

FREQUENCY OF CERVICAL INTRAEPITHELIAL NEOPLASIA AND CARCINOMAS IN WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS

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Bacterial vaginosis is one of the most frequent disorders of vaginal ecosystem in women during their reproductive life. The first concept in the pathogenesis of bacterial vaginosis points out that the unfamiliar initial influence on vaginal ecosystem decreases lactobacillus concentration, which results in the increase of vaginal secretion pH and multiplication of Gardnerella vaginalis and other anaerobes.

The aim of this study was to compare the frequency of cervical neoplasia in women with and without bacterial vaginosis.

The research included 158 patients, between 18 and 51 years of age, at the Clinic of Gynecology and Obstetrics in Niš. Clinical processing of patients consisted of cytological, colposcopic and pathohistological examinations. Bacterial vaginosis was, beside clue cell detection with native preparation, diagnosed by the application of other Amsel's criteria: presence of characteristic vaginal secretion, positive amino test, values of vaginal secretion pH over 4.5.

The results showed that 23.42% of the patients had bacterial vaginosis. The greatest percent of the patients with bacterial vaginosis (24.32%) was between 20 and 34 years of age. In the examined population, 7.59% of the patients had positive pathohistological finding. The number of normal pathohistological findings was statistically significantly lower in the patients with bacterial vaginosis (75.68%) than in those without bacterial vaginosis (97.52%), ($\chi^2=16.28$, $p<0.001$). *Acta Medica Medianae* 2011;50(1):5-10.

Key words: bacterial vaginosis, cervical neoplasia, sexually transmitted diseases, frequency

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Introduction

Bacterial vaginosis is one of the most frequent disorders of female vaginal ecosystem during their reproductive life. The first concept in the pathogenesis of bacterial vaginosis points out that the unfamiliar initial influence on vaginal ecosystem decreases lactobacillus concentration, which results in the increase of vaginal secretion pH and multiplication of Gardnerella vaginalis and other anaerobes (1). Gardnerella vaginalis is the part of normal vaginal flora. It produces amino acids, and the anaerobes produce enzymes that dissolve the amino acids to amines: putrescin, kadaverin and trimetilamin. The amines increase the vaginal secretion pH, which influences the increased vaginal epithelium desquamation, which then increases the amount of vaginal secretion (2). Thus appears circulus vitiosus in

which the increased vaginal secretion pH influences a significant decrease or disappearance of lactobacilli and, consequently, the development of non-specific vaginosis.

The experiments proved that the existence of the strong bonds between epithelial vaginal cells is necessary for vagina acidity maintenance. Epithelial vaginal and exocervical cells acidify vaginal lumen with the active proton secretion mechanism and, partly, with the V-H+-ATP-asis activity localized in the apical part of the plasma membrane. The basic proton secretion activity is the constitutive activity in life, and is supplemented by the estrogen-regulated acidification (3). Under the physiological conditions, normally present lactobacilli that function mutually with vaginal epithelium, colonizing it and making it resistant to other microorganisms, prevent ascendant or systemic infections (4). It is considered that otherwise very spread sexually transmitted diseases, such as acute and chronic Chlamydia infections, gonococci infections, as well as bacterial vaginosis although not referred to as the sexually transmitted disease in the true sense participate in cervical carcinoma oncogenesis in the co-infection with humane papillomaviruses (5).

The hypothesis of possible causal connection between bacterial vaginosis and cervical intraepithelial lesions was first posed by Pavić, in 1982 (6). He assumed that nitrosamines as the product of anaerobic bacteria have mutagenic effect on the cell DNA, and also pointed out the possible synergic activity of nitrosamines, viruses and hormones (7). Oncogenetic process is relatively slow and develops according to biological continuity from the basic intraepithelial neoplasia to invasive carcinoma. Already formed neoplasia can itself retreat spontaneously. The probability of such activity is lower in case of higher level of neoplasia. Carcinoma in situ progresses to an invasive carcinoma in about 70% of cases (8,9).

In favor of the more precise determination of cervical intraepithelial neoplasia frequency level in women with bacterial vaginosis, we set the aims of this study.

Aim

The aim of the research was to:

- examine the frequency of bacterial vaginosis in sexually active women,
- examine the frequency of cervical neoplasia in the same group of women,
- compare the frequencies of cervical neoplasia in women with and without bacterial vaginosis.

Methods

The examination included 158 women, between 18 and 51 years of age, the patients at the Clinic of Gynecology and Obstetrics in Niš.

Clinical processing of the patients consisted of cytological, colposcopic and pathohistological examinations.

