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Association between cervical intraepithelial neoplasia progression and cervicovaginal microbiota status — narrative review

Marta Balajewicz-Nowak[1,2], Monika Kabzinska-Turek[1,2], Violetta Hosiawa[1,2]

[1] Department of Gynecology and Obstetrics, Faculty of Medicine, Jagiellonian University Medical College, Kraków, Poland
[2] Clinical Department of Gynecological Oncology and Gynecology, University Hospital in Kraków, Kraków, Poland

Corresponding author: Marta Balajewicz-Nowak, M.D., Ph.D.
Clinical Department of Gynecological Oncology and Gynecology, University Hospital in Kraków
ul. Jakubowskiego 2, 30-688 Kraków, Poland
Phone: +48 12 400 27 00; E-mail: marta.balajewicz@uj.edu.pl

Abstract: In 2022, an estimated 660 000 women were diagnosed with cervical cancer worldwide and about 350 000 women died from the disease. Human papillomavirus (HPV) infection is one of the most common sexually transmitted infections. However, only a small percentage of high-risk (HR) HPV infections progress to cervical precancer and cancer. In this study, we presented the role of the cervicovaginal microbiome (CVM) in the natural history of HPV infection. Non-viral factors associated with the outcomes of HR-HPV infections have not been fully elucidated. While smoking, hormonal contraceptive use, and parity are associated with developing precancer and cancer, systemic and local immune responses are thought to be important for clearance and control of infection. In addition, specific host immune regulatory alleles are associated with risk of cervical cancer development. There is a strong association between changes in vaginal microbiota and persistent HPV infection, and improving vaginal microbial environment would reduce the risk of developing cervical cancer.

Keywords: human papilloma virus, cervical intraepithelial neoplasia, cervicovaginal microbiome.

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Introduction

The main cause of cervical cancer is persistent infection with high-risk types of human papillomavirus (HPV), an extremely common family of viruses that are transmitted through sexual contact. Cervical cancer occurrence is a process through ultimately progressing cervical intraepithelial neoplasia (CIN) to invasive cancer. During this process CIN lesions may regress, persist, or progress. Several studies revealed notable differences between vaginal microbiota among patients with

precancerous lesions and healthy females. Although, it remains unclear which specific vaginal microenvironment promotes or inhibits disease progression. Microbiota are the range of microorganisms that may be commensal, symbiotic, or pathogenic in particular environment. Each site of human the body has particular microbiota which accounts for definite role in human health. Dysbiosis, disruption of microbiota homeostasis, can threaten health condition due to increasing host susceptibility to infections. It is shown that different factors including life style, hygiene, age, genetic conditions, diet, environmental factors, diseases and exposure to antibiotics can affect microbiota.

Cervicovaginal bacterial composition

The vaginal microbiota has been classified into five different community state types (CST) including: *L.crispatus* (CST I), *L.gasseri* (CST II), *L.iners* (CST III), *L.jensenii* (CST V). CST IV contains a heterogenous group which divided into two subgroups (CST IV-A and CST IV-B). CST IV-A has the modest proportion of *Lactobacilus spp*. And low propotions of anaerobic bacteria while CST IV-B has higher proportion of *Atopobium*, *Prevotella*, *Parvimonas*, *Gardnerella*, *Megasphera*, *Ruminococcaceae*, *Mobiluncus*, *Sneathia*, and depletion of Lactobacillus spp. In healthy females, CST I and V are dominant microbiota. During infection with HPV, CST II dominate and enhance clearance of HPV infection. *L.iners* with other species such as *Bacteroides*, *Fusobacterium*, *Veillonela, Actinomycetes*, *Bifidobacterium*, *Peptococcus*, *Peptostreptococcus*, *Propionibacterium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Gardnerella vaginalis* and *Prevotella bivia* also exist at low proportions. The vaginal microbiota is dynamic process, due to frequent alteration from one microbiota to another in the same woman during her lifetime, usually from CST III to CST IV [1–4] (Fig. 1).

CST	Dominant species
I	*Lactobacillus crispatus*
II	*Lactobacillus gasseri*
III	*Lactobacilus iners*
V	*Lactobacillus jensenii*

IV-A	BVAB1 Gardnerella vaginalis Atopobium vaginae
IV-B	Gardnerella vaginalis BVAB1 Atopobium vaginae Prevotella
IV-C	Lactobacillus spp. Gardnerella vaginalis Atopobium vaginae BVAB1

C0	Prevotella
C1	Streptococcus dominated
C2	Enterococcus dominated
C3	Bifidobacterium dominated
C4	Staphylococcus dominated

Fig. 1. CSTs — community state types. There are 5 major types, each dominated mainly by a specific type of microbe: CST I Lactobacillus crispatus; CST II Lactobacillus gasseri; CST III Lactobacillus iners; CST V Lactobacillus jensenii; CST IV is characterized by low numbers of Lactobacillus spp. with the increase in diversity of anaerobic bacteria [5].

