Gynecology and Obstetrics Clinical Medicine 3 (2023) 207–212

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Review Article

Trichomonas vaginalis and human papillomavirus: Association with the microbiota and burden on the cervix

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ARTICLE INFO

Keywords:
Human Papillomavirus
Trichomonas vaginalis
Vagina microbiota
Co-infection

ABSTRACT

Trichomoniasis and HPV infection are common non-viral and viral sexually transmitted diseases worldwide. Emerging evidence shows that the female genital tract and its microbiota are greatly affected by these pathogens. However, the relationship between Trichomonas vaginalis, the vaginal microbiome, and High-risk (HR)-HPV infection is complex and multifaceted. Studies have proven that concurrent infections of HIV and HPV increase the risk of cervical cancer. With this basis, a question arises: How does the concurrent infection of trichomonas vaginalis and HPV affect genital tract health? Does this concurrent infection enhance or inhibit the development of cervical lesions? This review aims to bring light to these questions. This review also covers the association of trichomonas vaginalis and HPV with the microbiota of the genital tract.

1. Background

Sexually transmitted infections (STIs) have become a significant problem worldwide. According to the World Health Organisation (WHO), about a million people acquire STIs yearly, with most infections asymptomatic. The most common causatives of STIs are linked to eight pathogens, which are syphilis, trichomonas vaginalis, human immunodeficiency virus (HIV), human papillomavirus (HPV), Herpes simplex virus, hepatitis B, chlamydia, and gonorrhoea. HIV, herpes simplex virus, hepatitis B, and HPV have treatment options that suppress symptoms but not cure. In contrast, the other four including Trichomonas vaginalis have treatment options that cure the cause.[1]

Trichomoniasis is a common, curable, sexually transmitted disease, and it is caused by trichomonas vaginalis (*T. vaginalis*).[2,3] Trichomonas vaginalis is a protozoan flagellate which colonises the urogenital tract.[4,5] Trichomonas vaginalis is not a reportable disease, implying that most infections are asymptomatic.[6] However, there are specific symptoms associated. The frequent symptoms observed in women include vaginitis(mainly), dyspareunia, pruritis, et cetera.[5] In the clinical field, the preferred treatment for trichomoniasis, according to the recent treatment guidelines for sexually transmitted diseases, is metronidazole.[7]

The virulence of the *T. vaginalis* parasite is due to the double-stranded RNA Trichomonas vaginalis virus (TVV) inhabiting the parasite.[8,9] The symbiotic infection of *T. vaginalis* with TVV is said to lead to severe clinical manifestations of trichomoniasis.[10–12] Several studies have demonstrated that *T. vaginalis* can increase the risk of acquisition of other STDs such as HIV, HPV, et cetera.

HPV is one of the most common causes of sexually transmitted diseases in both men and women worldwide. It is thought to be a widespread sexually transmitted viral disease.[13] Papillomaviruses are members of the Papovaviridae family. There have been more than 200 types of HPV associated with humans, and they have been divided into

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Publishing services by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.gocm.2023.10.002
Received 10 August 2023; Received in revised form 15 October 2023; Accepted 27 October 2023
Available online 10 November 2023

low and high oncogenic risk types.[14,15] HPV infections are not reportable since they usually do not manifest. HPV infection, however, may lead to warts, oropharyngeal cancers, and anogenital or cervical cancers (Fig. 1).[16]

Cervical cancer, a prominent type of HPV-related gynecological tumour, is one of the leading causes of death in women.[17,18] Cervical cancer is caused by the persistent infection of HPV.[19] High-risk HPV(HR-HPV) sub-types are proven to be the primary causative agent of cervical lesions. However, it is insufficient for developing cervical intraepithelial neoplasia (CIN) and cervical cancer.[20,21] Microbiota of the female genital tract has been said to be associated with HPV and the progression from cervical lesions to cancer.[22] Also, co-infection with other STIs may assist in developing cervical lesions and progression to cancer.[23]

There is evidence linking *T. vaginalis* and HPV, with research suggesting that *T. vaginalis* may contribute to the development of cervical cancer. This comprehensive review aims to elucidate whether the co-infection of *T. vaginalis* and HPV has a positive or negative impact on cervical-related diseases in women. Additionally, this review examines the association between these pathogens and the microbiota, both individually and during co-infection.

