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# CHARACTERISTICS OF VAGINAL AND VULVAR MICROBIOTA IN PATIENTS WITH VULVAR PRECANCEROUS LESIONS AND VULVAR SQUAMOUS CELL CARCINOMA

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## Abstract

**The aim** of the study was to analyze the composition of the microbiota of the vagina and vulva in patients with precancerous lesions of the vulva in comparison with healthy controls and in women with vulvar cancer. **Materials and methods.** 286 women with vulvar lesions aged from 25 to 70 years and 60 gynecologically healthy women (30 under 50 years and 30 after 50 years) were included in the study. Patients with vulvar lesions were divided into 5 groups: 87 women with high-grade intraepithelial neoplasia of the vulva (VHSIL) dependent on the human papilloma virus (HPV) (Group 1 - G1), 154 women with differentiated vulvar intraepithelial neoplasia (dVIN) independent of HPV (Group 2 – G2), 9 patients with Paget's disease (Group 3 – G3),, 8 women with melanoma *in situ* of the vulvar skin (Group 4 – G4), and 28 patients with vulvar squamous cell carcinoma (VSCC) (Group 5 – G5). **The results of the study** showed that among patients with precancerous lesions of the

vulva, the vaginal microbiota of persons with VHSIL is characterized by an increase in facultative and obligate anaerobes, fungi of the genus *Candida spp.,* as well as *Ureaplasma spp., Mycoplasma hominis,* compared to patients with dVIN. A characteristic feature of the vaginal microbiota of dVIN patients was a high concentration of *Prevotella spp.*Healthy vulvar skin of patients with precancerous lesions resembled the vaginal and skin microbiome composition of control groups, including genera of *Prevotella, Lactobacillus, Gardnerella, Staphylococcus* and *Corynebacterium*. The affected vulvar skin of patients with VHSIL showed an increase in taxa from the genus *Prevotella spp., Fusobacteria spp.* and *Peptostreptococcus spp.* Affected vulvar skin of patients with dVIN was characterized by enrichment of taxa from the genus *Prevotella sgp., Fusobacteria spp., Atopobium vaginae* and depletion of *Corynebacterium spp., Lactobacillus spp., Staphylococcus spp., Peptostreptococcus spp.* compared to unaffected skin. **Conclusions.** The study showed a change in the composition of the vaginal microbiota in precancerous diseases and cancer of the vulva. A deeper understanding of the complex interaction between the host and microorganisms in the vaginal area is promising for identifying new strategies in the field of cancer prevention and treatment.

**Keywords: vulvar squamous intraepithelial neoplasia; high-grade intraepithelial neoplasia of the vulva; differentiated intraepithelial dysplasia of the vulva; Paget's disease of the vulva; melanoma of the skin of the vulva; microbiota of the vulva; vaginal microbiota; precancerous lesions of the vulva.**

In most Western countries, the prevalence and prognosis for patients with vulvar cancer (VC) have remained largely unchanged over the past two to four decades [1, 2, 3]. In terms of epidemiology, there is a general consensus that vulvar cancer arises by two distinct pathways, one associated with human papillomavirus infection and the other independent of it [4]. In low-income countries, HPV-related disease is the cause of most cases of VCs and affects mainly premenopausal women [5]. In high-income countries, on the contrary, the majority of VCs are independent of HPV and occur in older women. High-grade squamous intraepithelial lesions (VHSIL) or conventional vulvar intraepithelial neoplasia (VIN) are precursors to HPV-associated VC, whereas differentiated VIN (dVIN) is usually considered the main precursor to HPV-independent VC. Various risk factors have been described, which are involved in the pathogenesis of the pathology, but with very different levels of evidence. In general, the epidemiology of VC and precancerous lesions of the vulva is still poorly understood. The following 19 risk factors or categories of risk factors were investigated in the

studies: HPV infection, familial clustering of HPV-associated cancer, other sexually transmitted diseases, sexual behavior, cervical intraepithelial neoplasia, sclerosing lichen, autoimmune diseases, menstrual and reproductive factors, taking oral contraceptives and menopausal hormone therapy, metabolic syndrome, diabetes and body mass index, food products, alcohol consumption, smoking, human immunodeficiency virus and acquired immunodeficiency syndrome (HIV-AIDS), organ transplantation, breast implants, Fanconi anemia, previous abnormal cervical cytology, education [6].

