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АСОЦИЈАЦИЈА ПОМЕЃУ БАКТЕРИСКАТА ВАГИНОЗА И СКВАМОЗНИТЕ ИНТРАЕПИТЕЛНИ ЛЕЗИИ НА ГРЛОТО НА МАТКАТА

ASSOCIATION BETWEEN BACTERIAL VAGINOSIS AND SQUAMOUS INTRAEPITHELIAL LESIONS OF THE UTERINE CERVIX

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Abstract

Introduction. Bacterial vaginosis is polymicrobial, primarily anaerobic infection, previously called non-specific vaginitis or vaginitis accompanied by Gardnerella vaginallis. It is a result of an imbalance between different types of bacteria in the vagina. The aim of the study was to determine the association between bacterial vaginosis and squamous intraepithelial lesions of the uterine cervix.
Methods. This cross-sectional study was conducted in a series of 338 sexually active women with cytologically diagnosed squamous intraepithelial lesion of the uterine cervix at the University Clinic of Gynecology and Obstetrics in Skopje in the period from October 2014 to October 2015. The age of the patients ranged from 20 to 59 years (35±10.49). All women underwent cervical biopsy with endocervical curettage for histopathological analysis and cervical biopsy for detection and HPV typing. Criteria for diagnosis of bacterial vaginosis was the presence of ≥20% clue cells of ePapanicolaou smear.
Results. Bacterial vaginosis was detected in 19.5% (66/338) of the examined women. The most affected was the young population under the age of 30 years. The results showed an association between bacterial vaginosis and squamous intraepithelial lesions of the uterine cervix (p=0.032). There was no association between bacterial vaginosis and the grade of lesion of the uterine cervix (p=0.118), nor with HPV infection (p=0.570). But, however an association was found between HPV infection and squamous intraepithelial lesions of the uterine cervix (p=0.001).
Conclusion. The most common risk factor for squamous intraepithelial lesions of the uterine cervix are persistent high-risk HPV infections. Bacterial vaginosis is the most common co-infection.

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Keywords: bacterial vaginosis, intraepithelial lesions, clue cells, human papillomavirus

Апстракт

Вовед. Бактериската вагиноза е полимикробна, примарно анаеробна инфекција, претходно наречена неспецифичен вагинитис, или вагинитис придружен со Gardnerella Vaginallis. Последица е на нарушена рамнотежа меѓу различни типови бактерии во вагината. Целта на студијата е се одредување на поврзаноста меѓу бактериската вагиноза и сквамозните интраепителните лезии на грлото на матката.
Методи. Студија на пресек (cross-sectional study), спроведена врз серија од 338 сексуално активни жени со цитолошки наод на сквамозна интраепителна лезија на грлото на матката, на Универзитетската клиника за гинекологија и акушерство во Скопје, во периодот од октомври 2013 година, до октомври 2014 година, на возраст од 20 до 59 години (35±10,49). Кај сите пациентки е направена цервикална биопсија со ендоцервикална киретажа за хистопатолошка анализа и цервикална биопсија за детекција и за ХПВ типизација. Критериум за поставување дијагноза на бактериска вагиноза е присуството на ≥20% "патоказни" (clue) клетки на Папаниколау тестот.
Резултати. Бактериска вагиноза детектирана кај 19,5% (66/338) од испитаните жени. Најафектирана е младата популација на возраст под 30 години. Резултатите покажаа асоцијација меѓу бактериската вагиноза и сквамозните интраепителните лезии на грлото на матката (п=0,032). Не е најдена поврзаност на бактериската вагиноза со степенот на лезија на грлото на матката (п=0,118), ниту пак, со ХПВ инфекцијата (п=0,570). Детектирана е асоцијација меѓу ХПВ инфек-

цијата и сквамозните интраепителните лезии на грлото на матката (p=0,001).
Заклучок. Најчест ризик фактор за појава на сквамозните интраепителните лезии на грлото на матката се перзистентните високо-ризични ХПВ инфекции. Бактериската вагиноза е најчеста коинфекција.