2. Vaginal microbiota, HPV and Trichomonas vaginalis

2.1. Vaginal microbiota and health

The health of the female genital tract, i.e., the vagina, dramatically depends on vaginal microbiota. A healthy vagina is associated with low microbial diversity and has proven to be dominated by the Lactobacilli species. The species has long been thought to play an essential role in defence against pathogens using two mechanisms. Lactobacillus creates biofilms and barriers by adhering to the mucus and, as a result, competes and prevents colonisation from pathogens. It produces protective antimicrobial compounds, including bacteriocin-like substances, biosurfactants, hydrogen peroxide, and lactic acid.[24]

The vaginal microbiota has been classified into five community state types (CST) according to the relative abundance of bacteria. Lactobacilli species dominate CST I, II, III and V- four communities; the other (CST IV) comprises anaerobic microorganisms with a few lactic acid bacteria.[25,26] The lactobacilli species in most women mainly dominate the microbiota of a healthy vaginal tract and are essential to preventing infections. However, not all Lactobacilli may be vital for protecting the genital tract. Some species have been associated with the aggravation of diseases; hence, though dominant, they may not all be protective.[27]

In rethinking what is linked to vaginal health, the decreased presence of Lactobacillus species may not necessarily mean disease in some women, such as people of colour, as the CST IV group mostly dominates them.[28] These women have healthy genital tracts until an external stimulus causes an imbalance, leading to disease formation.

2.2. Vaginal microbiota affecting trichomonas vaginalis

As established, the Lactobacillus species relates to the healthy condition of the genital tract. How, then, do *T. vaginalis* and the microbiota interplay with each other in terms of acquisition and pathogenesis?

The pathogenesis of trichomoniasis involves the adhesion of *T. vaginalis* to the epithelial cells of the genital tract, leading to the inflammatory responses of the genital tract.[3,29,30] Studies have related the pathogenesis of trichomoniasis to the microbiota.[6, 30,31]

2.2.1. Lactobacilli species

The adherence of *T. vaginalis* to ectocervical cells is influenced by Lactobacilli species, as demonstrated by Phukan et al.[32] In their study, the adherence of strains of *T. vaginalis (B7RC2 and G3)* was experimented with Lactobacilli species (*L. gasseri* ATCC 9857 and CBI3(*L. plantarum/L. pentosus*)). Adhesion of strong T. vaginalis strain was inhibited by lactobacilli gasseri. However, this is strongly dependent on cell contact and dose-species dependent. L. gasseri produces extracellular vesicles (EV), contributing to the microbiome's function. Treatment with L. *gasseri* inhibited *T. vaginalis* cytoadhesion.[33,34]

2.2.2. Non-lactobacilli species

The risk of *T. vaginalis* acquisition is higher in the presence of specific bacteria in the microbiome. Jarrett et al.[35] in their cohort of HIV-negative sex workers, found that the richness in the difference of vaginal bacteria increased the risk of *T. vaginalis* acquisition. Prevotella amnii and Sneathia sainguinegens species increased the risk of *T. vaginalis* acquisition twofold; moreover, though of borderline significance, the bulleidia species was also linked to a two-fold risk increase of *T. vaginalis* acquisition risk. Upon the infection of *T. vaginalis*, CST IV bacteria may be in greater abundance, as per Brotman and co-workers,[36] Hinderfeld et al.[37] in the line of adhesion of *T.* vaginalis to host cells, concluded that Bacterial vaginosis (BV)-associated bacteria acted as pathobionts that enhanced *T.* vaginalis' adherence to the host cells. Gardnerella vaginalis can create a persistent biofilm that aids the binding of the *T.* vaginalis strain. BV-associated bacteria increased the adherence of *T.* vaginalis to mucin cells, overpowering the mucus barrier faster than expected (Fig. 2). Moreover, in the presence of the BV-associated bacteria, the *T.* vaginalis