The stability and composition of the vaginal microbiome plays an important role in determining the patient's innate immune response and susceptibility to infections, including HPV. A decrease in the number of lactobacilli is associated with the persistence of HPV and the severity of the lesion. Potential effects of the vaginal microbiome associated with changes in vaginal pH, bacteriocin production, mucosal and epithelial integrity disorders, oxidative stress, and effects on cellular targets such as p53, pRB, and survivin are synergistic with HPV exposure [7]. There are very few studies that would study the state of the microbiome of the vagina and vulva in precancerous diseases and vulvar cancer. Although some authors indicate a correlation between the development of gynecological malignant neoplasms and changes in the microbial community, namely a decrease in the number of commensal bacteria and an increase in the number of anaerobic bacteria [8, 9].

Therefore, the aim of our study was to analyze the composition of the microbiota of the vagina and vulva in patients with precancerous lesions of the vulva in comparison with healthy controls and in women with carcinoma of the vulva.

**Research materials and methods**

To solve the task, the study of the flora of the vagina and skin of the vulva was carried out in patients with precancerous lesions and cancer of the vulva and gynecologically healthy women who sought medical help at the National Cancer Institute (Kyiv, Ukraine) and the "Verum" clinic (Kyiv, Ukraine) in 2017–2023.

In total, 286 women with lesions of the vulva aged from 25 to 70 years and 60 gynecologically healthy women (30 under the age of 50 and 30 after 50 years) were included in the study. Patients with lesions of the vulva were divided into 5 groups: G1 - 87 women with VHSIL; G2 - 154 women with DVIN, G3 - 9 patients with Paget's disease of the vulva grade Ia (adenocarcinoma *in situ* of the skin of the vulva), G4 - 8 women with melanoma *in situ* of the skin of the vulva, and G5 - 28 patients with VSCC. Healthy women were divided into groups CG1 and CG2 according to age <50 years and ≥ 50 years. Patients were included in the study after obtaining written informed consent in accordance with the principles of the

Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine. The diagnosis was established on the basis of anamnesis of the disease, complaints, clinical examination, vulvoscopy and the results of morphological examination of pathologically changed tissues.

The Femoflor 16 (DNA Technologies, Russia) set was used to study the state of the microflora. This is a test system for determining the quantitative and qualitative composition of the vaginal flora by detecting the DNA of bacteria using the polymerase chain reaction (PCR) method "in real time". Femoflor 16 allows you to determine 25 indicators, including control of material intake, total bacterial mass and 23 groups of microorganisms.

Statistical processing and data analysis were carried out using the software Statistica 7.0 for Windows and Microsoft Excel. The work uses standard methods of descriptive and comparative analysis. Mean (M), the standard error of the mean (±SEM) and odds ratio (OR) were calculated. The reliability of parametric values was assessed according to the Student's criterion. Comparison of non-parametric features was performed using the analysis of linkage tables using the Pearson Chi-square test.Odds ratio (OR), 95% confidence interval (95% CI) were determined. The obtained data were presented as OR [95% CI]. A value of $p<0.05$ was considered statistically significant.

**Research results and their discussion**

Analysis of the content of facultative anaerobes in the vagina showed that the microbiota of the vagina of patients with VHSIL differed from that of controls by a higher specific gravity of *Enterobacterium spp.* 11.62 times (31.03% vs. 2.67%; OR 4.8808 [2.3583-10.1013], $p<0.01$), *Streptococcus spp.* – 14.21 times (37.93% vs. 2.67%; OR 6.6282 [3.2446-13.5406], $p<0.01$), *Staphylococcus spp.* – 42.53% vs. 0.00% ($p<0.01$). No significant difference was observed between the composition of the vaginal microbiota of subjects with dVIN and controls. Enterobacterium spp taxa were found in the vaginal microbiota of VSCC patients 8.65 times more often than in controls – 57.69% vs. 6.67% (OR 16.1538 [3.2115-81.2547], $p<0.01$) (Table 1).

In women with precancerous diseases of the vulva, the highest content and diversity of facultative anaerobes was observed in individuals with VHSIL.

