Influence of human papillomavirus infection on the vaginal microbiome of women with immunocompetency

Influência da infecção por papillomavirus humano no microbioma vaginal de mulheres imunocompetentes

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ABSTRACT

Introduction: The influence of vaginal infections on the natural history of human papillomavirus (HPV) is still unclear. **Objective:** To determine if patients with low-grade squamous intraepithelial lesions (LSILs) and HPV have more vulvovaginitis than patients with normal liquid-based cervical cytology who were negative for HPV. **Methods:** This is a cross-sectional study including 322 patients who underwent cervical exams. One hundred and sixty-seven of these patients had LSILs on cervical cytology and were simultaneously hybrid capture 2 (HC2)-positive for HPV, and the remaining 155 patients were negative for malignancies and intraepithelial lesions by cytology and HC2-negative for HPV. The prevalence of vaginal infections in both groups was compared using the χ^2 test without Yates' correction. **Results:** Among the patients with HPV and LSILs, the most common vaginal infection was vaginosis (8.98%) compared to candidiasis (12.9%) in the patients without LSILs and HPV. No significant differences were found in the prevalence of vaginosis between the two groups (p=0.53). Candidiasis was statistically more prevalent in patients without LSILs and HPV (p<0.001). **Conclusion:** An association was found between the presence of *Candida* and the absence of HPV. Although vaginosis was more frequent among patients with LSILs and HPV, it was not statistically significant.

Keywords: papillomavirus infections; Papanicolaou test; vaginitis.

RESUMO

Introdução: A influência das infecções vaginais na história natural do papillomavirus humano (HPV) ainda é incerta. **Objetivo:** Determinar se pacientes com lesões intraepiteliais escamosas de baixo grau (LIEBG) e HPV têm mais vulvovaginites que aquelas com citologia cervical em meio líquido normal e testes negativos para HPV. **Métodos:** Este é um estudo transversal, que incluiu 322 mulheres que fizeram exames de colo. Cento e sessenta e sete destas tinham LIEBG na citologia oncótica e foram simultaneamente positivas para HPV na captura híbrida 2 (CH2). As outras 155 tiveram citologias negativas para neoplasia intraepitelial e malignidade e eram CH2 negativas para HPV. A prevalência de infecção vaginal nos dois grupos foi comparada usando o teste do χ^2 sem correção de Yates. **Resultados:** Entre as pacientes com HPV e LIEBG, a infecção vaginal mais comum foi a vaginose (8,98%), enquanto que, no grupo sem LIEBG e sem HPV, foi a candidíase (12,9%). Nenhuma diferença estatisticamente significante foi encontrada na prevalência de vaginose entre os dois grupos (p=0,53). Candidíase foi estatisticamente mais prevalente nas pacientes sem LIEBG e HPV (p<0,001). **Conclusão:** Foi encontrada uma associação entre a presença de *Candida* e a ausência de HPV. Embora a vaginose tenha sido mais frequente em pacientes com LIEBG e HPV, esse dado não foi estatisticamente significante.

Palavras-chave: infecções por papillomavirus; teste de Papanicolaou; vaginite.

INTRODUCTION

The cervicovaginal milieu is a very complex environment, where glandular secretions, exfoliated epithelial cells and many microorganisms, both pathogenic and nonpathogenic, interact. Many infections can occur in the lower female genital tract and disrupt this intricately balanced vaginal ecosystem[1]. Moreover, a disrupted cervicovaginal environment due to a change from a protective bacterial population to a non-protective population can affect an individual's susceptibility to other infections[2].

The most common infectious disorders in the cervicovaginal milieu are related to *Candida sp*., *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Chlamydia trachomatis* and human papillomavirus (HPV)[1]. Among these, only those caused by *Trichomonas*, *Chlamydia* and HPV are considered sexually transmitted infections (STIs). HPV is more prevalent in sexually active young women, and its manifestations vary from latency to intraepithelial lesions and carcinomas in the lower female genital tract[3].