Клучни зборови: бактериска вагиноза, интраепителни лезии, "патоказни" клетки, хуман папилома вирус

Introduction

Bacterial vaginosis is the most common vaginal syndrome in a woman's reproductive period. Cervical cytological abnormalities are found more in women with abnormal vaginal flora, which suggests a possible association between bacterial vaginosis and squamous intraepithelial lesions of the uterine cervix [1,2].
Bacterial vaginosis is polymicrobial, primarily anaerobic infection, previously called nonspecific vaginitis or vaginitis accompanied by Gardnerella vaginalis. It is a result of an imbalance between different types of bacteria in the vagina. Instead of the normal predominance of Lactobacillus, different types of microorganisms can be found in the vagina, such as: Gardnerella vaginalis, Mycoplasma hominis, Ureoplasma urealyticum, Peptostreptococcus, Mobiluncus species, Prevotella species and Bacteroides [3,4].
Risk factors that may be associated with bacterial vaginosis are: smoking, use of intrauterine device, frequent vaginal discharge, promiscuity and early first intercourse [5].
Bacterial vaginosis can be associated with some sexually transmitted infections, such as infection with Chlamydia trachomatis, Neisseria gonorrhoeae, with the Human Immunodeficiency Virus and Human papilloma virus [6-8].
The diagnosis of bacterial vaginosis can be established by using Nugen's scoring system, Amsel's clinical criteria, modified Amsel's criteria, microbiological criteria or by the presence of clue cells of the Papanicolaou (PAP) smear [9,10].
The diagnosis of bacterial vaginosis only by the presence of clue cells in a wet sample or the presence of ≥20 clue cells of the PAP smear as inclusion criteria is an accurate method and a good predictor of bacterial vaginosis [11].
Clue cells are cells of the vaginal epithelium overlapping with Gardnerella and other microorganisms, which look like to be sprinkled with powdered sugar [12].
Previous studies that investigated the association between bacterial vaginosis and squamous intraepithelial lesions of the uterine cervix have provided conflicting results and different conclusions; from the existence of a strong association [13] to the lack of association between bacterial vaginosis and squamous intraepithelial lesions of the cervix [14].
The aims of the study were: to determine the prevalence of bacterial vaginosis, to determine the most affected age group of patients, to determine the association between bacterial vaginosis and HPV infection, to determine the association between bacterial vaginosis and squamous intraepithelial lesions of the uterine cervix and the grade of the lesion and to determine the association between HPV infection and squamous intraepithelial lesions of the cervix.

Material and methods

This was a cross-sectional study conducted in a series of 338 sexually active women with cytological diagnosed squamous intraepithelial lesion of the uterine cervix at the University Clinic of Gynecology and Obstetrics in Skopje in the period from October 2014 to October 2015. The age of the patients was between 20 and 59(35±10.49). All women underwent cervical biopsy with endocervical curettage for histopathological analysis and cervical biopsy for detection and HPV typing. Criteria for diagnosis of bacterial vaginosis was the presence of ≥20% clue cells of the Papanicolaou smear.
From a total of 350 women with cytological diagnosed squamous intraepithelial lesion of the uterine cervix, 12 were with histopathological finding of invasive squamous cell carcinoma of the uterine cervix.

Criteria of inclusion

The study included 338 sexually active women with squamous intraepithelial lesion of the uterine cervix of the PAP smear.

Criteria of exclusion

The study did not include: pregnant women, women with previous surgery of the uterine cervix (cervical conization, carbon dioxide laser vaporization), women receiving antibiotic therapy and women with histopathological diagnosis of invasive squamous cell carcinoma of the uterine cervix.

Methods of examination

All 350 women underwent cervical biopsy with endocervical curetage for histopathological analysis and cervical biopsy for detection and HPV typing.
All samples for cytology were taken using Thin Prep PAP smear cytology and were analyzed in the Laboratory of the University Clinic of Gynecology and Obstetrics in Skopje by a doctor-cytopathologist. Cytological results were classified according to the revised

