination covering external areas, colposcopy, vaginal pH measurement, sampling for vaginal biocoenosis assessment purposes and cytological sampling.

Results: It was demonstrated that diagnostic conformity for *Candida sp* accounted for only 17.2%, changes of bacterial flora for only 4% and – in the case of *Trichomonas vaginalis* – for only 3.9%. According to observations, bacterial infections and candidiases have been more frequently diagnosed during vaginal biocoenosis examining comparing to cytological screening, whereas infections with *Trichomonas vaginalis* have been more frequently diagnosed in cytological screening.

Conclusions: Lack of 100% correlation between the vaginal biocoenosis test and cytological result according to the Bethesda system means that assessment of vaginal microflora in phase-contrast microscopy should not be abandoned.

Keywords: Bacterial infections, *Candida sp*, cervical cytology, *Trichomonas vaginalis*, vaginal biocoenosis

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INTRODUCTION

Cervix is the site of frequent lesions in females, especially in those in reproductive age. Inflammations are the most common health problem of the female genital organ. The potential causes of non-infectious cervicitis include local injury, chemical irritation, radiation, systemic inflammation or neoplasm. Sexually transmitted infections are more common etiologic agent of cervicitis and may be caused by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, herpes simplex virus (HSV) or human papilloma virus (HPV). Non-infectious diseases include *Mycoplasma genitalium*, *Candida spp* and bacterial vaginosis [1,2].

The standard diagnostic sequence of cervical infections includes medical interview, physical examination and microscopic examination. Physical examination consists in abdominal palpation, vaginal examination with vulva abnormality assessment, colposcopy (assessment of vaginal mucosa and ectocervix), vaginal pH assessment, amine test (with 10% KOH solution) and sampling for macroscopic examinations. Vaginal purity degree is assessed from vaginal swabs whereas the ectocervical and cervical canal smears are subject to cytological screening according to PAP or Bethesda systems (screening test). Detailed infection diagnosis covers also microbiological examinations or HPV test [3-6].

At present, the gynaecologists have been increasingly frequently switching from vaginal biocoenosis assessment towards cervical cytology results to obtain information on the type of infection. Exfoliative cervical cytology is a screening test for dysplastic intraepithelial lesions and ectocervical cancers. One should emphasize however that one of the four parts of the new Bethesda classification specifies such inflammatory lesions as: *Trichomonas vaginalis*, *Candida*, *Actinomyces*, *Chlamydia*, cellular changes consistent with HSV infection and changes of bacterial flora. The gynaecologists however may perform vaginal biocoenosis assessment individually and diagnose its abnormalities in a relatively short timeframe. Any identified abnormal vaginal discharge obliges to determine its colour and cohesion. At the first stage, vaginal pH should be assessed using the litmus paper. Normal pH ranges between 3.8 and 4.5. pH<4.5 excludes bacterial vaginitis. In such cases diagnosis should be switched towards fungal aetiology confirmation. pH>4.5 result obtained in the test may reveal pathogenic bacterial flora or trichomoniasis [7-12].

Thus, the purpose of this study was to analyse the association between lesions revealed during vaginal biocoenosis assessment in correlation to lesions described in the studies

dedicated to cytological assessment of ectocervical smear.

MATERIALS AND METHODS

The study group included 1991 female patients aged 17 – 85 years (average age of 37.4). The patients underwent gynaecological examination covering external areas, colposcopy, vaginal pH measurement, sampling for vaginal biocoenosis assessment purposes and cytological sampling.

Upon cervical imaging with colposcope, ectocervical surface and adjoining discharge was assessed in detail. Macroscopic ectocervical image revealed both normal condition and the following lesions: inflammation, erosion, hyperplasia, polyp, ulceration, condylomas, deformation, necrosis and tumour.

Vaginal discharge was sampled from the posterior fornix using a sterile swab (tupfer) and adequately prepared: thin layer of vaginal discharge was equally distributed on a surface of dry and defatted slide, dripped with 0.9% NaCl solution and covered with cover slip. The obtained smears were assessed using the Olympus IX41 phase contrast microscope according to the Jirovec – Peter – Malek classification.

