

Evaluation of risk factors for anal human papillomavirus infection in heterosexual women diagnosed with human papillomavirus associated cervical dysplasia

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Ethics Committee Approval

The study was approved by the Pamukkale
University Medical Ethics Committee (May 5,
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All procedures in this study involving human
participants were performed in accordance with
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Conflict of Interest

No conflict of interest was declared by the
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Abstract

Background/Aim: Cervical dysplasia is a well-recognized precursor to cervical cancer, and human papillomavirus (HPV) infection is the primary causative agent in its development. The intricate relationship between cervical and anal HPV infections remains understudied. There have been no established risk factors determined for anal HPV infection in women without a history of anal intercourse. This study aims to address this critical knowledge gap by evaluating the risk factors for anal HPV infection in a homogeneous population of heterosexual women with HPV-associated cervical dysplasia.

Methods: This retrospective cohort study was carried out in a single tertiary center and comprised women between the ages of 30 and 65. Women diagnosed with either low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL) and without a history of anal intercourse were included in the analysis. Participants without a histological or colposcopic diagnosis were excluded from the analysis. Women with a history of prior cervical therapeutic intervention, previous cervical or genital dysplasia, known immunosuppressive disorders, current immunosuppressive medication use, a past cancer diagnosis, or a history of HPV vaccination were also excluded. Anal sampling was performed for HPV infection within the first year after the initial diagnosis of cervical dysplasia. Patient characteristics including smoking status were extracted from patient files.

Results: Overall, 186 women who met the inclusion criteria were tested for active anal HPV infection of the anal canal. Active anal HPV infection was found in 96 (51.6%) of the patients. In women with active anal HPV infection, 31 (32.3%) were found to have only HPV 16/18 genotypes, and 22 had HPV16/18 along with other high-risk types. When risk factors were analyzed, only current smoking was found to be associated with anal HPV infection in this group of women. Overall, 40.6% of the women with active anal HPV infection were smokers; however, only 25.6% of the women without anal HPV infection were current smokers ($P=0.029$).

Conclusion: Women had a high risk of active anal HPV infection during the diagnosis of cervical intraepithelial neoplasia. Current smoking was the only identifiable risk factor for anal HPV infection in women without anal intercourse history.

Keywords: anal cancer, cervical intraepithelial neoplasia, human papillomavirus, screening

Introduction

Human papillomavirus (HPV) is one of the most prevalent sexually transmitted infections worldwide, representing a main cause of cervical and anal cancer [1]. While the association between HPV and cervical dysplasia in women has been extensively studied, the simultaneous presence and impact of anal HPV infection in this population, specifically among heterosexual women diagnosed with HPV-associated cervical dysplasia, have received comparatively limited attention [2].

Cervical dysplasia is a well-recognized precursor to cervical cancer, and HPV infection is the primary causative agent in its development [3,4]. However, the intricate relationship between cervical and anal HPV infections remains understudied, even though the co-occurrence of these infections can have profound implications for disease progression and management. Emerging evidence suggests that women with cervical dysplasia may be at an elevated risk for anal HPV infection due to shared risk factors, such as sexual behavior and immune status [5]. The potential consequences of concurrent cervical and anal HPV infections, including an increased risk of invasive cervical cancer and anal cancer, underscore the importance of elucidating the risk factors and epidemiological patterns associated with this dual infection.

In the literature, most studies on the presence of anal HPV have been conducted on men or HIV-positive patients. Research in women, especially those that include anal HPV screening along with cervical cancer screening, is quite limited. Moreover, there have been no established risk factors for anal HPV infection in women without a history of anal intercourse. This study aims to address this critical knowledge gap by evaluating the risk factors for anal HPV infection in a homogeneous population of heterosexual women with HPV-associated cervical dysplasia. The analysis in this specific population will shed light on the risk factors and natural history of these infections. These results may also provide critical insights for our understanding of concurrent anal HPV infection and, therefore, provide a foundation for the development of anal cancer screening in high-risk patients.

Materials and methods

Study population

This retrospective cohort study was carried out in a single tertiary center. Approval was obtained by the Institutional Ethics Review Board from the same center (Pamukkale University Medical Ethics Committee May 5, 2019 / 05). The study cohort comprised women between the ages of 30 and 65.

The study's inclusion criteria were: women over 30 years of age who had previously tested positive for high-risk cervical human papillomavirus (HR HPV) prior to their colposcopy admission, and those with a histological diagnosis of either low-grade squamous intraepithelial lesion (LSIL) or high-grade squamous intraepithelial lesion (HSIL). LSIL cases underwent conservative management, while standard cervical excision procedures were performed for initial HSIL diagnoses.