In patients with VHSIL, taxa *Enterobacterium spp.* were found in the vagina 3.68 times more often than in persons with dVIN (31.03% vs. 8.44%; OR 4.8808 [2.3583-10.1013], $p<0.01$), *Streptococcus spp.* – 4.49 times (37.93% vs. 8.44%; OR 6.6282 [3.2446-13.5406], $p<0.01$), *Staphylococcus spp.* – 4.68 times (42.53% vs. 9.09%, OR 7.4000 [3.6935-14.8218], $p<0.01$). Women with VSCC were distinguished from patients with VHSIL and

dVIN by a high specific gravity of such facultative anaerobes as representatives of *Enterobacterium spp.* – 57.69% against 31.03% and 8.44%, that is, it was, respectively, greater in 1.86 (OR 2.5641 [1.0736-6.1239], p<0.04) and in 6.84 (OR 12.5148 [4.9128-31.8803], p<0.01) times (see Table 1).

Table 1 - The content of facultative anaerobes in the vaginal microbiota in patients with precancerous and cancerous lesions of the vulva, n (%)

| Group | Facultative anaerobes | | |
|---|---|---|---|
| | *Enterobacterium spp.* | *Streptococcus spp.* | *Staphylococcus spp.* |
| G1 (VHSIL), n=87 | 27(31,03) $^{c1,2,5}$ | 33(37,93) $^{c1,2}$ | 37(42,53) $^{c1,2}$ |
| G2 (dVIN), n=154 | 13(8,44) $^{1,5}$ | 13(8,44) $^{1}$ | 14(9,09) $^{1,3}$ |
| G3 (Paget's disease of the vulva), n=9 | 1(11,11) | 2(22,22) | 3(33,33) $^{2}$ |
| G4 (melanoma *in situ*), n=8 | 2(25,00) | 1(12,50) | 1(12,0) |
| G5 (VSCC), n=28 | 15(57,69) $^{c2,1,2}$ | 3(11,54) | 4(15,38) |
| CG1, n=30 | 2(6,67) | 1(3,33) | 0(0,00) |
| CG2, n=30 | 2(6,67) | 3(10,00) | 4(13,33) |
| Note.$^{c1, c2, 1, 2, 3, 4, 5}$ – statistically significant difference with the indicators of the groups CG1, CG2, G1, G2, G3, G4, G5 (p<0,05). | | | |

The highest content and diversity of obligate anaerobes in the vaginal microbiota were also recorded in women with VHSIL, among which representatives of *Prevotella sgp.* prevailed. (67 (77.01 %)), *Gardnerella vaginalis* / *Prevotella bivia* / *Porphyromonas spp.* (51 (58.62 %)), *Mobiluncus spp.* / *Corynebacterium spp.* (51 (58.62 %)) and *Atopobium vaginae* (46 (52.87 %)). In women with dVIN, the vaginal microbiota was also dominated by *Prevotella spp.* (133 (86.36%)), *Gardnerella vaginalis* / *Prevotella bivia* / *Porphyromonas spp.* (73 (47.40 %)), *Mobiluncus spp.* / *Corynebacterium spp.* (73 (47.40 %)) and *Atopobium vaginae* (66 (42.86 %)). The vaginal microbiome of patients with VSCC was characterized by less diversity of obligate anaerobes, but *Gardnerella vaginalis* / *Prevotella bivia* / *Porphyromonas spp.* were detected in 18 (69.23%) individuals, and *Prevotella spp.*. and Peptostreptococcus spp. - in 15 (57.69%) individuals (Table 2).

Table 2 - Percentage content of obligate bacterial vaginosis-associated anaerobes in the vaginal microbiota of women with precancerous and cancerous lesions of the vulva, n(%)