For the purposes of cytological sampling the colposcopic cervical image was acquired. Ectocervical and cervical canal cells were sampled using the Cervex-Brush-type gynaecological swab brush. The material sampled on the brush was then distributed onto the slide and immediately fixed by treating with Cytifix. The slides were stained with Papanicolaou method and assessed using the valid cytological slide screening method according to the Bethesda system with the Olympus CX40 microscope.

According to the GCPs (Guidelines for Good Clinical Practice), this research was approved by the Bioethical Commission of the Medical University of Bialystok (Resolution No.: R-I-002/36/2013).

Statistical research was performed using the STATISTICA 10 PL software. Description of the study group was performed using the basic descriptive statistics. Correlations between the researched parameters were verified with the Spearman's correlation test. Data is presented in the contingency tables. The critical level for all tests of significance was <0.05.

RESULTS

Vaginal biocoenosis assessment and cytological screening results.

In vaginal biocoenosis assessment, its normal condition was demonstrated in 95.1% of female patients. Presence of *Candida sp.* fungi was

observed in 216 female patients (10.9%), abnormal bacterial flora in 74 female patients (0.3%) and *Trichomonas vaginalis* in 6 female patients (Table 1).

Analysis performed on the basis of cytological smear results according to the Bethesda system revealed normal smear in 24% of female patients. As many as 1544 female patients screened in 1991 (76%) were diagnosed with abnormal condition associated with non-cancerous lesions and epithelial cell abnormalities. Non-cancerous lesions (CI according to the Bethesda system) were present in 62.9% of female patients and included infections (5%) and pH changes in inflammations (57.9%). The second diagnostic group consisted in

epithelial cell abnormalities(13.1% of diagnoses, CII according to the Bethesda system) with such diagnosed lesions as: ASC-US (atypical squamous cells of undetermined significance) 207 female patients, ASC-H (atypical squamous cells- cannot exclude HSIL) in 8 female patients, LSIL (low-grade squamous intraepithelial lesion) in 37 female patients, atypical glandular cells of cervical canal (AGC) in 8 female patients and squamous carcinoma in 1 female patient. HSIL (high-grade squamous intraepithelial lesion) and other glandular cell abnormalities were not observed (Table 1, Figure 1).

Table 1. Specification of the screened female patients in terms of vaginal biocoenosis assessment and cytological diagnosis according to the Bethesda system

	Number of female patients (N=1991)	
	N	(%)
Vaginal biocoenosis		
Normal	1695	85.1%
<i>Candida sp</i>	216	10.9%
Bacteria	74	0.3%
<i>Trichomonas vaginalis</i>	6	3.7%
Cytological diagnosis according to the Bethesda system		
Normal smear	447	24.0%
Infection	100	5.0%
<i>Trichomonas vaginalis</i>	(17)	(17%)
<i>Candida sp.fungi</i>	(51)	(51%)
<i>Actinomyces</i>	(5)	(5%)
<i>Bacterial flora lesion</i>	(27)	(27%)
Reactive cellular changes associated with inflammation	1153	57.9%
ASC-US	207	10.4%
ASC-H	8	0.4%
LSIL	37	1.8%
HSIL	0	0%
Squamous carcinoma	1	0.1%
Atypical glandular cells of cervical canal (AGC)	8	0.4%