Vaginal dysbiosis

Vaginal *Lactobacillus spp.* is important for maintenance of the cervical epithelial barrier function as it can protect from the invasion of HPV to the basal keratinocytes through maintenance of low pH and bacteriocin production. In general, the Lactobacillus genus shows high abundance in cervicovaginal microbiota and was first described in 1892 by Döderlein. Lactobacillus species like L. crispatus, L. gasseri, and L. jensenii are able to produce lactic acid and hydrogen peroxide (H_2O_2), which inhibit the growth of other bacteria and viruses. Furthermore, *Lactobacillus spp.* produce lactic acid and create an acid environment. *Lactobacillus spp.* is the most important component of cervicovaginal microbiota, playing an essential role. They contribute to the reinforcemenet of the host immune system against several primary and opportunistic pathogens. The most common species in HPV negative women are *L. crispatus* (CST I) and *L. gasseri* (CST II) whereas CST III and CST IV are frequently observed in HPV positive women and may be associated with development of premalignant cervical lesion. The homeostasis of cervicovaginal microbiome is maintained via interaction with the local microenvironment. When this homeostasis is disrupted, leading to a condition known as dysbiosis. Dysbiosis, situation of disruption of microbiota homeostasis, can be prompted development of cancer through epithelial barrier disruption, metabolic dysregulation, abnormal cellular proliferation, genome instability, chronic inflammation, and angiogenesis. Dysbiosis can threaten health condition due to increasing host susceptibility to infections. It is shown that different factors including women's reproductive cycle, use of oral contraceptives, sexual activity, vaginal douching, lactation, diabetes mellitus, stress, life style, age, host genetic, diet, environmental factors, type of birth delivery, infant feeding methods, diseases, and exposure to antibiotics can affect microbiota. Some women with dysbiosis develop symptoms such as abnormal vaginal discharge, inflammation, odor, and pruritus, being diagnosed, under these conditions, with bacterial vaginosis. Although some women show symptoms, a great part of them are asymptomatic. However, both symptomatic and asymptomatic women are more likely to acquire HIV, HPV, and other infections. Recently, next-generation sequencing (NGS) has been applied in clinical research on the vaginal microbiota. NGS method directly amplifies and sequences a particular region of rRNA gene using DNA extracted from the clinical specimen without the need for cell culture. NGS based methods have allowed massive parallelization of bacterial DNA sequencing and facilitate the rapid identification of thousands of vaginal microbiota within a few days. This method additionally permit to verify presence of intracellular pathogens. Cervicovaginal dysbiosis can disturb the responses of host immune system by triggering inflammations, conductive to high risk HPV infections. The increases diversity of microbiome leads to more production of cytokines and chemokines, amplifying the inflammation, and causing cell damage. The dysregulated immune response can create appropriate microenvironment for persistent HPV infection and tumor development.

Chronic inflammation is a promoter of cancer. Some bacterial vaginosis associated bacteria such as *Atopobium* can activate the proinflammatory transcription factor nuclear factor -kB (NF-kb), tumor necrosis factor α (TNF α), IL-6, IL-8, and granulocyte-macrophage colony stimulating factor (GM-CSF).Inflammatory conditions cause the tissue damage and facilitate penetration of HPV virus to basal keretinocytes. In preliminary results vaginal microbiota dominated by anaerobic or facultative Lactobacillus bacteria, combined with a deficiency in lactic acid bacteria, can trigger an increase in the ratio of L-lactic acid to D-lactic acid produced by vaginal epithelial cells. Of the two acid lactic isomers (D- and L-lactic acid), D-lactic acid is more protective against