| Group | *Gardnerella vaginalis / Prevotella bivia / Porphyromonas spp.* | *Prevotella spp.* | *Sneathia spp./Leptotrihia spp. / Fusobacterium spp.* | *Megasphaera spp. / Veillonella spp. / Dialister spp.* | *Lachnobacterium spp. / Clostridium spp.* | *Mobiluncus spp. / Corynebacterium spp.* | *Peptostreptococcus spp.* | *Atopobium vaginae* |
|---|---|---|---|---|---|---|---|---|
| G1 (VHSIL), n=87 | 51 (58,62) c1,3 | 67 (77,01) c1,5 | 17 (19,54) 2 | 36 (41,38) c1,2,3 | 30 (34,48) c1,2,3 | 51 (58,62) c1,3,4 | 31 (35,63) c1,2,3 | 46 (52,87) c1,3,4 |
| G2 (dVIN), n=154 | 73 (47,40) c2,3 | 133 (86,36) c2,4,5 | 9 (5,84) 1 | 7 (4,55) 1 | 8 (5,19) 1 | 73 (47,40) c2,3,4,5 | 0 (0,00) 1 | 66 (42,86) c2,3,4,5 |
| G3 (Paget's disease of the vulva), n=9 | 0 (0,00) 1,2,5 | 6 (6,67) c2 | 0 (0,00) | 0 (0,00) 1 | 0 (0,00) 1 | 0 (0,00) 1,2 | 0 (0,00) 1,5 | 0 (0,00) 1,2 |
| G4 (melanoma *in situ*), n=8 | 2 (25,00) | 2 (25,00) 2 | 0 (0,00) | 0 (0,00) | 0 (0,00) | 0 (0,00) 1,2 | 0 (0,00) 5 | 0 (0,00) 1,2 |
| G5 (VSCC), n=28 | 18 (69,23) c2,3 | 15 (57,69) c2,1,2 | 4 (15,38) | 0 (0,00) | 0 (0,00) | 0 (0,00) 2 | 15 (57,69) c2,3,4 | 0 (0,00) 2 |
| CG1, n=30 | 4 (13,33) | 3 (10,00) | 3 (10,00) | 2 (6,67) | 2 (6,67) | 3 (10,00) | 0 (0,00) | 1 (3,33) |
| CG2, n=30 | 5 (16,67) | 2 (6,67) | 3 (10,00) | 1 (3,38) | 0 (0,00) | 0 (0,00) | 0 (0,00) | 0 (0,00) |

Note. $^{c1, c2, 1, 2, 3, 4, 5}$ – statistically significant difference with the indicators of the groups CG1, CG2, G1, G2, G3, G4, G5 ($p<0,05$).

As can be seen from the table. 2, differences in the spectrum of vaginal obligate anaerobes in women with precancerous diseases of the vulva were more numerous in patients with VHSIL than in patients with dVIN *Sneathia sgp.* / *Leptotrihia spp. / Fusobacterium spp.* 3.35 times (19.54% vs. 5.84%, OR 3.9127 [1.6609-9.1273], $p<0.01$), *Megasphaera spp.* / *Veillonella spp.* / *Dialister spp.* – 9.09 times (41.38% vs. 4.55%, OR 14.8235 [6.2101-35.3839], $p<0.01$), *Lachnobacterium sgp.* / *Clostridium spp.* – 6.64 times (34.48% vs. 5.19%,

OR 9.6053 [4.1557-22.2011], p<0.01) and *Peptostreptococcus spp.* - by 35.63% (35.63% vs. 0.00%, p<0.01). *Prevotella spp* taxa were found in the vaginal microbiota of patients with VSCC less often than in patients with dVIN (57.69% vs. 86.36%, OR 0.1822 [0.0760-0.4365], p<0.01), *Mobiluncus spp.* / *Corynebacterium spp.* – 0.00% vs. 47.40% (p<0.01), *Atopobium vaginae* – 0.00% vs. 42.86% (p<0.01).

The number of fungi of the genera *Candida spp.*, *Ureaplasma spp.*, *Mycoplasma hominis* in the vaginal microbiota of women with VHSIL was 37.83 times higher than that of individuals with dVIN, respectively (35.63% vs. 4.55%, OR 11.6250 [4.8410-27.9161], p<0.01), 2.94 times (34.48% vs. 11.69%, OR 3.9766 [2.0530-7.7028], p<0.01) and 1.71 times (31.03% vs. 18.18%, OR 2.4300 [1.3047-4.5259], p<0.01) (Table 3).