Cytological sample was taken by scratching the joint between flatly layered and cylinder cervical epithelium, using a wooden spatula on ectocervix, and a stick with a cotton wool tip on endocervical canals, which includes the whole transformational zone and enables the obtaining of a real sample. Immediately after sample taking, we made a smear fixated with cytological fixative and colored by standardized method according to Papanikolau (10,11). Papanikolau test results were presented in the class system for reporting cervical smear.

Colposcopic examinations were done on Karl Zeiss colposcope, using the standard enlargements and the green filter, cervical smearing with acetic acid and Lugol's solution in order to notice the abnormal zones – epithelial transformation – as white spaces. (12). Colposcopy helped in accomplishing the aimed biopsy.

Pathohistological examination included taking biopsy from suspect colposcopic changes (acid-white epithelium, mosaic, punctuation, leucoplakia, and atypical vascularization) (13).

Cervical biopsy results were reported as normal pathohistological finding, as mild neoplasia – CIN I, middle – CIN II, severe – CIN III, carcinoma in situ – CIS, and invasive cervical carcinoma - ICC (14).

Native preparation was used for diagnosing the bacterial vaginosis according to the presence of "clue cells", epithelial cells covered with lots of Gardnerella vaginalis. The smear was suspended into a heated saline, and with a pipette put in drops onto the glass slides and covered with opaque glass. Besides the presence of the "clue cells", bacterial vaginosis was diagnosed applying the other Amsel's criteria: the presence of the increased homogeneous secretion was determined by inspection, litmus paper was used in determining the secretion pH over 4.5, and the presence of the positive amine "smell" test was determined by exposing the vaginal secretion 10% KOH that leads to amine release and "rotten fish" smell appearance.

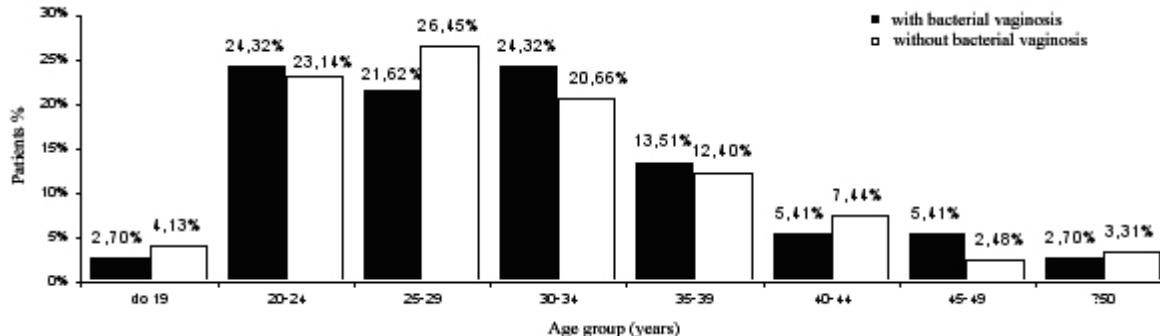
Statistical data processing was done in MS Office Excel program, and estimations were done in SPSS program, version 15.0. The examined parameters were given as frequencies and percents. Pearson's Hi square test is used as non-parameter test in the comparison of the frequencies.

Results

The number of patients according to age groups and the presence of bacterial vaginosis is given in Graph 1.

23.42% of the patients had bacterial vaginosis. The results showed that 31 of 37 patients with bacterial vaginosis belonged to the group between 20 and 40 years of age (Graph 1).

The results of the analysis of several pathohistological findings according to the presence of bacterial vaginosis are given in Table 2.



Graph 1. Percentage frequency of women by age group and the presence of bacterial vaginosis

Tabela 1. Frequency of pathohistological findings according to the presence of bacterial vaginosis

| Pathohistological finding | With bacterial vaginosis | | | Without bacterial vaginosis | | | Total | |
|---------------------------|--------------------------|---------|---------|-----------------------------|--------|-----------|-------|---------|
| | n | %HP | %VP | n | %HP | %VP | n | %VP |
| Regular | 28 | 19,18% | 75,68% | 118 | 80,82% | 97,52%*** | 146 | 92,41% |
| CIN I | 3 | 60,00% | 8,11% | 2 | 40,00% | 1,65% | 5 | 3,16% |
| CIN II | 2 | 100,00% | 5,41% | 0 | 0,00% | 0,00% | 2 | 1,27% |
| CIN III | 2 | 66,67% | 5,41% | 1 | 33,33% | 0,83% | 3 | 1,90% |
| CIS | 1 | 100,00% | 2,70% | 0 | 0,00% | 0,00% | 1 | 0,63% |
| ICC | 1 | 100,00% | 2,70% | 0 | 0,00% | 0,00% | 1 | 0,63% |
| Total | 37 | 23,42% | 100,00% | 121 | 76,58% | 100,00% | 158 | 100,00% |

The number of normal pathohistological findings was statistically significantly lower in the patients with bacterial vaginosis (75.68%) than in those without it (97.52%), ($\chi^2=16.28$, $p<0.001$).