The influences of bacterial vaginosis, trichomoniasis and candidiasis on the natural history of HPV have been well studied, but the results remain unclear. Some studies have reported an association between *Candida* and papillomavirus[3], but most studies have found no relationship[1,4]. *Trichomonas* is also uncertainly associated with HPV; some studies have indicated a positive relationship[5,6], while other studies have not[1,4].

However, the biggest controversies lie in the study of the relationship between bacterial vaginosis (BV) and HPV. BV, which is caused by a decrease of *Lactobacilli* and a predominance of anaerobic bacteria in the vaginal flora, is among the most common causes of vaginal complaints in women of childbearing age[7]. Many studies have shown a positive association between BV and HPV, even more consistently than those observed for other vaginal infections. King et al.[1], in a longitudinal multi-site investigation including 756 women with human immunodeficiency virus (HIV) and 380 uninfected women at high-risk for HIV, found that bacterial vaginosis was associated with increased prevalence and incidence of HPV and delayed clearance of infection, even with paired risk factors.

Study carried out at the Hospital Geral de Fortaleza (HGF) – Fortaleza (CE), Brazil.

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A meta-analysis by Gillet et al.[7] also showed a positive association between BV and uterine cervical HPV infection, even when the number of sexual partners was matched.

Several hypotheses that support this association have been postulated. In BV-negative women, hydrogen peroxide-producing *Lactobacilli* dominate the vaginal microbiome and are part of the main defense mechanisms. Loss of these protective microorganisms and other changes in the vaginal milieu related to BV could facilitate the survival of other sexually transmitted agents and are risk factors for developing vaginal infections[7]. Other alterations in vaginal fluids induced by BV can explain this association, such as reduced levels of secretory leukocyte protease inhibitor (SLPI), increased levels of mucin-degrading enzymes and changes in the production of immunological factors, such as cytokines[8-10].

Most studies on this topic have found an association between BV and HPV but have not established a cause-effect relationship. Whether HPV-positive women are more likely to develop BV is unclear[7].

Although an association between BV and HPV has been suggested, there is no evidence that BV increases the risk of squamous intraepithelial lesions (SILs) or cervical cancer[11-13]. Bacterial vaginosis seems to affect only the viral incidence and prevalence but not the persistence of HPV infection. This explains the lack of effect of BV infection on the development of SILs[3,14]. In a cohort study by Mao et al.[15], a time lag analysis suggested that HPV infection usually precedes BV detection. Therefore, patients with papillomavirus have an increased risk for vaginosis. However, there are a few studies evaluating the influence of viral infections and subclinical lesions on the cervicovaginal milieu and the possibility of an increased risk of acquiring other infections.

OBJECTIVE

The purpose of the current study was to determine if patients with HPV-induced, low-grade squamous intraepithelial lesions (LSILs) have a higher prevalence of vaginitis.

METHODS

This is a retrospective cross-sectional study performed using a database of cervical pathologies from a private laboratory in Fortaleza, Brazil, between January 2009 and May 2012. The cervical cytology results were collected. Women with a history of prolonged corticotherapy or transplants were excluded. Furthermore, negative tests for HIV, hepatitis B and C and VDRL were established as inclusion criteria. A total of 322 women were included.

This study was approved by the Research Ethics Committee of the Hospital Geral de Fortaleza, Brazil.

All cervical cytologies were assessed with a liquid-based Pap test, using the SurePath (BD Diagnostics, Burlington, NC, USA) and ThinPrep (Hologic, Inc., Bedford, Massachusetts, USA) assays. The Bethesda System 2001 was used to conduct the cytological diagnosis[16].

The presence of at least 20% of clue cells in the smear was diagnosed as vaginosis[17]. The presence of blastoconidia or pseudohyphae was diagnosed as candidiasis.

HPV infection was determined using the hybrid capture 2 (HC2) test (Qiagen AG, Garstligweg 8, CH-8634, Hombrechtikon, Switzerland), which was performed using the same material obtained for the cervical cytology assays. The material was processed according to the manufacturer's guidelines for the identification of high-risk HPV-DNA (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Quantitative results were also obtained and expressed in relative light units (RLU), indicating the viral load. Each test was processed with positive and negative controls in triplicate.

