A comparison of vaginal biocoenosis examination with exfoliative cervical cytology

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ABSTRACT

Introduction: Gynecologists have been increasingly frequently switching from vaginal biocoenosis assessment towards cervical cytology results to obtain information on infection type. 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 in bacterial flora. Gynecologists, however, can perform vaginal biocoenosis assessment individually and diagnose its abnormalities in a relatively short timeframe.
Purpose: To analyse associations between lesions revealed during vaginal biocoenosis assessment in correlation to lesions described in studies dedicated to cytological assessments of ectocervical smear.
Materials and methods: The study group included 1991 female patients scheduled for follow-up cytological screening in a gynecological office. Patients underwent gynecological 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 our observations, bacterial infections and candidiases were more frequently diagnosed during vaginal biocoenosis examinations comparing with cytological screening; whereas, *Trichomonas vaginalis* infections were more frequently diagnosed in cytological screening.
Conclusions: Lack of 100% correlation between the vaginal biocoenosis test and cytological results 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

The cervix is a site of frequent lesions in females, especially in those of 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 a 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 of 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 ectocervical and cervical canal smears are subject to cytological screening according to PAP or Bethesda systems (screening test). Detailed infection diagnosis also covers microbiological examinations or HPV tests [3-6].

Gynecologists have been increasingly frequently switching from vaginal biocoenosis assessment towards cervical cytology results to obtain information on infection type. 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. Gynecologists, however, can perform vaginal biocoenosis assessment individually and diagnose its abnormalities in a relatively short timeframe. Any identified abnormal vaginal discharge should then be assessed to determine its colour and cohesion. In the first stage, vaginal pH should be assessed using lithmus paper. Normal pH ranges between 3.8 and 4.5. A pH<4.5 excludes bacterial vaginitis. In such cases, diagnosis should be switched towards fungal etiology confirmation. A pH>4.5 result 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 studies dedicated to cytological assessments 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 gynecological examination covering external areas, colposcopy, vaginal pH measurement, sampling for vaginal biocoenosis assessment purposes, and cytological sampling.

Upon cervical imaging with a colposcope, the ectocervical surface and adjoining discharge was assessed in detail. The macroscopic ectocervical image revealed both normal conditions 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 the surface of a dry and defatted slide, dripped with 0.9% NaCl solution and covered with a 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 gynecological swab brush. The material sampled on the brush was then distributed onto the slide and immediately fixed by treating with Cytofix. The slides were stained using the Papanicolaou method and assessed using the valid cytological slide screening method according to the Bethesda system with the Olympus CX40 microscope.

In accordance with 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 STATISTICA 10 PL software. Description of the study group was performed using basic descriptive statistics. Correlations between the researched parameters were verified with Spearman's correlation test. Data is presented in 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. The 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 smears in 24% of female patients. As many as 1544 (76%) female patients screened, out of 1991, were diagnosed with an 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 the female patients and included infections (5%) and pH changes in inflammations (57.9%). The second diagnostic group consisted of 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) in 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%
Candida sp	216	10.9%
Bacteria	74	0.3%
Trichomonas vaginalis	6	3.7%
Cytological diagnosis according to the Bethesda system		
Normal smear	447	24.0%
Infection	100	5.0%
Trichomonas vaginalis	*(17)*	*(17%)*
Candida sp.fungi	*(51)*	*(51%)*
Actinomyces	*(5)*	*(5%)*
Bacterial flora lesion	*(27)*	*(27%)*
Reactive cellular changes associated with inflamation	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%

