



REVIEW ARTICLE

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SYSTEMATIC REVIEW OF THE INCIDENCE OF HUMAN PAPILLOMAVIRUS IN WOMEN WITH BACTERIAL VAGINOSIS

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ABSTRACT

Diseases affecting the female reproductive system are becoming more prevalent worldwide, among which are the bacterial vaginosis caused by the bacterium *Gardnerella vaginalis* and cervical cancer caused by the Human Papilloma Virus (HPV). may corroborate for significant pathological consequences. It aims to correlate *Gardnerella vaginalis* Bacterial Vaginosis with the development and clinical manifestations of HPV through a bibliographic analysis on the general characteristics of Bacterial Vaginosis and HPV, their interactions with the immune system and the correlation of microorganisms causing epithelial lesions. The bacterium *Gardnerella vaginalis* may be responsible for facilitating HPV to enter epithelial lesions of the basal and parabasal layers, thus increasing the chances of risk of developing cervical cancer, since from the reduction of *Lactobacillus* spp. susceptibility to injury and irritation of the vaginal mucosa corroborating HPV virus entry. With the increasing prevalence in recent years of various gynecological diseases, much is discussed about prophylactic and educational measures thus changing the reality of women's health.

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INTRODUCTION

The female reproductive system confers a form in which the vagina and cervix are considered complex organs, as it concentrates a considerable amount of aerobic and anaerobic bacterial species, calling this complex the "vagina's natural microbiota". Such species sometimes are the cause of cervicovaginal inflammation, often accompanied by a foul-smelling discharge.

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Factors that contribute to this event are established, such as pregnancy, menopause, vaginal pH variations, surgery, immune disorders, chemotherapy, high numbers of sexual partners, IUD use, spermicides, broad spectrum antibiotics, poor hygiene habits, vaginal douche habit, coitus frequency, lack of a vaginal immune response, among others, may be possible to modify bacterial flora, and cause saprophytic germs to become pathogenic (GUPTA, KAKKAR, BHUSHAN, 2019). In terms of pathologies linked to the vaginal flora, it stands out in the Bacterial Vaginosis (BV) considered worldwide, as one of the diseases responsible for vaginal infection in sexually active women, and in reproductive age,

allowing sex hormones to be involved in its pathogenesis (VERWIJS *et al.*, 2019). Another pathology that must be highlighted is the cervical cancer, as it is the sixth cancer that causes women to die, in which about 90% of malignant cell growth is due to the clinical manifestation of the HPV virus (NERI, ANDRADE, SILVA, 2019). This study aims to correlate *Gardnerella vaginalis* Bacterial Vaginosis with the development and clinical manifestations of HPV, since they are considered a risk to women's health and body homeostasis.

METHODOLOGY

This is a literature review work based on published materials, such as periodical articles from websites such as PubMed, Scielo and Google Scholar, dissertations and theses in order to correlate and map the history of infections caused by *Gardnerella vaginalis* and Virus of the Human papilloma.

DISCUSSION

Bacterial Vaginosis: The vaginal microbiota is a very complex ecosystem where equilibrium microorganisms are found. The microorganisms living in the microbiota are the aerobic, anaerobic bacteria and fungi of the genus *Candida*. Bacteria that predominate in the vaginal ecosystem belong to the family of *Lactobacillus* sp. The composition of the resident microbiota consists of microorganisms that although coexist with women non-offensively, are sometimes able to become sufficiently aggressive, being responsible for important infectious complications (PEREIRA, 2018). However, mostly sexually transmitted exogenous microorganisms will only cause genital infection after interacting with the resident microflora and overcoming the mechanisms of vaginal defense. Resident or temporary microorganisms that populate the female genital tract are fundamental to the balance of the vaginal environment (NETO, 2011). While talking about the vagina's natural microbiota, we notice an abundance of *Lactobacillus* sp species besides the hormonal system itself, such as estrogen - female hormone - which stimulates the proliferation of stratified epithelial cells, where high glycogen levels will be produced, which will be metabolized. *Lactobacillus* sp will form lactic acid and other organic acids that maintain vaginal pH from 4.0 to 4.5 (PEREIRA, 2018; BACKES *et al.*, 2019). This acidic pH, associated with Hydrogen Peroxide (H₂O₂), also produced by *Lactobacillus* sp, confer the natural protection of the vagina, inhibiting the growth of microorganisms. The release of this substance is important for vaginal balance, thus inhibiting the anaerobic bacterial growth (FALCONI-MCCAHILL, 2019).