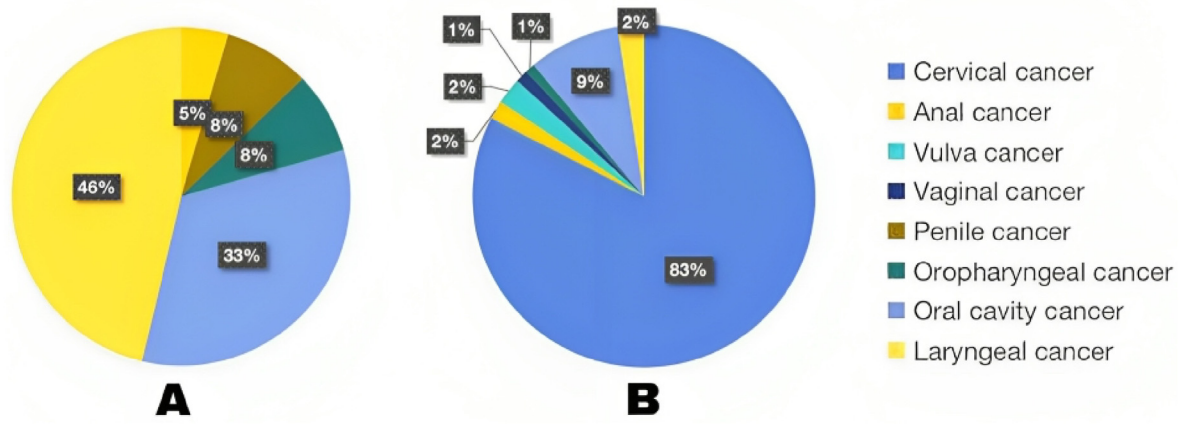

Fig. 1. Crude incidence of HPV-related cancers in China. The crude incidence of HPV-related cancers in males(A) and the crude incidence of HPV- related cancers in females (B).
Source: Bruni L, Albero G, Serrano B, Mena M, Collado JJ, Gómez D, Muñoz J, Bosch FX, de Sanjosé S. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in China. Summary Report 10 March 2023. [Date Accessed: 14 October 2023]