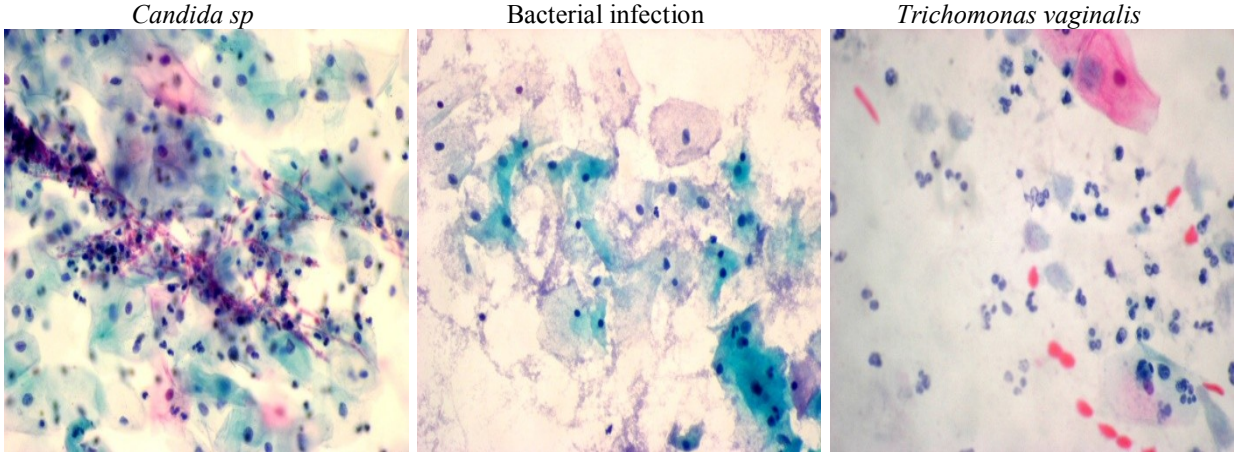

Figure 1. The most common cervical infectants – microscopic images of a slide stained using the PAP method. Magnification x 400

Analysis of chosen parameters and vaginal biocenosis assessment results.

In the analysis of the results 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 biocenoses). Over the age of 60, this percentage decreased slightly. Incorrect vaginal bacterial flora was observed more frequently in younger patients. In women aged 10-19, up to 20.6% of them were diagnosed with fungi and a change in 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 and 70-89, 10% in women aged 30-49, and 5% in women aged 50-69. 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).

The correlation of vaginal pH with vaginal biocenosis was statistically significant ($p<0.000$). Acidic vaginal pH was predominant in the diagnosis of normal vaginal biocenosis (66.8% of patients) and *Candida sp* (75.9%). On the other hand, the basic pH of the vagina was mainly related to changes in the bacteria of the 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		***Candida sp***		**Bacteria**		***Trichomonas vaginalis***		
Age										
10-19	34	24	(70.6%)	7	(20.6%)	3	(8.8%)	0	(0%)	
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%)	**0.006**
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%)	
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%)	0.525
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%)	
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%)	0.613
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 is marked in bold.