Bethesda classification [15,16], such as: Atypical Squamous Cells of Undetermined Significance-ASC-US, Atypical Squamous Cells cannot exclude HSIL-ASC-H, Low-grade Squamous Intraepithelial Lesion-LSIL (productive HPV infection, Cervical Intraepithelial Neoplasia grade 1-CIN1), High-grade Squamous Intraepithelial Lesion-HSIL (CIN2, CIN3, CIS) and invasive squamous cell carcinoma.
Criterion for diagnosis of bacterial vaginosis was the finding of ≥20% clue cells in the visual field of the PAP smear under an optical microscope at 1000 times magnification.
The first step in PAP testing was taking and collecting the cytological material. The material for cytological analysis was taken with a special "blue" cyto brush, which simultaneously collects material from exocervix and endocervix. It was collected in special plastic collectors for Thin Prep PAP smear (Hologic, USA), containing 20 ml PreservCyt solution. The name of the woman and outpatient's number were written on each collector.
The second step was preparation of a microscopic slide in three stages: making smear, smear fixing and coloring the smear.
The third step of the PAP test was examination of the slides under an optical microscope (Olympus BH-2, USA). The microscopic examination was done with 100 times magnification of the lens and 10-fold increase in the microscope eyepiece. Criterion for diagnosis of bacterial vaginosis was the finding of ≥20% clue cells in the visual field of the slide of the PAP smear.
Samples for histopathological analysis were taken to the University Clinic of Gynecology and Obstetrics in Skopje and were analyzed at the University Clinic of Radiotherapy and Oncology in Skopje, at the Department of Histopathology and Clinical Cytology by an experienced expert in pathohistology. According to morphology of the bioptic samples, the cervical findings were characterized as: normal finding (non-specific cervicitis), Low-Grade Squamous Intraepithetlial Lesion-LGSIL (Flat condyloma, cervicitis chronic virosa, mild dysplasia), High-Grade Squamous Intraepithelial Lesion-HGSIL (moderate dysplasia, severe dysplasia, carcinoma in situ) and invasive squamous cell carcinoma.
The analysis of the samples from the cervical biopsy, detection and HPV typing were made at the University Clinic of Gynecology and Obstetrics in Skopje, in the Laboratory for HPV typing.
The first step in HPV testing was the isolation of DNA from the collected cells of the cervical biopsies.
The second step was detection of HPV DNA using multiplex polymerase chain reaction (PCR).
The third step was genotyping using the reverse hybridization. It is a method that is based on the hybridization of specific DNA probes that are immobilized on nitrocellulose or nylon tapes.

Statistical Methods

Data were analyzed by a specific software for databases (Excel). Statistical analysis of the established statistical series was made with the statistical program SPSS, version 12.0.
The structure of numerical signs was analyzed by determining the measures of central tendency (arithmetical mean) and measures of dispersion (standard deviation).
Analysis of the relationship (the existence of association) between two sets of attribute variables was performed using the Chi-square test.
Statistical significance was defined as a p value <0.05.

Results

Of the 338 examined patients aged from 20 to 59 years (35±10.49) 144(42.6%) were aged 20-29 years, 92(27.2%) 30-39 years, 60(17.8%) 40-49 years and 42(12.4%) were aged 50-59 years (Table 1).

Table 1. Distribution of patients according to age groups

Age group	Number	%
20-29	144	42.6
30-39	92	27.2
40-49	60	17.8
50-59	42	12.4
Total	**338**	**100**

Of the total of 338 patients, bacterial vaginosis was found in 66 (19.5%) patients.
The frequency of bacterial vaginosis according to age group in 66 patients is shown in Figure 1.

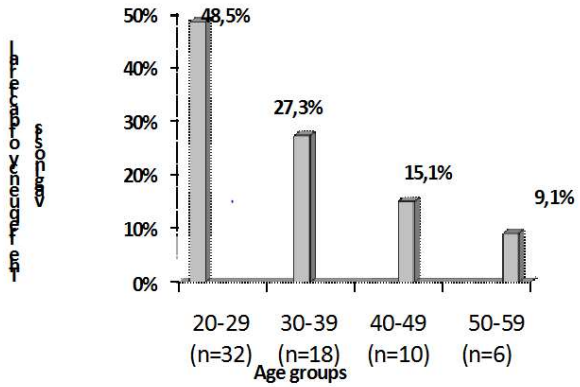

Fig. 1. Frequency of bacterial vaginosis according to age group in 66 patients

The association between bacterial vaginosis and HPV infection is shown in Table 2.