Participants without a histological or colposcopic diagnosis were excluded from the analysis. Women with a history of prior cervical therapeutic intervention, previous

cervical or genital dysplasia, known immunosuppressive disorders, current immunosuppressive medication use, a past cancer diagnosis or a history of HPV vaccination were also excluded. Collected data for each participant included age, number of sexual partners, age at first sexual encounter, parity status, smoking habits, and HPV genotypes upon admission. Based on histopathological examination results, patients were classified into either the LSIL or HSIL group. Presence of the HPV infection was grouped into three sections: only HPV16/18; only other high-risk HPV; and HPV 16/18 along with other HPV types.

Specimen collection and HPV genotyping procedure

Anal sampling was performed within the first year after the initial diagnosis of cervical dysplasia. To obtain these samples, Dacron swabs were gently inserted into the anal canal and rotated in a circular manner. Subsequently, these swabs were preserved in a liquid transport solution and sent to the microbiology laboratory. The Advanced XL NA Purification-EZ1® device from Qiagen Inc. in Valencia, CA, was used for DNA extraction. HPV DNA amplification was carried out using the 14 Real-TM Quant kit from NLM in Settala MI, Italy. In cases where the initial sample was deemed inadequate for analysis, additional anal swabs were collected. Laboratory staff was blinded to the cervical HPV results of the patients.

Statistical analysis

Statistical analyses were conducted using SPSS 1.0.1 and R software (with the EasyR plugin). The distribution of continuous variables was assessed using the Shapiro-Wilk test to determine normality. Parametric t-tests were applied to normally distributed continuous variables, while nominal variables were analyzed using Pearson's chi-square or Fisher's exact tests when applicable. Continuous variables were presented as the mean (SD), and categorical variables were reported as the number of cases and their respective percentages. A *P*-value of less than 0.05 was considered statistically significant.

Results

Overall, 186 women who met the inclusion criteria were tested for active anal HPV infection of the anal canal. All patients had previously undergone colposcopic evaluation after being referred from the national HPV-based cervical cancer screening program. A diagnosis of high-grade or low-grade cervical lesions was extracted from the histopathological examination reports of the cervical biopsy materials and the colposcopic examination results. Since patients were initially referred after the cervical cancer-screening program, all of them had active cervical HPV infections. Baseline characteristics of the study population are presented in Table 1.

Table 1: Characteristics of the study population with respect to their cervical pathology

	Low-grade cervical intraepithelial lesion (n=64)	High-grade cervical intraepithelial lesion (n=122)	P-value
Age (years)	45.3 (9.3)	43.8 (8.8)	0.302
Parity	1.8 (0.9)	2.0 (1.0)	0.153
Presence of menopause	24 (37.5%)	40 (32.8%)	0.520
First coital age (years)	19.9 (3.8)	20.4 (4.3)	0.582
Active smoking	15 (23.4%)	47 (38.5%)	0.038

HPV: Human Papilloma Virus

Initially, 101 (54.3%) patients were referred with cervical HPV16/18 genotypes. Overall 96 (51.6%) women were

found to have active anal HPV infection. In women with active anal HPV infection, 31 (32.3%) were found to have only HPV 16/18 genotypes, and 22 had HPV16/18 along with other high-risk types. Distribution of the HPV genotypes in cervical and anal samples in women with and without active anal HPV infection is presented in Table 2.

The association of possible risk factors for active anal HPV infection including age, parity, presence of menopause, first coital age, presence of high-grade cervical lesion, and active smoking is summarized in Table 3. No association was found except for active smoking. Overall, 40.6% of the women with active anal HPV infection smoked; however, only 25.6% of the women without anal HPV infection were active smokers ($P=0.029$).

Table 2: Distribution of the HPV genotypes in cervical and anal samples in women with and without active anal HPV infection

	Active anal HPV infection (n=96)		No active anal HPV infection (n=90)	
	Cervical HPV test	Anal HPV test	Cervical HPV test	Anal HPV test
Only HPV 16/18	31 (32.3%)	22 (22.9%)	37 (41.1%)	0
Only other HPV types	43 (44.8%)	58 (60.4%)	42 (46.7%)	0
HPV 16/18 along with other HPV types	22 (22.9%)	16 (16.7%)	11 (12.2%)	0
Total	96 (100%)	96 (100%)	90 (100%)	0

HPV: Human Papilloma Virus

Table 3: Evaluation of risk factors for active anal HPV infection

	Active anal HPV infection (n=96)	No active anal HPV infection (n=90)	P-value
Age (years)	44.3 (9.3)	44.3 (8.6)	0.980
Parity	1.9 (1.1)	2.0 (0.9)	0.962
Presence of menopause	35 (36.5%)	29 (32.2%)	0.543
First coital age (years)	19.7 (4.1)	20.9 (4.2)	0.073
Presence of high-grade cervical lesion	66 (68.8%)	56 (62.2%)	0.349
Active smoking	39 (40.6%)	23 (25.6%)	0.029*