Vaginosis arises with decreased *Lactobacillus* sp (SILVA *et al.*, 2019). As this barrier falls, the anaerobic bacteria present in the ecosystem begin to proliferate. *Gardnerella vaginalis* is a bacterium that is part of the normal vaginal microbiota, being detected about 20 to 80% of sexually active women, but in small colonies compared to the others (PEREIRA, 2018; SILVA *et al.*, 2019). The alteration of this flora happens due to several factors such as the lack of hygiene of the woman, the excessive sexual intercourse or relationship with several partners, where the man or woman can be the vector of bacteria, the use of antibiotics, preventive IUD, another factor that can also be related is hygienic exacerbation, as personal hygiene products can significantly alter the physicochemical characteristics of the vagina, thus leading to the death of

Lactobacillus sp, thus increasing anaerobic bacteria such as *Gardnerella vaginalis*, *Mobiluncusbacteroides* and *Mycoplasma hominis*, leading women to search for professional care because of the appearance of a characteristic BV odor (FALCONI-MCCAHILL, 2019). BV is distinguished by the appearance of vaginal discharge that has different colors such as whitish, yellowish or grayish, having a milky, homogeneous appearance, with bad odor caused by the release of organic acids and amines, such as putrescine and cadaverine, due to alkalinization of vaginal secretion (BACKES, 2019). Because of the absence of inflammation, the condition is not called vaginitis, because in some situations they exist without pain, without itching, without dyspareunia, without redness of the vulva or vagina, and without leukocytes that are toxic, just a microbial deviation to anaerobic pathogens (SILVA *et al.*, 2019). However, there are several complications when *Gardnerella* proliferates and may increase the risk of acquiring and transmitting sexually transmitted infections (STIs), as the vagina is very unprotected and hyperemic, and is associated with obstetric and gynecological adverse outcomes, including spontaneous abortion, premature labor, preterm membrane rupture, post-cesarean endometritis, intrauterine chorioamnionitis infection, upper genital tract infections, and pelvic inflammatory disease (REBOUÇAS *et al.*, 2019).

In addition to the clinical problems, asymptomatic patients may appear and because of this will not be concerned to consult a doctor but may cause negative psychosocial reflexes due to the unpleasant odor, leading to embarrassment because she will be associated to a bad woman hygiene (PEREIRA, 2018). Much concern is currently being raised about these conditions, as the variation in bacterial species observed among BV cases may explain the limited ability of some antibiotics to heal this condition among pregnant women, especially when the frequency of inflammatory and irritative symptoms of these diseases are observed on vaginal walls in BV due to the absence of polymorphonuclear leukocytes (MENDLING *et al.*, 2019). In the diagnosis of BV, physical examination and laboratory tests of Amsel and the Nugent Score are performed. The Amsel test is performed on four criteria in relation to vaginal discharge, first is observed the coloration and its appearance. Second step is to check the pH if it is higher than 4.5. In the third step is the Whiff test (amine test) that checks the odor. The last step is to check the Clue Cells where is observed the presence of guide cells, which are cells covered by bacteria in fresh examination or smear using Gram method. For confirmation in the diagnosis, it must be positive in at least three of these criteria (SILVA *et al.*, 2019). The Nugent Score consists of observing the smeared slide by the Gram method and counting the bacteria present (morphotypes) and will be counted from 1 to 4 according to the occurrence per field, the numbers will be summed obtaining their final score for establish the outcome. If normal it will show (0-3), intermediate (4-6) and if positive (7-10). The image below shows how accurate the diagnosis can be in counting and sampling *Gardnerella vaginalis* bacteria (PEREIRA, 2018). Another laboratory method used for the diagnosis of BV, although not a bacterioscopic method is the Pap smear. Cytopathologists commonly report the presence of clue cells in smears stained by this method, based on the observation of squamous cells covered by bacteria that thus assume a violet stain (FIGUEIREDO, 2006). The Bethesda System 2001 brings as criteria for the diagnosis of BV, the presence of a blade bottom consisting of a thin layer of

cocobacilli that live in this system and recognize and destroy invaders, however when there is a failure the body is subject to contamination by viruses, bacteria and other parasites (LIMBERGER *et al.*, 2012). The presence of clue cells and the remarkable absence of lactobacilli using the term VB compatible vaginal flora modification (FIGUEIREDO, 2006).