The patients were divided into two groups. The first group included women who exhibited negative cytology for malignancies and SILs and were HC2-negative for HPV. The second group included women with LSILs and evidence of HPV infection. There were 155 patients in the first group and 167 patients in the second group.

The prevalence of vaginal infections was compared between the groups. Only HPV-positive patients with LSILs were assessed, whereas patients with latent infections or high-grade lesions were excluded because of their greater activity and increased viral replication[18].

The statistical analyses were performed with GraphPad InStat, 3.10 version (GraphPad Software Inc., San Diego, California, USA). The prevalence of vaginal infections in both groups was compared using the χ^2 test without Yates' correction to determine the p-value and the 95% confidence interval (95%CI).

RESULTS

The mean age of the HPV-positive group was 29.2 years, with a standard deviation of 10.2 years. The mean age for the HPV-negative women was 35 years, with a standard deviation of 11.52 years. Patients with HPV were significantly younger ($p<0.0001$).

In the patients that were HPV positive, the most common vaginal infection was vaginosis (15 cases; 8.98%) (**Figure 1**), whereas candidiasis was the most common infection in the patients that were HPV negative (20 cases; 12.9%) (**Figure 2**). The infections identified in

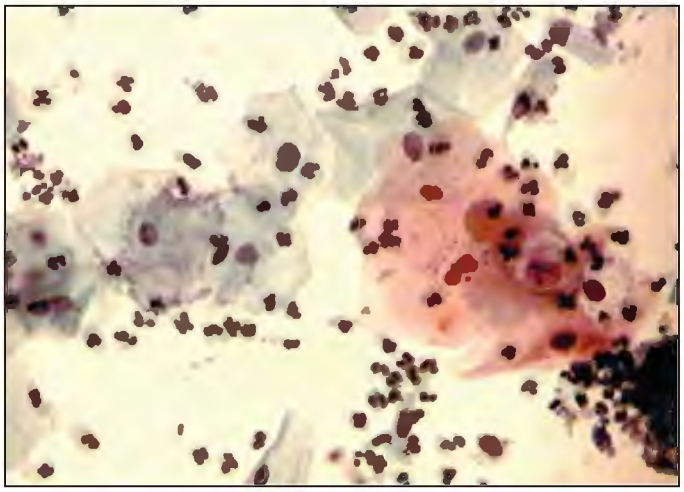

Figure 1 – Cytological analysis of a specimen exhibiting a low-grade squamous intraepithelial lesion (and positive hybrid capture 2 for high-risk human papillomavirus) with clue cells, suggesting bacterial vaginosis (SurePath 400x).

the HPV-positive patients with LSILs and the HPV-negative patients without lesions are detailed in **Figures 3 and 4**, respectively.

The difference in the prevalence of vaginosis between patients with and without HPV was not statistically significant (p=0.53). However, the frequencies of *Candida* infection were significantly different between groups (p<0.001), as shown in **Figure 5**.

DISCUSSION

Women with HPV were statistically younger (p<0.0001), which is consistent with the peak incidence of the disease. Dunne et al.[19] reported that the highest prevalence of HPV was among women aged from 20 to 24 years, followed by a gradual decline in prevalence to 59 years of age.

Gardnerella was the most frequently identified bacteria using cytology in the HPV-positive patients (8.98%), whereas in the HPV-negative patients, *Candida* was the most frequently identified pathogen (12.9%), which is consistent with the findings of Murta et al.[20], who also observed that *Candida* was the most frequent agent in a group without HPV (23.9%; 13.8% in HPV-positive patients; p<0.001).

These findings are consistent with the hypothesis that the local cervicovaginal milieu plays a role in an individual's susceptibility to HPV infection because women who carry *Candida* spp. are likely to possess a healthy *Lactobacillus*-dominated vaginal microbiome, in contrast to women with bacterial vaginosis[21]. Dols et al.[22] showed that, in women with HPV, the prevalence of *Lactobacillus crispatus* was significantly reduced and that there was a shift in the composition of the *Lactobacillus* microbiota following HPV infection. The leading hypothesis concerning these associations is that the absence of protective lactobacilli increases the biological susceptibility of acquiring STIs upon exposure[7]. Women with a loss of *Lactobacillus*-predominant vaginal microflora are more likely to acquire HPV. In contrast, women who have a vaginal flora dominated by *Lactobacillus* species tend to have fewer HPV infections but are more likely to develop candidiasis.