infections. Differently from other *Lactobacillus* species, *Lactobacillus iners* has a small genome and is unable to produce D-lactic acid and H2O2. This shift can elevate the expression of extracellular matrix metalloproteinase inducers and the activity of metalloproteinase-8. In study Liu *et al.* identify characteristic microbial signatures associated with high grade CIN. They noted a gradual increase in the proportions of specific area as *Gardnerella*, *Dialister*, and *Prevotella* as precancerous cervical lesions developed, with CST IV characterized by a dominance of these bacteria, showing increased susceptibility. Diversity analysis indicates an increase in the complexity of vaginal microbiota with disease progression. Both species diversity and richness in the CIN3 group were higher than those in the other group. *Lactobacillus* has the capacity to improve antiviral defenses and modulate inflammation mediated damage. *Lactobacilii* stimulate epithelium cells to produce surfactant proteins. *L.gasseri* can significantly increase interferon α and interferon β production in HPV-positive cervical epithelial cells and reduce the expression of the pro-inflammatory cytokines. *Fanyhessea vaginae* and *Sneathia amnii* causes more abundant cytokines release including IL-6, IL-8, monocyte chemoattractant protein-1 (MVP-1), macrophage inflammatory protein 3α (MIP3 α). It's reported that high IL-1/IP-10 ratio in bacterial vaginosis is associated with lower rate of HPV infection clearance. Gardnerella vaginalis and spp. Can induce THP-1 macrocytes to differentiate to the M1 phenotype and *Lactobaccillus* promotes M2 macrophages polarization. M1 macrophages increase reactive oxygen species (ROS) levels and M2 macrophages increase integrity of epithelial barrier. Additionally, *Lactobacillus* play anti-inflammatory role, promoting the differentiation of CD4+T cells toward immunosuppressed Treg cells. Reduction of *Lactobacilli* and the less acid environment may act as a precancerous factor, activating pathway related to cell proliferation and angiogenesis in the cervicovaginal epithelium. Non-Lactobacillus dominance was associated with several proinflammatory, chemotactic and haematopoietic immune response cytokines. L. iners can produce inerolysin, a cholesterol-dependent cytolysin. Inerolysin is a pore-forming toxin like the vaginolysin protein secreted by Gardnerella which forms pores in vaginal epithelium, compromising its integrity and favoring viral infections [6–8].

In addition, enrichment of anaerobic bacteria such as *Gardnerella vaginalis*, *Peptostreptococcus anaerobius*, and *Porphyromonas uenonis* were detected in women wit squamous intraepithelial neoplasia (SIL) and cervical cancer. The depletion of *L. crispatus* and increased abundance in anaerobic bacteria such as *Gardnerella vaginalis*, *Peptostreptococcus anaerobius*, and *Porphyromonas uenonis* was significantly more common in women with SIL and cervical cancer. *Gardnerella vaginalis* is a gram-variable facultative anaerobe, and its abundance in the vaginal environment increases dramatically during bacterial vaginosis and has been reported as a risk factor for cervical disease. Therefore, there is a strong association between changes in vaginal microbiota and persistent HPV infection, and improving vaginal microbial environment would reduce the risk of developing cervical cancer. CST dominated by *Fusobacterium spp.* are associated with an immunosuppressive microenvironment, characterized by higher levels of IL-4 and TGFβ-1 and by a shift from a Th1 to a Th2 immune response. *Peptostreptococcus anaerobius* is a gram-positive anaerobic coccus involved in female genital tract infections such as bacterial vaginosis and pelvic inflammatory disease as well as high-grade cervical lesions. *Porphyromonas uenonis*, a gram-negative anaerobes, is mainly found in the gastrointestinal tract, oral cavity, and genital tracts. However, the significance of its association with cervical disease is still unknown. Among common bacterial species, infections of *Atopobium vaginae*, *Dialister invisus*, *Finegoldia magna*, *Gardnerella vaginalis*, *Prevotella buccalis*, and *P. timonensis* were significantly associated with the risk for high-grade squamous intraepithelial neoplasia and cervical cancer. *Atopobium vaginae* is highly specific

for bacterial vaginosis, similarly to *Gardnerella vaginalis*, and enrichment of *Atopobium vaginae* is associated with HSIL. Dominance of *Atopobium vaginae* in vaginal microbiota represents an important risk factor for development of cervical neoplasia. *Dialister invisus* is a gram-negative coccobacillus and is related with new HPV-type infection within a year in women with normal cytological results. *Finegoldia magna* usually appears on the skin and mucous membranes and is associated with vaginosis, as well as wound infections, soft tissue abscesses, bone infections, and infectious endocarditis. *Prevotella buccalis* and *Prevotella timonensis* are belonged to the genus *Prevotella*, gram-negative anaerobic rods which have been isolated from the upper respiratory tract, oral cavity, and urogenital tract. Abundance of *Prevotella* is associated with HPV persistence and inversely related to the abundance of *Lactobacillus*.

Cervicovaginal dysbiosis can disturb the responses of host immune system by triggering inflammations, conductive to high risk HPV infections. The increases diversity of microbiome leads to more production of cytokines and chemokines, amplifying the inflammation, and causing cell damage. The dysregulated immune response can create appropriate microenvironment for persistent HPV infection and tumor development. Additionally, dysbiotic bacterial communities and their metabolites can stimulate local immune cells, leading to production of various inflammatory cytokines and reactive oxygene species (ROS). Acute inflammation may be protective for HPV clearance. However, chronic inflammation and oxidative damage by ROS can exhibit genotoxic effects on epithelial cells, consequently leading to cell apoptosis and tumorogenesis. The dysbiotic microenvironment also contributes to cell proliferation and angiogenesis which are hallmark of cancer (Fig. 2).