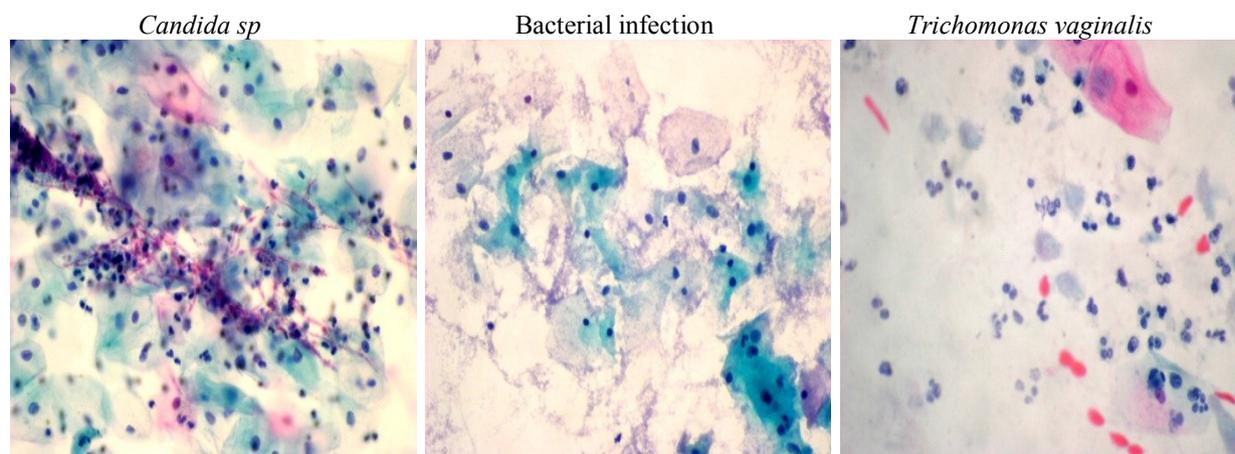


Figure 1. The most common cervical infectants – microscopic images of a slide stained using the PAP method. Magnification x 400

Analysis of chosen parameters and vaginal biocoenosis assessment results.

Analysis of the result of the vaginal biocoenosis study, the percentage of patients with normal bacterial flora was quite high (from 70.6% in women aged 10-19 years) and increased with the age of the patients up to 91.3% (percentage of patients aged 50-59 years with proper vaginal biocoenoses. After 60 years, this percentage decreased slightly. Incorrect vaginal bacterial flora was observed more frequently with younger patients. In women aged 10-19, up to 20.6% of them were diagnosed with fungi and the change of bacterial flora in 8.8% of patients. *Candida sp* in the vaginal biocoenosis study were also found in about 15% of women aged 20-29, 70-89, in about 10% in women aged 30-49, and in about 5% in women aged 50-69. The abnormal bacterial flora

was present in 2.2-4.4% of patients aged 20-69 years. *Trichomonas vaginalis* was observed in age groups from 20 to 69 years old. These were mostly single cases (Table 2).

Statistically significant was correlation of vaginal pH with vaginal biocoenosis ($p < 0.000$). Acidic vaginal pH was predominant in the diagnosis of normal vaginal biocoenosis (66.8% of patients) and *Candida sp* (75.9%). On the other hand, the basic pH of the vagina was mainly related to the change of the bacterial vaginal flora. As many as 94.6% of patients with this infection had abnormal vaginal pH. Basic vaginal pH was also predominant in women with vaginal trichomoniasis (66.7%) (Table 2).

There was no statistical significance between the infectious agent and the macroscopic image and diagnosis in the Bethesda system.

Table 2. Correlation between chosen parameters and vaginal biocoenosis assessment results

	N	Vaginal biocoenosis								p
		Normal		<i>Candida sp</i>		Bacteria		<i>Trichomonas vaginalis</i>		
Age										
10-19	34	24	(70.6%)	7	(20.6%)	3	(8.8%)	0	(0%)	0.006
20-29	502	404	(80.5%)	75	(14.9%)	22	(4.4%)	1	(0.2%)	
30-39	758	659	(86.9%)	72	(9.5%)	26	(3.4%)	1	(0.1%)	
40-49	397	337	(84.9%)	43	(10.8%)	16	(4.0%)	1	(0.3%)	
50-59	185	169	(91.3%)	11	(5.9%)	4	(2.2%)	1	(0.5%)	
60-69	93	83	(89.2%)	5	(5.4%)	3	(3.2%)	2	(2.2%)	
70-79	15	13	(86.7%)	2	(13.3%)	0	(0%)	0	(0%)	
80-89	7	6	(85.7%)	1	(14.3%)	0	(0%)	0	(0%)	
Vaginal pH										
acidic	1302	1132	(66.8%)	164	(75.9%)	4	(5.4%)	2	(33.3%)	<0.0001
basic	689	563	(33.2%)	52	(24.1%)	70	(94.6%)	4	(66.7%)	
Macroscopic image										
Normal	1591	1474	(92.7%)	62	(3.9%)	53	(3.3%)	2	(0.1%)	0.525
Inflammation	164	60	(36.6%)	99	(60.4%)	2	(1.2%)	3	(1.8%)	
Erosion	214	143	(66.8%)	52	(24.3%)	18	(8.4%)	1	(0.5%)	
Hyperplasia	3	3	(100%)	0	(0%)	0	(0%)	0	(0%)	
Polyp	14	11	(78.6%)	2	(14.3%)	1	(7.1%)	0	(0%)	
Ulceration	1	0	(0%)	1	(100%)	0	(0%)	0	(0%)	
Condylomas	1	1	(100%)	0	(0%)	0	(0%)	0	(0%)	
Deformation	2	2	(100%)	0	(0%)	0	(0%)	0	(0%)	
Necrosis	1	1	(100%)	0	(0%)	0	(0%)	0	(0%)	
Diagnosis in the Bethesda system										
Normal smear	447	423	(88.7%)	35	(7.3%)	18	(3.8%)	1	(0.2%)	0.613
Infection	100	54	(54%)	41	(41%)	4	(4%)	1	(1%)	
Reactive changes	1153	997	(86.5%)	104	(9%)	49	(4.3%)	3	(0.2%)	
ASC-US	207	173	(83.6%)	31	(15%)	2	(1%)	1	(0.4%)	
ASC-H	8	7	(87.5%)	0	(0%)	1	(12.5%)	0	(0%)	
LSIL	37	33	(89.2%)	4	(10.8%)	0	(0%)	0	(0%)	
Squamous carcinoma	1	1	(100%)	0	(0%)	0	(0%)	0	(0%)	
AGC	8	7	(87.5%)	1	(12.5%)	0	(0%)	0	(0%)	