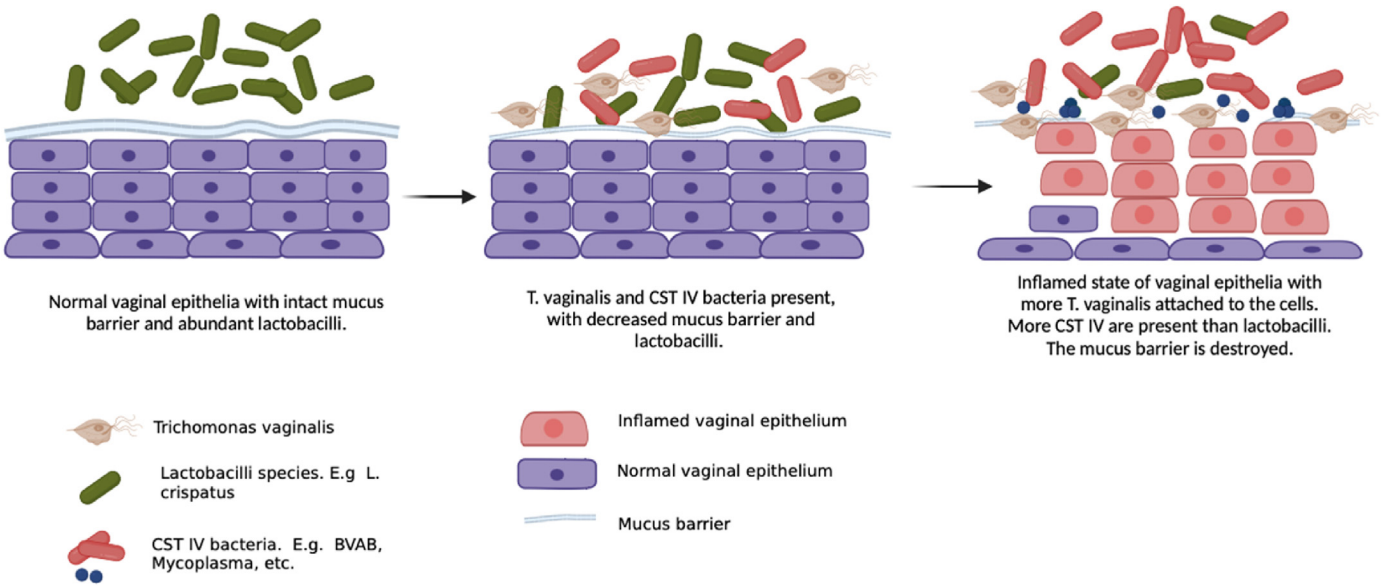

Fig. 2. Graphical representation of the association between Trichomonas vaginalis and the microbiota. The figure shows normal epithelia with a normal mucus barrier and lactobacilli. In the presence of trichomonas vaginalis, CST IV bacteria increases. In the presence of CST IV bacteria, the mucus barrier is overpowered easily allowing the *T. vaginalis* to attach to the epithelial cells leading to inflammation of the cells.

strain with the lowest adherence (i.e., G3) increased significantly in its adherence ability, almost catching up to the strong-adhering *T*. vaginalis strain(B7RC2).

Most women who go through trichomoniasis treatment get rid of the infection. However, there have been metronidazole-resistant cases. Verwijs et al.'s[38] study demonstrate oral metronidazole's impact on the vaginal microbiota. This study showed an increased percentage of Lactobacilli species in successful treatments of trichomoniasis. Gardnerella species was found in raised portions in unsuccessful or rather persistent cases. As previously established that Gardnerella vaginalis can create a biofilm that aids in *T. vaginalis* adhesion, Verwijs et al. also accredited the persistence (treatment failure) to the biofilm-creating ability of *G. vaginalis*.

2.3. HPV-related dysbiosis

Vaginal microbiome and HPV infection are thought to have a particular association.[22,45] It begs whether persistent HPV infections lead to the diversity of microbiota or whether the variety of microbiota is responsible for the persistent HPV infection, which leads to cervical dysplasia.

2.3.1. HPV-acquisition dysbiosis

HR-HPV acquisition has been correlated to the cervicovaginal microbiome in a study with over 600 subjects. Huang X et al.[39] concluded that it is not the microbiota diversity or the typical microbiota but agents specific to each squamous intraepithelial lesion (SIL) that might positively influence the acquisition of HR-HPV types independent of abundance. A "core" microbiome was found in all the samples. Lactobacillus (35.2 % of the total abundance), Burkholderia (24.5 %), and Pseudomonas (16.8 %) were present among HPV -16, 52 and 58 SIL groups, and it concluded that HPV 16 might be associated with Coleofasciculus and Oribacterium specifically, HPV 52 to Motilibacter and Kaistia, and Paludibaculum and Litorilinea associated only with HPV 58.

Likewise, Chao XP et al.[40] found high-risk HPV acquisition to be associated with the increased variety of microbiome species. The study, which involved 151 women (65 HPV positive and 86 HPV negative), showed results of dominant Lactobacillus species in both groups, with increased proportions of the following: Gardnerella, Atopobium, Megasphaera, Alloscardovia, and Sneathis, in the HPV-positive group. However, their main finding was that anaerobic bacteria such as Bacteroides plebeius, Acinetobacter lwoffii, and Prevotella buccae were significantly more found in the HPV- positive group—also, Lin W et al.[41] demonstrated that women with the infection of Gardnerella vaginalis showed a higher rate of HR-HPV acquisition than those with dominant lactobacillus species. In this study, the HPV-positive women had decreased lactobacillus species and increased Gardnerella and Prevotella species.

2.3.2. HPV-persistent dysbiosis

The persistence of HR-HPV infection has also been linked to alterations in the microbiome of the genital tract. It is known that clearance of HPV may occur within six months to a year of infection; however, some persist over a year, leading to pathological changes in the cervix.

Bi Q et al.[42] stated in the study that HPV persistence is linked with cervicovaginal dysbiosis. Healthy and transient HPV infection had high Lactobacillus species. However, persistent HPV infection showed decreased proportions of the lactobacillus species and increased portions of Bacteroides, Acinetobacter, Sphingomonas, Pseudomonas and Prevotella. Lactobacillus iners was strongly related to transient HPV infection.