Predominant age group of bacterial vaginosis: Abnormal vaginal discharge is one of the most common reasons in women of reproductive age, causing many to serach for medical attention for a gynecological complaint. Among the most common causes is bacterial vaginosis (BV), which is responsible for 22% to 50% of cases of vaginal infection (KAMGA, NGUNDE, AKOACHERE, 2019). Bacterial vaginosis is an extremely prevalent and recurring condition, being a cause of vaginal infections among sexually active women between 15 to 45 years old. Although not a reportable disease in most of the time, as they are mostly seen on Pap tests, or sometimes women only buy the antibiotic without confirming the pathology through tests, the data available in a study conducted at a hospital in New Guinea show whereas the prevalence of bacterial vaginosis among non-pregnant women ranges from 15% to 30% and 50% for pregnant women, since there are constant microbiota imbalances in pregnancy. However, most cases of vaginosis are asymptomatic and remain unreported and untreated (VALLELY *et al.*, 2019). A study by Kanga, Ngunde, Akoachere (2019) at the Central Clinic in Tiko, an African city located in Camões, the analysis did not bring constraints or any factors that interfered with the woman's personal life, thus demonstrating the humanized side of the research. The survey of sociodemographic data is of paramount importance to observe the predominant line of infections by Gardnerella vaginalis. It is noticed that the age group from 25 to 34 years old has a higher prevalence of BV, because in this group women have an active sexual life. Important fact to be highlighted is the prevalence of BV in married women, but from what the study shows is strictly linked to education, because most women attended only secondary education, where they did not receive the discipline of sex education corroborating the misinformation aboutand genital care.

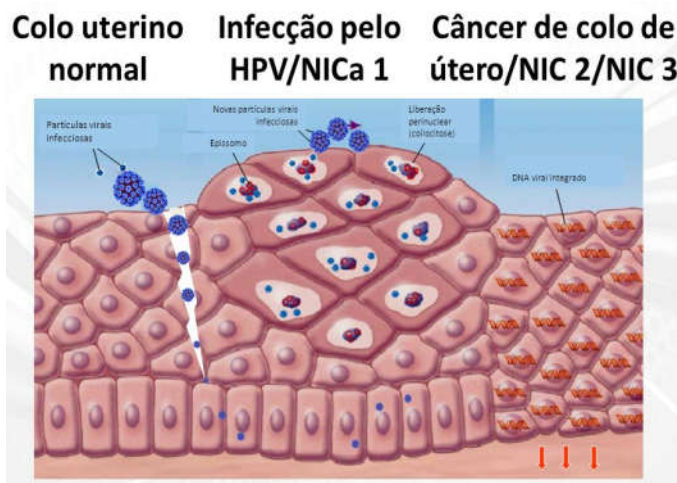
Table 1. Incidence of Bacterial Vaginosis in Women

Parameter	Age and situation	Number of cases
AGE	15 – 19	5
	20 – 25	14
	25 – 29	27
	30 – 34	27
	35 – 39	21
	≥ 40	6
MARITAL STATUS	Married	62
	Single	38
SCHOLARITY	No teaching	1
	Elementary	55
	Junior High	24
	High school	14
	Universityeducation	6

Source: Adapted from KAMGA, NGUNDE, AKOACHERE, 2019.