p- value of statistical significance calculated using the Spearman's correlation test; Statistical significance was marked in bold.

Comparative analysis of vaginal biocoenosis assessment results with the infectants diagnosed according to the Bethesda system.

There were 238 cases of *Candida sp.* fungal infection diagnosed in vaginal biocoenosis assessment and cytological smear in total. Only in 41 women this diagnosis was confirmed in both tests, which accounted for 17.2% of cases. As many as 73.5% of fungal infections revealed in the vaginal biocoenosis test were not confirmed by cytological smear. Only in 9.3% of women the *Candida sp.* infection was diagnosed in cytological smear according to the Bethesda system.

Abnormal bacterial flora was observed in 101 female patients, of which 69.3% were diagnosed in the vaginal biocoenosis test, whereas 26.7% of these infections were determined in cytological smear according to the Bethesda system. Only 4% of these infections were confirmed in both tests.

Trichomonas vaginalis was more frequently diagnosed in cytological smear (20 female patients) comparing to vaginal biocoenosis test (5 female patients). Diagnosed *Trichomonas vaginalis* infection in both tests was confirmed only in 1 case (Figure 2).

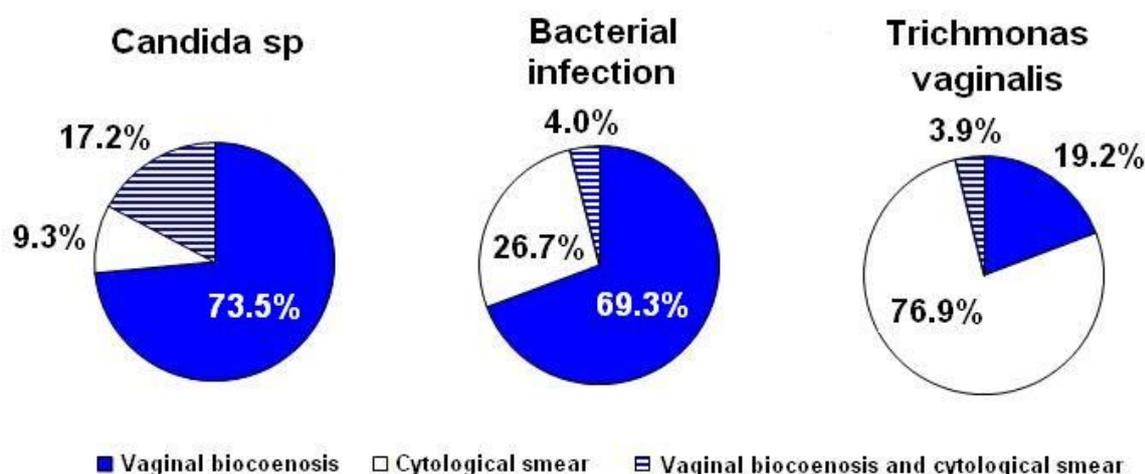


Figure 2. Comparison of vaginal biocoenosis test results with infectant diagnosed according to the Bethesda system depending on type of test.