Table 3 - The presence of fungi of the genus *Candida spp.*, as well as *Ureaplasma spp., Mycoplasma hominis* in the vaginal microbiota of women with precancerous and tumor lesions of the vulva, n (%)

| Group | *Candida spp.* | *Ureaplasma spp.* | *Micoplasma hominis* |
|---|---|---|---|
| G1 (VHSIL), n=87 | 31(35,63) $c^{1,2}$ | 30(34,48) $^{c1,2,3}$ | 27(31,03) $^{c1,2,3}$ |
| G2 (dVIN), n=154 | 7(4,55) $^{1}$ | 18(11,69) $^{c2,1}$ | 28(18,18) $^{c2,1}$ |
| G3 (Paget's disease of the vulva), n=9 | 1(11,11) | 0(0,00) $^{1}$ | 0(0,00) $^{1}$ |
| G4 (melanoma *in situ*), n=8 | 1(12,50) | 0(0,00) | 0(0,00) |
| G5 (VSCC), n=28 | 3(11,54) | 1(3,85) | 0(0,00) |
| CG1, n=30 | 2(6,67) | 3(10,00) | 1(3,33) |
| CG2, n=30 | 4(13,33) | 0(0,00) | 0(0,00) |
| Note. $^{c1, c2, 1, 2, 3, 4, 5}$ – statistically significant difference with the indicators of the groups CG1, CG2, G1, G2, G3, G4, G5 (p<0,05). | | | |

The presence of fungi of the genus *Candida spp.*, as well as *Ureaplasma spp., Mycoplasma hominis* in the vaginal microbiota of women with VSCC probably did not differ from that in controls and in women with precancerous diseases of the vulva.

The bacterial microbiome on the skin of a healthy vulva included the genera *Lactobacillus, Corynebacterium, Staphylococcus* and *Prevotella,* which indicates the transmission of microorganisms of vaginal, skin, and intestinal origin (Fig. 1). The major bacterial species found in the non-lesioned and non-lesioned skin of patients with vulvar VHSIL and dVIN were similar to those observed in healthy vulvar skin and were not likely to

differ. Most often, these were bacterial genera, which included *Prevotella spp.*, *Lactobacillus spp.*, *Staphylococcus spp.* and *Gardnerella spp.* (see Fig. 1).