In a longitudinal study of the vaginal microbiome and HPV, Usyk M et al.[43] found L. iners significantly associated with HPV clearance. At the same time, Gardnerella, combined with multi-bacterial diversity, was responsible for HPV progression and the formation of CIN.

Wei B et al.[44] shows that Gardnerella, Atopobium and Distalis were linked to persistent infection of HPV and the pathogenesis of cervical lesions. They observed a reduction in the Lactobacilli species in the disease groups, which is consistent with the studies mentioned above. Actinobacteria (Gardnerella and Atopobium) were found in increased proportions in the disease groups. Although not as significant as the increase of Actinobacteria, there was also an increase in the Bacteroidetes and Fusobacteria. A surprising find-out of this study was that L. iners was correlated with the severe progression of disease in the cervix, which was contradictory to previous studies. However, other works support this finding about L. iners due to its ability to disrupt the microbiota and may not be considered a "protective" lactobacilli-dominated community.[27,45]

Furthermore, Lactobacilli may be an intrinsic factor in the clearance of HPV infection and the regression of cervical lesions. Evidence shows that L. gasseri had the fastest remission of HPV DNA in patients a year

after infection.[45] Also, whereas Lactobacilli-depleted communities (CST IV) associated with BV often correlate persistence, Lactobacilli-depleted communities with a mixture of aerobic and anaerobic bacteria such as Pseudomonas, streptococcus, etc., were prevalent in HPV clearance.[46] Mitra A et al.[47] expand on the vaginal microbiota and the regression of untreated CIN2; Lactobacilli-rich microenvironments had a higher regression rate at 12 months than Lactobacilli-lacking microenvironments. The recent relationship between cervical cancer and the vaginal microbiome has been explained in detail by other review articles.[20,48]

HPV and its association with the vaginal microbiota is still much more complex than known. The clearance of HPV and inhibition of the growth of cervical cancer cells is linked to the lactobacilli species, though it is still widely understudied.[49] Future studies in this aspect can focus on the treatment abilities of Lactobacilli species regarding the clearance of HPV and the inhibition of the growth of cancer cells.

3. Co-infection of Trichomonas vaginalis and HPV

It is known that infection with trichomonas vaginalis increases the risk of HIV acquisition.[50] What happens then if there is a concurrent infection of HPV and *T. vaginalis*? Does it lead to disease progression or regression?

3.1. Trichomonas vaginalis and HPV acquisition

In a study of the Tanzanian population to find the association between Trichomonas vaginalis and HR-HPV, Lazenby GB et al.[51] found that women with *T. vaginalis* were at greater risk of HR-HPV infection, specifically HPV 16. HPV 16 is one of the leading causes of changes in cytology and, even worse, progression to cancer. It is suggested that *T. vaginalis* plays a role in the progression of cervical lesions. Similarly, Rodriguez et al.[52] concluded that *T. vaginalis* was strongly associated with HR-HPV, just like BV; *T. vaginalis* was incredibly significant in HPV -18, 45,66 or 68. In this study, women with *T. vaginalis* had a high prevalence of HR-HPV— also, Donders GG et al.[53] found that *T. vaginalis* is associated with low-risk HPV(LR-HPV) and HR-HPV.

A plausible mechanism for this co-infection may involve *T. vaginalis*, in conjunction with CST IV bacteria, compromising the tight junctions of human ectocervical cells. This compromise could enhance the paracellular permeability (PcP), resulting in the easier acquisition of STIs, such as HPV. The process of enhancing PcP can be triggered by both *T. vaginalis* and CST-IV, through dephosphorylation of significant protein components of tight junctions and increased phosphatase activity.[54]

3.2. Trichomonas vaginalis and risk of cervical lesions

Trichomonas vaginalis has been associated not only with the acquisition but also with cervical lesions. A Finnish longitudinal study[55] noted the risk for precancerous lesions and cancer due to *T. vaginalis* infection to be high and significant for one year. A pooled analysis study in rural China confirmed an association between HR HPV and CIN with past or current *T. vaginalis* infection; Feng RM et al.[56] concluded that the risk of HR HPV increases with present *T. vaginalis* infection compared to those with past (treated) *T. vaginalis* infection; past *T. vaginalis* has no association with HR-HPV. These results suggest an interaction of HPV, *T. vaginalis* and CIN2+.