Discussion

Earlier studies have demonstrated 30 to 40% of bacterial vaginosis frequency in women during their reproductive age (2), which is the value slightly higher than ours. We should bear in mind that the frequency of bacterial vaginosis and other sexually transmitted diseases significantly depends on the characteristics of the examined population, as well as on the applied diagnostic methods. The frequency of bacterial vaginosis in other authors' studies significantly increased over years. Every age group, five years older than the previous, had about 5% higher prevalence of bacterial vaginosis. Our researches have the trend of increase from 18th to 24th year, with the decrease in the group from 25th to 29th year, and repeated increase until the 34th year, and linear decrease from 35th to 50th year of age (Graph 1).

Over 40% of women from 40 to 45 years of age, according to the same data source, had bacterial vaginosis (2). The highest percent of our patients (24.32%) (Graph 1) with bacterial vaginosis were in much younger age group, from 20 to 34 years, which can refer to early sexual activity of our patients and the presence of other risk factors responsible for the appearance of bacterial vaginosis, although it is not considered as sexually transmitted infection in the true sense.

In favor of the bacterial vaginosis as a sexually transmitted disease there are:

- Higher frequency of Gardnerella vaginalis in sexually active adolescents than in virgins (34% to 17%) (13).
- Higher frequency in women with more than five sexual partners (40%) than in those without sexual experience (29%) (15).
- High frequency of bacterial vaginosis in women with vaginal trichomoniasis (40.7%-86%) (16-20).
- Higher frequency of Chlamydia trachomatis in women with bacterial vaginosis than in

those without it (11-21.6% to 8-14.6%) (9,17,21).

- Higher rate of HPV infection in women with bacterial vaginosis (22-25), as well as higher frequency of cervical intraepithelial neoplasia and carcinoma (45% to 29%) (26).

Therefore, an increasing number of potential biological sexually transmitted carcinogens are associated with continuous increase of the frequency of precursors, such as bacterial vaginosis and cervical intraepithelial neoplasia and carcinoma in younger women. For the stated since 1973, it is accepted that cervical carcinoma is a venereal disease in a wider context, so today we talk more and more about "sexually transmitted disease" caused by bacteria, viruses, protozoa and fungi (8, 26-28).

In our study, the results of the statistical analysis of the pathohistological findings according to the existence of bacterial vaginosis reported significantly lower number of normal pathohistological findings in the patients with bacterial vaginosis compared to those without it ($p<0.001$). All the pathohistological findings (CIN I, CIN II, CIN III, CIS and ICC) were more frequent in the patients with bacterial vaginosis, but without statistically significant difference (Graph 2).

Pavić's hypothesis about the association between bacterial vaginosis and cervical displasia was confirmed by numerous authors.

Guiljor et al. (1985) examined the connection between cervical infection and cervical neoplasm. They found the cervical infection in 67% of women with CIN, the third group of vaginal secretion in 33% of women with CIN and 31% of women in the control group. Gardnerella vaginalis was isolated in 47% of women with CIN and 33% of the women in the control group. Bacterial vaginosis was diagnosed, according to clinical criteria, in 45% of women with CIN and 29% of those without it. They also concluded that there was a possibility for synergistic interaction between abnormal cervico-vaginal microflora and HPV infection in the development of cervical neoplasia, especially since the women with neoplasia have greater transitional zone surface of metaplasia, as well as the frequent coexisting sexually transmitted infections (29).

Mardh (1991) reported that one of the long-term consequences of vaginitis and vaginosis could also be cervical intraepithelial neoplasia

because of the oncogenic potential of nitrosamine produced by anaerobic bacteria causally associated with bacterial vaginosis (30).

Kharsany et al. (1993) found significantly higher prevalence of bacterial vaginosis in women with cervical intraepithelial neoplasia compared to the control group (31).

Platz-Christensen et al. (1994) concluded that CIN-I and CIN-II were statistically significantly more frequent in women with bacterial vaginosis, and that the relative risk of CIN-III was five times higher if a woman had bacterial vaginosis (32).

Neuer and Menton (1995) confirmed that women with cervical intraepithelial neoplasia had higher, statistically significant, prevalence of bacterial vaginosis compared to the control group (33).

Barrington et al. (1997) accepted Mardh's statement (1991) (32) reporting the local vaginal nitrosamine production in bacterial vaginosis to participate in CIN creation (34).