Immune System and Human Papilloma Virus Replication: The HPV virus has been the subject of many studies and debates, having as its etiological agent the Human Papilloma Virus which is characterized as an STI, infecting men and women. It is worth mentioning that a large portion of HPV serotypes prefer the female genital tract, in which the authors report that the HPV virus has female genital tract cell tropism

(LIMBERGER *et al.*, 2012). The Human Papilloma Virus (HPV), a viral species with more than 174 types of genomic differentiation characterized by numerous researches, are classified according to their pathogenic risk and their cancer risk, since not all cause serious diseases, since a large part of the world's population is infected with this virus (DE LIMA *et al.*, 2019). Among the clinical manifestations of HPV's considered low-risk are the low-grade variations in cervical cells, warts and papillomatosis, whereas high-risk HPVs have manifestations such as cancers, especially those of the cervix, anogenital, vulva, penis, vagina, anus and a small incidence of head and neck cancers due to warts, rarely on the tongue, tonsils and oropharynx. In literature, they classify the low-risk types, HPV 6, 11, 40, 42, 43 and 44, are associated with genital warts and other warts found in the body. Types that can cause cancer include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 and contribute to 96.6% of invasive cervical cancer diagnosticate in Global sphere. HPV types 16 and 18 that cause about 66% of cervical cancer cases (BORGES *et al.*, 2019). The evolution of the virus depends on several factors, some of them are hormonal and immune. The shield of the human body is the immune system, since the childhood, the human being is protected by it against infections by cells in this system that recognize and destroy invaders, however when there is a failure the body is subject to contamination of viruses, bacteria and other parasites. HPV attacks basal epithelial cells, being a virus of isometric structure, double stranded DNA, circular genomic configuration and 8,000 base pairs (LIMBERGER *et al.*, 2012).



Source: LIMBERGER *et al.*, 2012 adapted from Goodman

Figure 1. HPV infection in cervical cells

In the above image it is clear that the contact of the human papilloma virus with the squamous epithelium is a complex of viral engineering, as in the image below they can remain in the skin for a while until there is a wound that generates exposure of the basal layer, the viruses then adhere to the plasma membrane of these immature cells and dump their material into the cytoplasm (LIMBERGER *et al.*, 2012). Thus, it is worth noticing that the virus has preferential basal layers because they are in a constant process of metabolization and growth, so its genetic material enters the nucleus where replication happens, however infected the young cell can not mature and due to desquamation the cell begins to rise to the surface with the larger nucleus containing little cytoplasm, with replication and mitoses the virus can change cell's morphophysiology so it does not undergo apoptosis

process thus causing a high risk injury being characterized as cancer in situ and may progress to invasive cancer (BORGES *et al.*, 2019; LIMBERGER *et al.*, 2012). The HPV virus already installed binds to heparin sulfate (proteoglycans) through the protein L1 that is the most abundant in its capsid releasing its material inside the cell, and can replicate its viral genome in approximately fifty copies, thus changing the morphophysiology of the cell. The increasing virus subculture occurs through the disintegration of the cytokeratin-weakening E4 protein that will be removed along with the superficial keratin layers, the vast majority of cells do not suffer apoptosis due to the virus DNA replication mechanisms, this makes the local immune system not be alerted causing its death (PEDROSA, MAGALHÃES, PERES, 2019). The virus attacks undifferentiated keratinocytes located in the squamous stratified epithelium, within these cells innate immunity is triggered and becomes the first protection line against the virus, not being sufficient, the humoral immunity that is mediated by IgA and IgG immunoglobulin activity takes action in an attempt to recognize the virus to warn other cells of the virus's genetic "code", recognizing how the virus acts is easier to stop its reproduction, and even eliminate them from the body, but this is only possible if the patient has a resistant immunity, on the contrary it will be much more complicated to perform these processes, or they become slower or often do not happen due to lymphocytic cell failures (LIMBERGER *et al.*, 2012).

It is noticed that high echogenic HPV viruses express significantly the E6 and E7 proteins that are directly linked to cancer cells, which causes cell pathway deregulation, controlling the cell cycle, in the case of the phase S where there is a constant growth and replication of DNA and apoptosis since once the infected and traumatized cell cannot die it undergoes oxidative stress making it a malignant cell (DE LIMA *et al.*, 2019). The correlation of immunity and HPV host with proinflammatory response of female genital Th1 response cytokines, with an increasing dendritic cell's density in the foreskin, and peripheral blood CD8 T cell responses where the immune system attempts to combat the HPV. Thus, an inflammatory immune response of the host mucosa to HPV is required for immune clearance, but this inflammatory response would recruit highly susceptible HIV cells to the genital tract, thereby increasing the susceptibility of women to AIDS (DE LIMA). *et al.*, 2019).