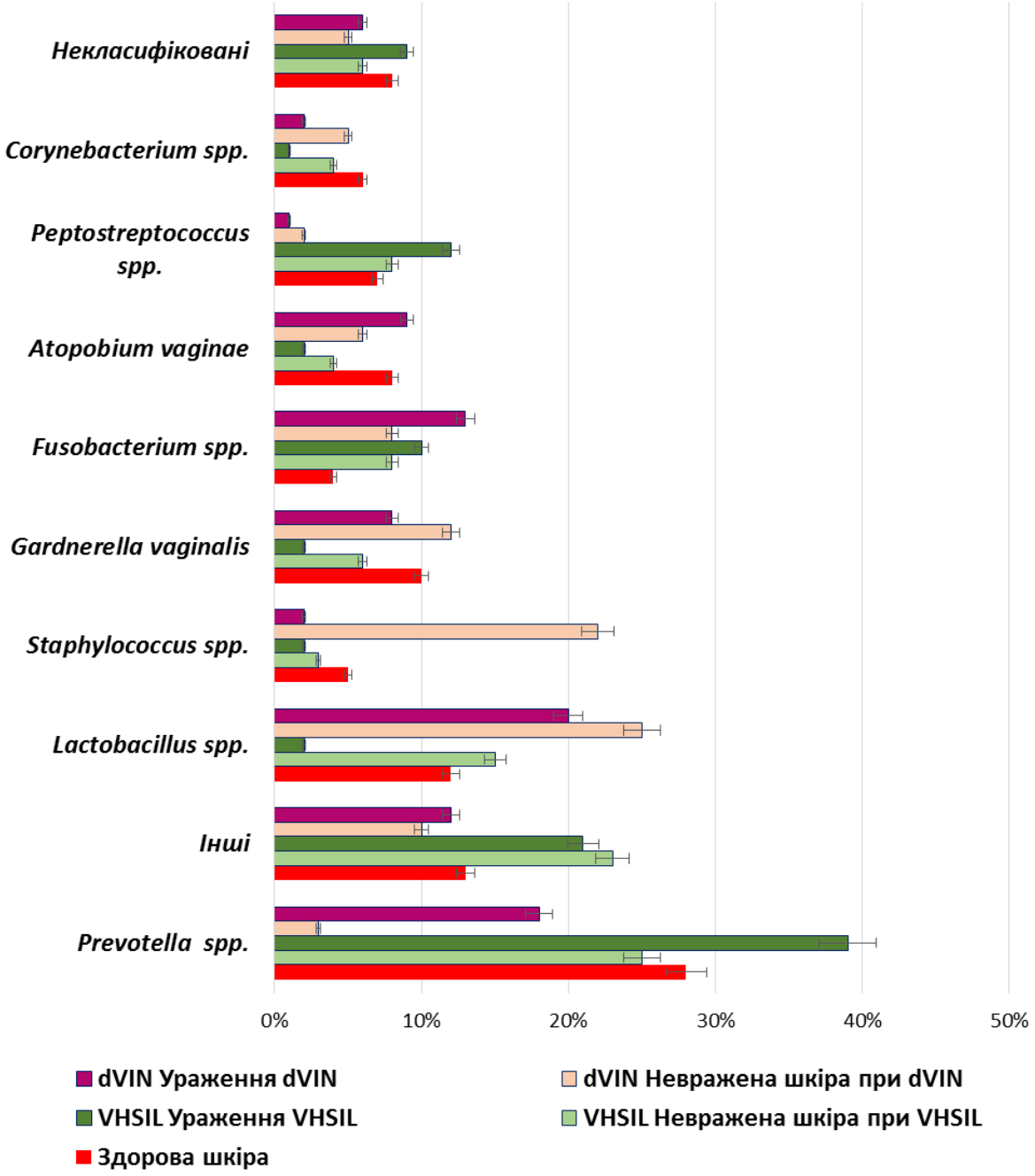


Figure 1. The relative number of bacterial genera inhabiting healthy skin in the vulva region of women in control groups, unaffected skin and affected skin in VHSIL and dVIN.

The affected skin of patients with VHSIL showed an increase in taxa from the genus *Prevotella spp.*, *Fusobacteria spp*. and *Peptostreptococcus spp.* Affected vulvar skin of

patients with dVIN was characterized by enrichment of taxa from the genus *Prevotella sgp, Fusobacteria spp., Atopobium vaginae* and depletion of *Corynebacterium spp., Lactobacillus spp., Staphylococcus spp., Peptostreptococcus spp.* compared to unaffected skin (see Fig. 1).

**Conclusions**

Among patients with precancerous lesions of the vulva, the vaginal microbiota of individuals with VHSIL is characterized by an increase in the vast majority of facultative and obligate anaerobes, fungi of the genus *Candida spp.,* as well as *Ureaplasma spp., Mycoplasma hominis*, compared to patients with dVIN. A characteristic feature of the vaginal microbiota of dVIN patients was a high concentration of *Prevotella spp.*

Healthy vulvar skin of patients with precancerous lesions resembled the vaginal and skin microbiome composition of control groups, including genera *Prevotella, Lactobacillus, Gardnerella, Staphylococcus* and *Corynebacterium*. The affected vulvar skin of patients with VHSIL showed an increase in taxa from the genus *Prevotella spp., Fusobacteria spp.* and *Peptostreptococcus spp.* Affected vulvar skin of patients with dVIN was characterized by enrichment of taxa from the genus *Prevotella sgp., Fusobacteria spp., Atopobium vaginae* and depletion of *Corynebacterium spp., Lactobacillus spp., Staphylococcus spp., Peptostreptococcus spp.* compared to unaffected skin.

The study showed a change in the composition of the vaginal microbiota in precancerous diseases and cancer of the vulva. However, our understanding of host defense mechanisms against these microorganisms remains limited. Additional studies are needed to elucidate the functional implications of these microbial communities for the vaginal microenvironment, particularly with regard to tumorigenesis. This will provide a more complete understanding of the functions of these bacteria in the initiation and progression of gynecological malignancies. And a deeper understanding of the complex interaction between the host and microorganisms in the vaginal area is promising for identifying new strategies in the field of cancer prevention and treatment.

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**Informed Consent Statement:** Written informed consent for treatment, use of the patients' personal data and their use was obtained from all examined women.

**Data Availability Statement:** All information is publicly available, data on a specific patient can be obtained upon request from the author.

**Conflict of Interest:** The author no conflict of interest.

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