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

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

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

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

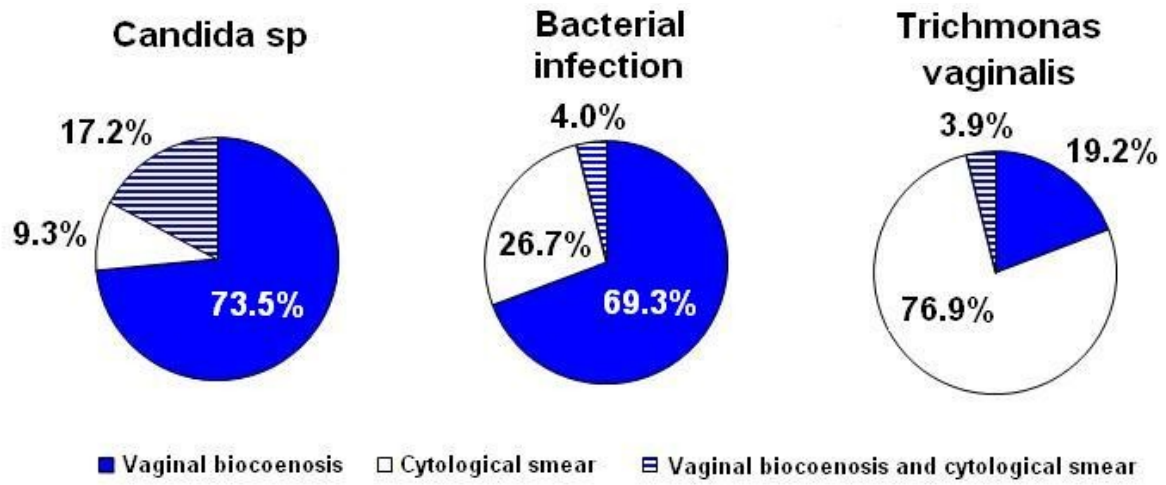

Figure 2. Comparison of vaginal biocoenosis test results with infectant diagnosed according to the Bethesda system depending on type of test.

DISCUSSION

One of key purposes of this study was to analyse associations between lesions revealed during vaginal biocoenosis assessment in correlation to lesions described in studies dedicated to cytological assessment of ectocervical smear. Diagnostic compliance for *Candida sp.* accounted for only 17.2%, bacterial flora abnormalities only 4%, and *Trichomonas vaginalis* 3.9%. It was observed that bacterial and *Candida sp.* infections were more frequently diagnosed during the vaginal biocoenosis test compared with cytological smear, whereas *Trichomonas vaginalis* infection was more frequently diagnosed through cytological smear. The vaginal biocoenosis test (i.e. phase-contrast cytology) displays numerous advantages, particularly in the differentiation of *G. vaginalis* and granulomas, in the assessment of lactobacillus count and morphology, detection of mobile forms of *Mobiluncus* in direct smear, assessment of cytological inflammation features, and assessment for the presence of *Trichomonas vaginalis,* and cytopathic effects of *C.albicans* [13,14]. It was also concluded that vaginal biocoenosis test supplementing 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, it is more expensive and time-consuming. Limitations to the vaginal biocoenosis test in the diagnosis of vaginitis and cervicitis primarily include a lack of epithelial cytoplasm staining, particularly pseudo-eosinophils observable in inflammations. Direct smears are usually quickly dryable, which results in degenerative lesions impeding inflammatory indicator diagnostics [16].

Gynecologists are encouraged to perform cytological screenings, used primarily to detect precancerous lesions and cervical cancer. The Bethesda system enables also diagnosing inflammatory lesions and identifying the inflammatory agent. Lack of 100% correlation of vaginal biocoenosis test results with the cytological results 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 the group of female patients eligible for vaginal biocoenosis assessment observed within recent years compromises their health by limited, frequently false negative information on the identified infectant provided to the health professional. The cheap and quick vaginal biocoenosis test in phase-contrast microscopy can be used as a working tool in any cytological laboratory; particularly in combination with colposcopy, it 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 from the female patient. It should not be performed, however, during antibiotic therapy or directly upon completion thereof. During menstruation, the patient should refrain from sexual intercourses for 48 hours and not use vaginal irrigation. No gynecological examination should be performed on the assessment day. Vaginal purity degree assessment may be performed by healthcare personnel trained in vaginal biocoenosis assessment, particularly in performing ectocervical examinations, also done by midwives.

This issue is referred to in 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 a primary healthcare midwife holding a certificate confirming exam completion conducted by the Central Coordinating Centre with a positive result or a certificate confirming the completion of a professional development course. The vaginal biocoenosis test could also be performed by midwifes; however, this should not relieve doctors from the obligation of performing screening tests with slides treated and stained using the Papanicolaou (PAP) method [17].

In 2013, the WHO issued its guidelines for screening and treatment of precancerous lesions for cervical cancer prevention [18]. It switched from preventive and diagnostic examinations in a standard sequence of cytology, colposcopy, biopsy, and histologically confirmed CIN towards an alternative method consisting of the "screen-and-treat" approach, under which the therapeutic decision is based on the result of the screening test and the treatment itself is initiated shortly thereafter, preferably immediately upon obtaining 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 states with an implemented screening program of a high quality index and based on histopathologically-verified cytological smear 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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