Retrospective study by Uthayakumar et al., conducted in England in 1998, had the aim to determine the association between bacterial vaginosis and cervical intraepithelial neoplasia. The examined group consisted of 300 women, referred to the hospital for having genital condyloma and not having any other sexually transmitted diseases. Uthayakumar et al. Determined that the women with bacterial vaginosis had higher risk of CIN changes (35).

Discacciati et al. (2006) determined the presence of bacterial vaginosis in 18% of women with SIL changes and 12% of women with normal finding, which was closest to our results (24.32% of women with CIN-I, CIN-II, CIN-III, CIS and ICC, and 19.18% of women with normal pathohistological finding had bacterial vaginosis (Graph 1)). The results were similar even when the level of SIL changes was taken into account. Bacterial vaginosis was found in 16% of women with mild SIL changes, and in 33% of women with severe SIL changes on the cervix (36).

Nam et al. (2009) achieved the results that, similarly to ours, showed that the incidence

of CIN changes was statistically significantly higher in women with bacterial vaginosis. However, multivariate logistic regression analysis did not determine that bacterial vaginosis significantly influenced the appearance of cervical intraepithelial neoplasia (37).

Unlike our results, Vetran et al. proved that bacterial vaginosis was not associated with SIL changes (40). Similar results that preceded these were obtained by Kos et al. (39).

According to the results obtained by Watts et al., bacterial vaginosis did not influence the prevalence and incidence of CIN changes (39).

Moscickia et al., too, did not find the association between bacterial vaginosis and CIN changes (39).

Frega et al. reported the results opposite to ours. Their aim was to estimate the association between CIN changes and bacterial vaginosis in 1.008 women divided into two groups: the first group consisted of 504 patients with CIN changes of different severity level, and the second group included 504 patients without CIN changes. In the first group, 36% of women had bacterial vaginosis, while in the second group, 49% of women had this infection. The obtained results reported no significant association between CIN changes and bacterial vaginosis (40).

In the study conducted in the population of American Indians and indigenous population of Alaska, as well as in non-indigenous populations of these regions, bacterial vaginosis was not a risk factor for CIN changes (9).

Conclusion

This study showed that in the population of sexually active women, between 18 and 51 years of age, 23.42% of the patients had bacterial vaginosis. In the examined population, 7.59% of the patients had positive pathohistological finding.

The number of normal pathohistological findings was statistically significantly lower in the patients with bacterial vaginosis (75.68%) than in those without it (97.52%), ($\chi^2=16.28$, $p<0.001$).

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UČESTALOST CERVIKALNIH INTRAEPITELIALNIH NEOPLAZIJA I KARCINOMA KOD ŽENA SA BAKTERIJSKOM VAGINOZOM I BEZ BAKTERIJSKE VAGINOZE

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Bakterijska vaginoza je jedan od najčešćih poremećaja vaginalnog ekosistema kod žena u reproduktivnoj fazi života. Prvi koncept patogeneze bakterijske vaginoze ukazuje da iz nepoznatog incijalnog uticaja na ekosistem vagine dolazi do smanjenja koncentracije laktobacila, što za posledicu ima povišenje pH vaginalnog sekreta i razmnožavanje Gardnerele vaginalis i ostalih anaeroba.

Cilj ovog istraživanja bio je da uporedi učestalost pojave cervikalne neoplazije kod žena sa i bez bakterijske vaginoze.

Istraživanjem je obuhvaćeno 158 ispitanica, starosti od 18. do 51. godine, bolesnica Klinike za ginekologiju i akušerstvo u Nišu. Klinička obrada ispitanica je podrazumevala citološko, kolposkopsko i patohistološko ispitivanje. Bakterijska vaginoza je, osim detekcije "clue cell" na nativnom preparatu, dijagnostikovana primenom ostalih Amselovih kriterijuma: prisustvo karakterističnog vaginalnog sekreta, pozitivnog amino testa, vrednosti pH vaginalnog sekreta iznad 4,5.

Rezultati istraživanja su pokazali da je 23,42% ispitanica imalo bakterijsku vaginozu. Najveći procenat ispitanica sa bakterijskom vaginozom (24,32%) bio je u starosnoj grupi od 20 do 34 godine. U ispitivanoj populaciji je 7,59% ispitanica imalo pozitivan patohistološki nalaz. Broj urednih patohistoloških nalaza bio je statistički značajno manji kod ispitanica sa bakterijskom vaginozom (75,68%) u odnosu na ispitanice bez bakterijske vaginoze (97,52%), ($\chi^2=16,28$, $p<0,001$). *Acta Medica Mediana 2011; 50(1):5-10.*

Ključne reči: bakterijska vaginoza, cervikalna neoplazija, polno prenosive bolesti, učestalost