Correlation between incidence of bacterial vaginosis and human papillomavirus: Changes in a woman's natural microbiota are directly related to human papillomavirus (HPV) infections, as women with high-risk papillomavirus infection may have a reduction in *Lactobacillus* spp., One of the components of the vaginal microbiota that is important peroxide producers, which may potentiate and increase the susceptibility of HIV contamination, important for maintaining cervical epithelial barrier function that inhibits HPV entry into basal keratinocytes (JUN-MO KIM & YOO JIN PARK, 2017). GB causes obstetric problems (abortion, premature birth) such as gynecological (pelvic inflammatory disease, endometritis, urinary tract infections (SILVA *et al.*, 2019). It is one of the major cofactors related to cancer by human papillomavirus (HPV) with an incidence up to 32%. *Gardnerella vaginalis* bacteria may be responsible for facilitating HPV entry into epithelial cells, causing lesions in the cervicovaginal epithelium in the basal and parabasal cells, thus increasing the risk of developing cervical cancer.

(PEDROSA *et al.*, 2019; BACKES *et al.*, 2019). The vaginal microbiota with HPV infection is more likely of being classified as a characterized state by an increased relative abundance of facultative and strict anaerobic bacterial species among them the prevalence of *Gardnerella vaginalis*, thus decreasing the abundance of *Lactobacillus* sp. (BORGES *et al.*, 2019). When there is an increasing colonization of BV-associated anaerobic bacteria, especially *Gardnerella*, there is a break in the barrier that facilitates HPV entry and other viral loads that may accelerate the process of epithelial lesion in the vaginal mucosa (JUN-MO KIM & YOO JIN PARK, 2017). In a study with 9165 women in Costa Rica, it was found that women with vaginal pH greater than 5 showing a clear picture of *Gardnerella vaginalis* infection were significantly associated with a 10-20% risk of positivity for menopausal HPV infection, because is during this period that women have drastic changes in the natural microbiota and the decrease of hormone production (MITRA *et al.*, 2016). Much is currently being discussed regarding the implementation of probiotics in the conventional treatment of bacterial vaginosis in order to improve and/or prevent recurrences of infection and to restore the normal conditions of *Lactobacillus* sp. As the vaginal microbiota does not have endogenous bacteria, these probiotics can be administered either orally or dermally, since besides carrying a microorganism, these medicines may be associated with drugs that help the immune system fight viral infections, especially the virus. HPV (TEIXEIRA, 2018). In fact, the vaginal microbiota plays a very important role in the protection against cervico-vaginal cancer, so it is necessary to research and understand the protection mechanism of these species or strains, protecting against HPV that can cause dysplasias or neoplasias (MITRA *et al.*, 2016).

Conclusion

Women's health is a sensitive issue and deserves attention from public health authorities in Brazil and around the world. With the increased prevalence in recent years of various gynecological diseases, many professionals started to alert such authorities about these problems in relation to microorganisms that directly affect women's health causing various disorders, including a BV and HPV. Considering how physical and chemical changes in the vagina's natural microbiota the correlation between bacteria and viruses can be highly detrimental to the health and well-being of women especially in the 20 to 35 age group, such changes are dangerous to internal and external factors that may trigger various disharmonic and homeostatic situations, another factor to be highlighted is the educational level and information that may corroborate to an early non-identification of HPV and Bacterial Vaginosis causing serious problems such as high-risk lesions and cervical cancer. Through foods, physical activity, hygiene and guidance with health professionals is possible to regulate the immune system and fight pathogenic microorganisms. It is also necessary to create integrated projects of health promotion in the ankle of women's health, aiming better living conditions, prioritizing the quality and treatment of diseases, especially, when in relation to the HPV and BV infection, changing the reality of women's health.

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