Table 2. Association between bacterial vaginosis and HPV infection

		Bacterial vaginosis			
		Present		Absent	
		n	(%)	n	(%)
HPV infection	Present n=250	47	(18.8)	203	(81.2)
	Absent n=88	19	(21.6)	69	(78.4)
	Total n=338	66	(19.5)	272	(80.5)

Of the total of 338 patients, 47(13.9%) were found to have bacterial vaginosis and HPV infection at the same time. Of the 250 HPV DNA positive patients, bacterial vaginosis was present in 47(18.8%) patients, and absence of bacterial vaginosis was observed in 203 (81.2%) of the HPV DNA positive patients.

The data analysis showed no association between bacterial vaginosis and HPV infection (chi-square test= 0.323, p=0.570, p>0.05).

Table 3. Prevalence of HPV DNA genotypes in 47 women with bacterial vaginosis

HPV DNA genotype	Type of infection	Bacterial vaginosis total (n=47)	
		n	%
16	single	17	(36.2)
	mixed	13	(6.4)
18	single	5	(10.7)
	mixed	1	(2.1)
31	single	12	(25.5)
	mixed	1	(2.1)
33	single	1	(2.1)
	mixed	0	(0)
45	single	1	(2.1)
	mixed	1	(2.1)
52	single	0	(0)
	mixed	1	(2.1)
6	single	2	(4.3)
	mixed	2	(4.3)

Distribution of bacterial vaginosis compared to histopathological diagnosis is shown in Table 4.

Table 4. Distribution of bacterial vaginosis compared to histopathological diagnosis

		Bacterial Vaginosis			
		Present		Absent	
		n	(%)	n	(%)
Histopathological diagnosis	Squamous intraepithelial lesion n= 264	58	(22.0)	206	(78.0)
	Normal finding n= 74	8	(10.8)	66	(89.2)
	Total n= 338	66	(19.5)	272	(80.5)

Of the total of 338 patients, bacterial vaginosis was found in 66(19.5%) patients. Of these 58(22.0%) were with histopathological finding of squamous intraepithelial lesion of the uterine cervix, and 8 with normal histopathological finding.

Data analysis showed an association between bacterial vaginosis and intraepithelial lesions of the uterine cervix (chi-square test=4.580, p=0.032, p<0.05).

Distribution of bacterial vaginosis in correlation with the grade of squamous intraepithelial lesion of the uterine cervix is shown in Table 5.

Of the total of 124 patients with histopathological finding of low-grade squamous intraepithelial lesions of the uterine cervix, bacterial vaginosis was found in

Table 5. Distribution of bacterial vaginosis in correlation with the grade of squamous intraepithelial lesion of the cervix

		Bacterial Vaginosis			
		Present		Absent	
		n	(%)	n	(%)
Grade of squamous intraepithelial lesion	Low grade SIL n= 124	22	(17.7)	102	(82.3)
	High grade SIL n= 140	36	(25.7)	104	(74.3)
	Total n= 264	58	(22.0)	206	(78.0)

SIL-squamous intraepithelial lesion

22(17.7%) patients, while of 140 patients with histopathological diagnosis of high-grade squamous intraepithelial lesion of the uterine cervix, bacterial vaginosis was found in 36 (25.7%) patients.
The data analysis showed no association between the presence of bacterial vaginosis and the grade of cervical lesion (chi-square test=2.438, p=0.118, p>0.05).

The distribution of the findings of HPV testing compared to histopathology findings is summarized in Table 6.
After the HPV testing, from the total of 338 patients 250(74.0%) were HPV DNA positive, while 88(26.0%) were HPV DNA negative. In patients with squamous intraepithelial lesion on the cervix, 215 (81.4%) were HPV DNA positive, while of the patients with pathologically normal findings 35(47.3%) were HPV DNA positive.
Data analysis showed an association between HPV infection and intraepithelial lesions of the cervix (chi-square test=34.987, p=0.001, p<0.05).