Similarly, a case-control study in Taiwan[57] concluded that the risk of cervical cancer was significantly increased in women with prior exposure to *T. vaginalis*. Though the risk of CIN was also increased in this study, it was not of statistical significance. It was observed that the reluctance of Taiwanese people to seek medical attention until they experienced severe symptoms was linked to this issue.

Yang S et al.[58] in a meta-analysis of the association of *T. vaginalis* and cervical cancer of about 7715 cases and 67598 controls, found that *T. vaginalis* is strongly associated with increased cervical cancer (OR=2.06). Also, mixed race, followed by black people, had the most significant association between *T. vaginalis* and cervical cancer (OR= 2.87 and 2.43, respectively), with Asians having the lowest association, which may imply that people of mixed race and black people with the co-infection of HPV and *T. vaginalis* might manifest cytological changes more than other races. Could mixed race and black individuals be at a higher risk due to their vaginal microbiota being predisposed to CST IV? This CST has been correlated with an increased risk of cervical dysplasia and susceptibility to HPV infection in the presence of *T. vaginalis*. Further investigation may be necessary.

The co-infection of HPV 16 and *T. vaginalis* increases the incidence of CIN 1, 2, 3 and invasive cervical cancer (ICC), as Yang M et al.[59] reported. However, the risk of CIN 2–3 was significantly increased in the co-infection but not for CIN 1 and ICC for HPV 16. In women with HPV 18, the co-infection of *T. vaginalis* did not increase the risk of cervical dysplasia or cancer, for that matter.

A recent meta-analysis also supports *T. vaginalis*' association with an increased risk of cervical lesions. The study included over 470,000 patients, with 8518 *T. vaginalis* positive. The study found that *T. vaginalis* patients had an increased risk of cervical dysplasia (from ASCUS to HSIL), with LSIL and HSIL having two times and 2.4 times increased risk, respectively, in the co-infection of *T. vaginalis* and HPV.[60]

A proposed mechanism for this increased risk of cervical dysplasia is that upon infection, T. vaginalis produces lytic enzymes that reduce the protective mucus layer, which can result in micro lesions, enhancing the virulence of the HPV and enabling the integration of its DNA into the host cell, which may cause DNA damage to the host cell and the start of the carcinogenic process.[51] (Fig. 3). Also, inflammation caused by *T. vaginalis* causes ruptures and lesions, facilitating the persistence and development of cervical dysplasia – another proposed mechanism.[30,58] Some studies have also suggested that *T. vaginalis* induces the production of nitric oxide (NO) in neutrophils of the cervical cells, resulting in DNA damage and the promotion of abnormal cells.[61,62] In HPV-infected cells in the cervix, the rise in NO results in early mRNA expression, lower pRb and p53 levels, poor p53 activity, and low apoptosis indices, increasing mutant cell survival and promoting carcinogenesis.[63] This may factor in the increased risk of cervical lesions and cancer. These proposed mechanisms, however, are still understudied; further studies could follow up and elucidate these mechanisms.

Further research is needed to investigate the cellular mechanisms between *T. vaginalis* and HPV. This will provide insights into how these infections interact and impact the cervix and its microbiota.

4. Strengths and limitations of existing literature

The existing literature had its strengths and limitations. Briefing on its strengths, the association of trichomonas and HPV was established across several studies. These studies had a vast and diverse study population, providing more reliable and substantial facts about the increased risk of cervical dysplasia and cervical cancer during this symbiotic infection. Further, the association of the microbiota, trichomonas vaginalis, and HPV has been uncovered to a great extent, though there is still more to discover. This association shows the relationship between *T. vaginalis* and Lactobacilli *regarding T. vaginalis'* adhesion to epithelial cells. Also, the treatment-resistance ability of *T. vaginalis* has been discussed, making finding new therapeutic measures possible. Furthermore, the association of the microbiota to HPV shows that lactobacilli may be a significant factor in treating HPV and even cervical cancer. However, existing literature has its limitations as well.