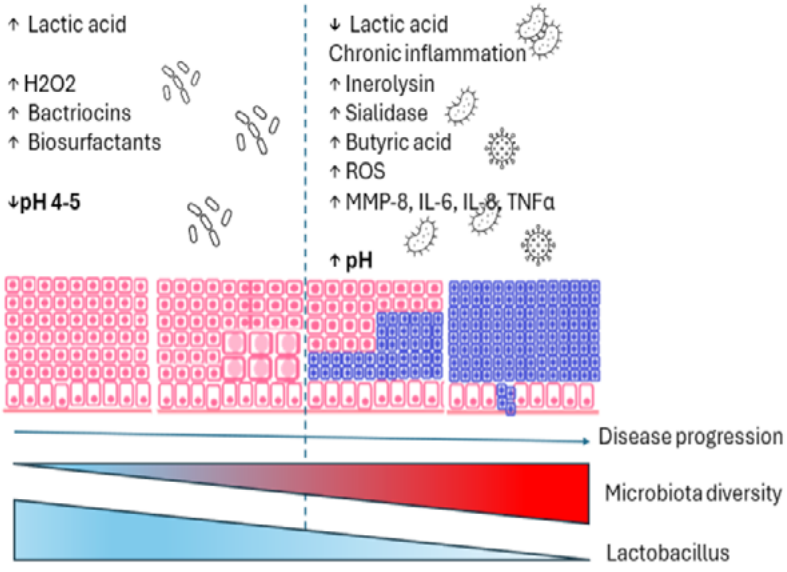

Fig. 2. Vaginal microbiota with Lactobacillus depletion, elevated vaginal pH, and genital chronic inflammation are associated with HPV persistence and progression. HPV infection converts vaginal bacterial community structure from CST III to CST IV, increasing vaginal bacterial richness and diversity, additionally HPV infection reduces the abundance of Lactobacillus species. Abbreviations: ROS (Reactive Oxygen Species), MMP (Matrix Metalloproteinases).

Cervicovaginal microbiota plays a significant role in creating the immune response responsible for HPV clearance. The bacterial or viral components are recognized by epithelial cells through Toll-like receptor (TLR), activating the innate immune response by releasing various pro-inflammatory cytokines. Macrophages and dendritic cells, as antigen presentic cells (APC), are then activated and recruit immune effector cells, such as Natural Killer cells. APCs also stimulate antigen-specific T cells and B cells to activate the adaptive immune response. Studies presents that dysbiosis CST IV cause chronic inflammation. Due to increased level of inflammatory cytokines (IL-1α, IL-1β, granulocytes-macrophage colony — stimulating factor, IL-10) in cervix and vagina the process of profilaration accelates and may promote cervical cancer development [7, 9–12].

Conclusions

Although human papillomavirus (HPV) is an important etiologic agent of cervical carcinogenesis, only in a certain women infected by it was associated with development of SIL and cervical cancer. Cervicovaginal environment is thought to be responsible for the development of cervical disease as well as the infection of high-risk HPVs. The vaginal microbiota coexists with various microorganisms in healthy cervicovaginal environment. Predominant *Lactobacillus* species could decrease the pH of the cervicovaginal environment, creating a chemical barrier to the both exogenous bacteria and viruses. Although the relationship between the change of microbiota and cervical carcinogenesis is not confirmed, significant changes in the vaginal microbial environment in women with SIL and cervical cancer are observed. The cervicovaginal microbiome is a dynamic network of microorganisms able to modulate a host's immune responses and promote an environment susceptible to viral infection acquisition and development of CIN. Recent studies showed the association between high-diversity cervical microbiota and HPV infection, SIL and cervical cancer. On the other hand, women with dominant Lactobacillus species (except for L. iners-dominant) can promote HPV clearance. Thus, specific bacteria or the high diversity microbiota may function as biomarkers for cervical lesions, and can as well be used to identify women at high risk of HPV chronic infection, SIL, and cancer. However, the precise crosstalk between the microbiota, carcinogenic agents and cervical epithelium, that promote or protect from cervical lesion formation are yet to be fully elucidated. The use of probiotics is demonstrated in vivo and in vitro for HPV clearance and significant SIL regression. Therefore, the manipulation of the microbiota by the use of probiotics may be a feasible option to induce HPV infection clearance, SIL regression, and stop progression to cervical cancer. Although the composition of the vaginal microbiota is affected by genetic and cultural background as well as lifestyle. We should take under consideration the possible cofactors like smoking, hormones and STDs on the progression of cervical lesions. Novel approaches to modulate vaginal microbiota from dysbiotic to optimal Lactobacillus-dominant community state could be beneficial and can lead to regress of lesions and improve therapeutic efficacy.

Authors' contributions

M.B.N. searched and identified appropriate articles, and wrote the manuscript; M.K.T. and V.H. revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

None declared.

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