DISCUSSION

One of key purposes of this study is to analyse the association between lesions revealed during vaginal biocoenosis assessment in correlation to lesions described in the studies dedicated to cytological assessment of ectocervical smear. Diagnostic compliance for *Candida sp.* accounted only for 17.2%, for bacterial flora abnormalities was only 4% and for *Trichomonas vaginalis* - 3.9%. It was observed that bacterial and *Candida sp.* infections are more frequently diagnosed during vaginal biocoenosis test comparing to cytological smear, whereas *Trichomonas vaginalis* infection is more frequently diagnosed in cytological smear. Vaginal biocoenosis test (i.e. phase-contrast cytology) displays numerous advantages in particular in differentiation of *G. vaginalis* and granulomas, in assessment of lactobacillus count and morphology, detection of mobile forms of *Mobiluncus* in direct smear, assessment of cytological inflammation features and in assessing the presence of *Trichomonas vaginalis* and cytopathic effect of

C. albicans [13,14]. It is also concluded that vaginal biocoenosis test supplementing the conventional cytology (PAP) enables reducing the frequency of false negative results for HPV infections [15]. Biological tests and molecular assays are more precise in infectant diagnosing however more expensive and time-consuming. Limitations to the vaginal biocoenosis test in vaginitis and cervicitis diagnosing include primarily lack of epithelial cytoplasm staining, in particular of pseudo-eosinophils observable in inflammations. Direct smears are usually quickly dryable that results in degenerative lesions impeding the inflammatory indicator diagnostics [16].

The gynaecologists are encourages to perform cytological screenings used primarily to detect precancerous lesions and cervical cancer. The Bethesda system enables also diagnosing the inflammatory lesions and identifying the inflammatory agent. Lack of 100% correlation of vaginal biocoenosis test result with the cytological result according to the Bethesda system means that assessing the vaginal microflora in phase-contrast microscopy should not be abandoned. Cytological

screening is insufficient to assess the infectant. Narrowing of the group of female patients eligible for vaginal biocoenosis assessment observed within the recent years compromises their health by limited, frequently false negative information on the identified infectant provided to health professional. Cheap and quick vaginal biocoenosis test in phase-contrast microscopy can be used as working tool in any cytological laboratory, in particular in combination with colposcopy, is a convenient tool to assess the inflammatory indicators and cause of infection [13]. Sampling the material for vaginal purity degree assessment requires no specific preparations at the side of the female patient. It should be not however performed only during antibiotic therapy or directly upon completion thereof. During menstruation the patient should refrain from sexual intercourses for 48 hours and do not use vaginal irrigation. No gynaecological examination should be performed on the assessment day. Vaginal purity degree assessment may be performed by healthcare personnel trained in vaginal biocoenosis assessment, in particular in the area of performing the ectocervical examinations also by midwives. This issue is referred to in the Ordinance No. /2013/DSOZ of the President of the National Health Fund of 27 November 2013. Cytological smear within the screening test can be sampled by primary healthcare midwife holding the certificate confirming the completion of the exam carried out by the Central Coordinating Centre with positive result or a certificate confirming the completion of the professional development course. Vaginal biocoenosis test could be also performed by midwives, however it should not relieve us from the obligation to perform screening tests with the use of slides treated and stained using the Papanicolaou (PAP) method [17].

In 2013, WHO issued its guidelines for screening and treatment of precancerous lesions for cervical cancer prevention [18]. It switches from preventive and diagnostic examinations in standard sequence of cytology, colposcopy, biopsy and histologically confirmed CIN towards the alternative method consisting in the "screen-a-treat" approach, under which the therapeutic decision is based on the result of screening test and the treatment itself is initiated shortly after, preferably immediately upon obtaining the positive screening results. Available screening tests cover the human papilloma virus (HPV) test, visual inspection with acetic acid (VIA) and cytological smear (PAP smear). The guidelines suggest however that the states having the screening programme of high quality index and based on histopathologically verified cytological smear implemented should continue the cytological screening with colposcopy or HVP test with colposcopy [18].

CONCLUSIONS

Lack of 100% correlation between the vaginal biocoenosis test and cytological result according to the Bethesda system means that assessment of vaginal microflora in phase-contrast microscopy should not be abandoned.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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