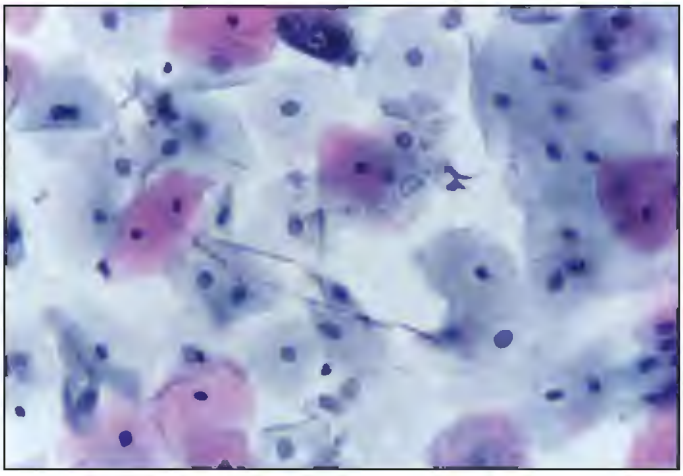

Figure 2 – Cytological analysis of a specimen negative for lesions (and negative hybrid capture 2 for high-risk human papillomavirus) exhibiting the presence of *Candida* morphotypes (SurePath 400x).

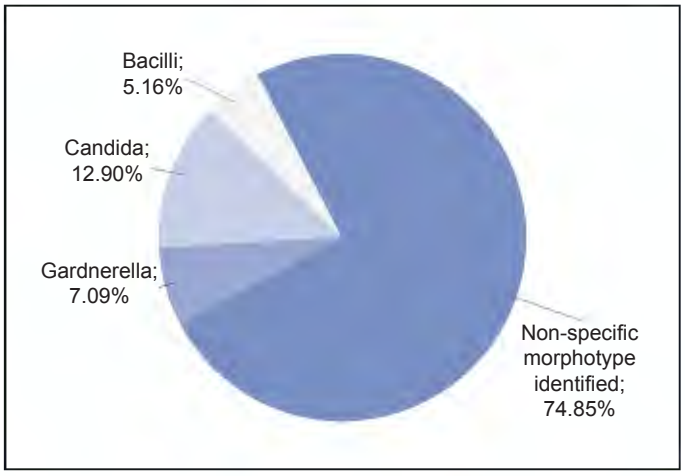

Figure 4 – Cytological findings obtained by liquid-based Pap tests in 155 women who were hybrid capture 2-negative for human papillomavirus and did not have malignancies or intraepithelial lesions.

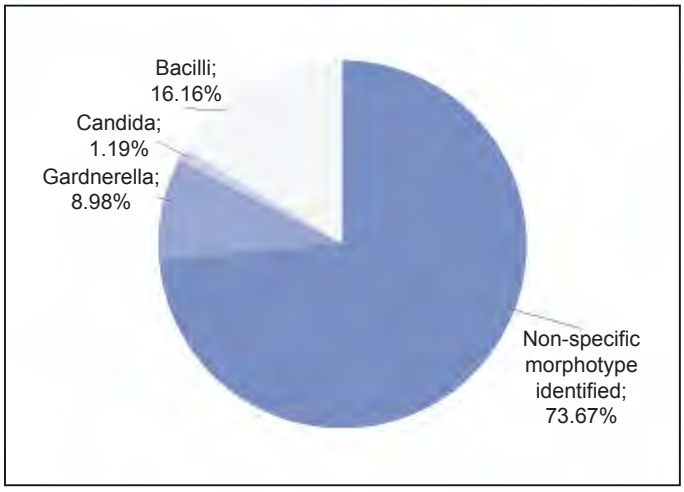

Figure 3 – Cytological findings obtained by liquid-based Pap tests in 167 women who were hybrid capture 2-positive for human papillomavirus and had low-grade squamous intraepithelial lesions.