Table 6. Distribution of findings of HPV testing compared to histopathological diagnosis

		HPV testing			
		HPV DNA positive		HPV DNA negative	
		n	(%)	n	(%)
Histopathological diagnosis	Squamous intraepithelial lesion n= 264	215	(81.4)	49	(18.6)
	Normal finding n= 74	35	(47.3)	39	(52.7)
	Total n= 338	250	(74.0)	88	(26.0)

Discussion

Early detection and treatment of intraepithelial lesions of the cervix play a key role in the prevention of cervical cancer [17].
Bacterial vaginosis can be associated with several obstetric and gynecological complications, such as preterm birth, chorioamnionitis, endometritis after cesarean section, pelvic inflammatory disease, postoperative infections after hysterectomy and with abnormal cervical finding [13,18,19].
In our study the prevalence of bacterial vaginosis was 19.5%. In previous published studies where bacterial vaginosis was detected by the presence of clue cells, as in our study, the prevalence ranged from 15.0-24.18% [20-23].
A higher percentage of bacterial vaginosis (up to 41.67%) was found in studies where the diagnosis of bacterial vaginosis was made on the basis of clinical Amsels' criteria [24]. The highest percentage of bacterial vaginosis (up to 49.02%) was is detected by Nugent's score, as the "gold standard" for diagnosis of bacterial vaginosis [25,26]. These data support the idea of detecting vaginal bacteriosis by the Nugent's score as the "gold standard" that should be used in the future.
In our study the highest rate of bacterial vaginosis of 48.5% (32/66) was found among the largest study group (young people under 30 years of age).

A high percentage of bacterial vaginosis among young people was also found in some previously published studies [27,28].
HPV infection plays a major role in changing the vaginal environment, helping the development of bacterial vaginosis [14]. On the other hand, women with bacterial vaginosis are more susceptible to getting and reactivation of HPV infection [29]. Many studies present conflicting results regarding the association between bacterial vaginosis and HPV infection [30-32]. In our study no association was found between bacterial vaginosis and HPV infection (p=0.570), which is similar to the results in some previously published studies [33-36].
The most common HPV DNA genotypes in women with bacterial vaginosis, in descending order, were HPV16 (42.6%), HPV31 (27.6%), HPV18 (12.8%), HPV6 (8.6%), HPV45 (4.2%), HPV33 (2.1%) and HPV52 (2.1%).
A number of previously published studies have presented a different relationship between bacterial vaginosis and intraepithelial lesions of the cervix. The found association between bacterial vaginosis and intraepithelial lesions of the cervix in our study (p= 0.032) was also found in some previously published studies [24,28,37-39]. In the study of Antovska *et al.* bacterial vaginosis was more common in the subgroup of women with invasive squamous cell carcinoma of the uterine cervix (6.3%) than in the subgroup with low-grade squamous intraepithelial lesions of the uterine cervix (2.1%) [40].

Several hypotheses are explaining the connection between bacterial vaginosis and intraepithelial lesions of the cervix. One of them assumes that in women with bacterial vaginosis mucin degradation enzymes grow. These enzymes such as sialidase (neuraminidase) play a key role in degradation of the protective layer of the cervical epithelium, causing microabrasion or changes in epithelial cells leading to destruction of the mucosal barrier protection [41].

In our study an association between bacterial vaginosis and the grade of the cervical lesion was found, and it was identical to some previously published studies [35,38]. In the future, it is necessary to conduct a study where the diagnosis of bacterial vaginosis will be made by using Nugent's score system or Amsel's clinical criteria.

The most common risk factor for pre-cancerous lesions of the cervix is infection with human papillomavirus (HPV), particularly with high-risk HPV genotypes [42,43]. In our study the prevalence of HPV infection was 74.0%. Our results showed that there was an association between HPV infection and intraepithelial lesions of the cervix (p=0.001).

Conclusion

This study showed prevalence of bacterial vaginosis was (19.5%); the most affected were the young people under the age of 30 years; there was no statistically significant association between bacterial vaginosis and HPV infection; there was an association between bacterial vaginosis and squamous intraepithelial lesions of the cervix; there was no association between bacterial vaginosis and the grade of the lesion of the uterine cervix and there was an association between HPV infection and squamous intraepithelial lesions of the uterine cervix.

Conflict of interest statement. None declared.

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