The first limitation of existing literature in this direction is the need for the cellular mechanisms involved when there is a co-infection of *T. vaginalis* and an HR-HPV. Though *T. vaginalis* is involved in the inflammatory response of the cervical and vaginal epithelial tissues, the direct pathway of *T. vaginalis* in the involvement of the carcinogenesis of the cervix is greatly lacking.

The second and final limitation is the lack of reports on the vaginal microenvironment during the co-infection of *T. vaginalis* and HPV. As

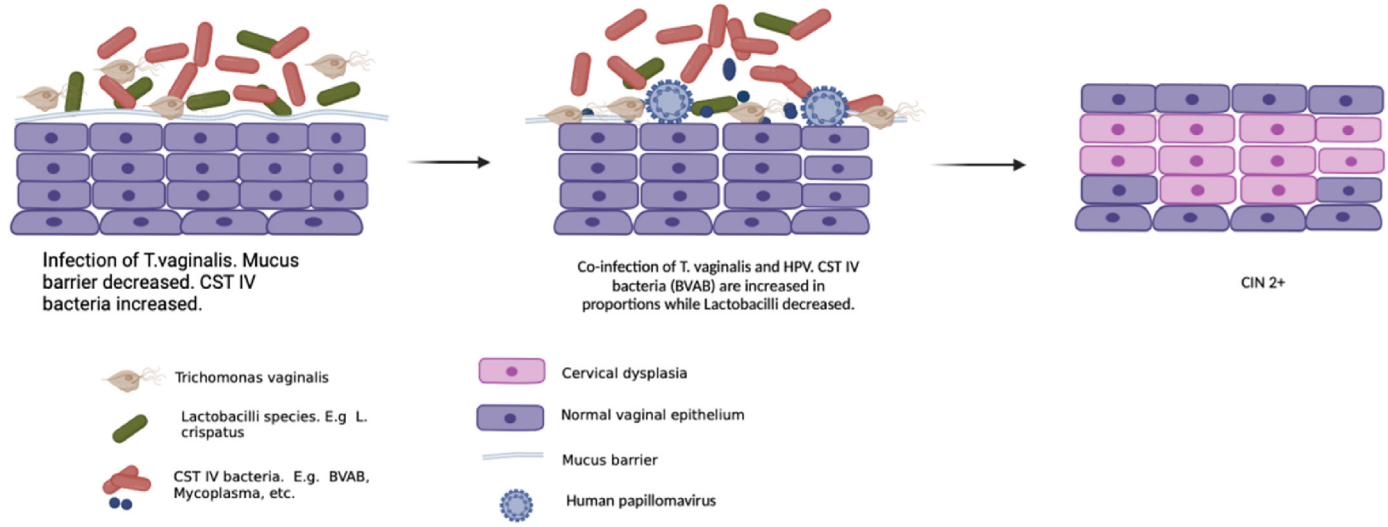

Fig. 3. Graphical representation of the co-infection of trichomonas vaginalis and HPV and the associations with the microbiota. During the infection of T. vaginalis, CST IV bacteria increases, mucus barrier is decreased, and as found by Hinderfield et al.,2019,[54] tight junctions of the cells are weakened enhacing paracellular permeability making it easier for co-infection of T. vaginalis and HPV. During the co-infection, CST IV bacteria increses. The co-infection of TV and HPV leads to an increase in the risk of cervical lesions.

established, the microenvironment of the vagina and cervix change under the disturbances present. However, studies have yet to present what occurs in the microenvironment during this symbiotic infection.

5. Conclusions

Trichomonas vaginalis and HPV are prevalent sexually transmitted diseases. There is an increased risk of developing cervical lesions during this symbiotic infection. Specific attention must be paid to the co-infection of HR-HPV and *T. vaginalis*, which leads to an even greater risk of cervical lesions. Their association with the microenvironment of the vagina greatly influence the pathogenesis of disease in individuals. Lactobacillus is critical in the protection of the microenvironment and female genital health.

Future studies can focus on the cellular mechanisms of the symbiotic infection of *T. vaginalis* and HPV in cervical carcinogenesis. Also, more attention should be paid to the changes in the vaginal microbiota that occur during the co-infection of HPV and *T. vaginalis. These may be* significant areas of study for deeper insight and help the therapeutic measures in the clinical field.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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