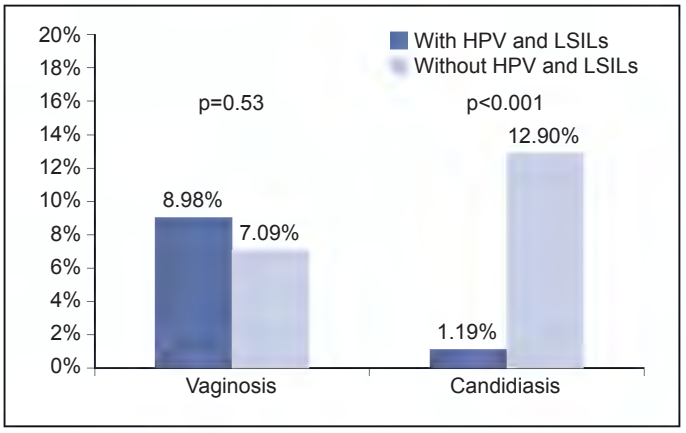

Figure 5 – Prevalence of vaginosis and candidiasis in 167 women who were hybrid capture 2 (HC2)-positive for human papillomavirus (HPV) and had low-grade squamous intraepithelial lesions (LSILs) and in 155 women who were HC2-negative for HPV and did not have malignancies or intraepithelial lesions.

The presence of *Candida* is not associated with an increased risk of acquiring HPV[23]. These findings are consistent with those of Watts et al.[24], who demonstrated that candidiasis was not related to high-risk papillomavirus infection in HIV-negative women.

There was no significant difference in the prevalence of BV between the two groups (with or without HPV).

Some studies have suggested that BV is associated with HPV acquisition. A meta-analysis of 12 studies including a total of 6,372 women indicated a positive association between BV and HPV infection, with an overall estimated *odds ratio* of 1.43 (95%CI 1.11–1.84)[7]. Nogueres et al.[3] found a positive association between *Gardnerella* and HPV. Vaginosis was observed in 9.1% of patients who were HC2-negative for HPV, while vaginosis was observed in 22.4% of HC2-positive patients (p=0.012). Guo et al.[25] reported that, compared to women without BV, women with BV had a lower clearance of HPV infection. All these findings can be explained by the hypothesis that susceptibility to HPV and the immune system's ability to clear HPV can be affected by vaginal bacterial infections, which disrupt the balance of the vaginal microbiota[7,26].

In our report, this association was not observed. Some of the limitations of the present study may explain the discordance among BV findings. There was a low prevalence of vaginal infections, which may be a result of our sampling from a private clinic, thus including patients with high social-economic status and fewer risk factors. Another limitation is that the microbiome evaluation was conducted only using Pap tests and disregarded other criteria such as the Nugent score and the Amsel clinical criteria.

There are other studies related to our findings regarding BV and HPV[4,27]. Zheng et al.[28] found a higher prevalence of BV in an HPV-positive group compared to a control group, which is similar to our results. However, this association was not statistically significant. In another study, bacterial vaginosis was found in 12% of subjects with LSILs and 4% of subjects with a normal Pap result; however, this result was not statistically significant either[29].

These data conflicts may be the result of the different methods applied in each study. A positive relationship between BV and HPV was observed in a population of sex workers, which was very different from our sample group[21]. Murta et al.[20] also found an association between vaginosis and papillomavirus; however, this study used a cytological diagnosis of HPV, which is significantly less sensitive than HC2.

Research on the alleged associations between HPV infection and vulvovaginitis may have an impact on clinical practice because these associations can serve as a basis for a simultaneous screening policy. A greater understanding of vaginal physiology and the interactions that occur in the cervicovaginal milieu can also increase our understanding of HPV physiopathology and, thereby, optimize prevention and treatment strategies[7].

CONCLUSION

The cross-sectional design allowed us to observe an association between *Candida* morphotypes and the absence of HPV. There was also a higher frequency of vaginosis in the papillomavirus group, but this finding was not statistically significant.

Because this was a cross-sectional study, we were unable to determine whether a change in the vaginal microbiota preceded HPV infection or vice versa. Therefore, further research is needed to clarify this relationship.

Conflict of interests

The authors report no conflict of interests.

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