HPV: Human Papilloma Virus

Discussion

Anal HPV infection is a major risk factor for anal cancer. Since HPV screening programs have become more frequently used worldwide, the investigation of anal HPV in the management of patients diagnosed with cervical HPV infection has also emerged as an important issue for investigating the future risk of anal cancer in this group of women. However, in this population, there is no universally defined risk factor for anal HPV infection apart from anal intercourse. In this study, we aimed to investigate several possible risk factors for active anal HPV infection in heterosexual women without an anal intercourse history who were referred because of active cervical HPV infection. Our results documented a high risk of active anal HPV infection in this group of women. Possible risk factors were evaluated including age, parity, presence of menopause, first coital age, presence of high-grade cervical lesion, and active smoking. Only smoking was found to be associated with active anal HPV infection in the first year of high-grade or low-grade cervical lesion diagnosis.

D'Hauwers et al. [6] previously reported a similarly high rate (56.3%) of active anal infection in women referred to colposcopy. Interestingly, that study documented a relatively low anal intercourse history (16.9%), implying that most of the women having anal HPV infection may have other risk factors. However, there has been a lack of evidence for defining the risk

factors of anal HPV infection in women with low-risk sexual behavior (heterosexual and/or without anal intercourse)

Over the years, with the increasing application of HPV-based cervical cancer screening programs, data related to concurrent anal HPV infection in this group of patients has also started to increase. Another interesting finding among these results is that discrepancies can be relatively commonly observed between anal and cervical HPV genotypes. For example, Guler et al. [7] showed a partial concordance rate of 58.3% between the anal and cervical HPV genotypes. This result can be interpreted as follows: Some women may present with relatively low risk cervical HPV genotypes; however, they may have an active anal HPV infectivity with HPV16/18 that has the highest oncogenic potential [8]. There has been no prospective data to allow us to comment on the long-term consequences of this discrepancy in HPV genotypes. Another research topic about which we do not have sufficient information regarding its long-term effects is the importance of persistence of the anal HPV infection. Valari et al. [9] indicated that 12 months following surgical procedures for cervical intraepithelial neoplasia, 53% of the women tested negative for HPV in the cervix; yet among these, 25% continued to show HPV presence in the anus. There is not ample information about the significance of this finding in terms of long-term risk of developing anal cancer.

HPV vaccination, which is the primary and probably the definitive preventive strategy for anal cancer, is still underutilized in many countries and might not be beneficial for older individuals [10]. Therefore, many researchers have been seeking an effective anal cancer screening strategy that has the ability to reach the success of cervical cancer screening protocols. A recent meta-analysis regarding the accuracy of anal cancer screening modalities in different groups at a higher risk of anal cancer concluded that triage of high-risk groups with HPV testing can reduce referral to anoscopy with adequate sensitivity and specificity rates [11]. The main limitation in this meta-analysis is that it mainly included men who have sex with men (MSM), which does not provide information about our study population. The argument can be made that there is also a need for risk defining information regarding the heterosexual women without anal intercourse history but who have high-risk cervical HPV infection. The most frequently encountered group that needs assessing of anal cancer risk is, in fact, this group.

Several studies have investigated the role of smoking in HPV-related anal disorders. Umutoni et al. [12] focused on HIV negative men who have sex with women (MSW) group and reported that active smokers have a higher risk of anal HPV prevalence and persistence compared to non-smokers. Another study on men, however, documented that smoking is a risk factor for high-grade anal lesions but not for HPV infection [13]. A Hawaiian HPV cohort study also found that smoking is associated with longer persistence of anal HPV infection [14]. To our knowledge, no study has investigated the risk of anal HPV infection in heterosexual women without a history of anal intercourse and have been referred with high-risk cervical HPV infection. The strength of our study is that we focused on a homogeneous group of women who recently attended a screening program. The advantage of such a population is that

besides cervical cancer screening, anal cancer screening can easily be performed via anal HPV screening.

Limitations

The limitation of this study is the lack of prospective follow-up of this group of women, which is needed to draw definitive conclusions regarding the value of anal HPV testing in triage procedures. However, we documented that current smoking is a risk factor for active anal HPV infection in our study population. Further studies are needed to understand the clinical value of high-risk anal HPV infection for both the prognosis of cervical dysplasia and for the long-term risk of anal cancer in women diagnosed with cervical intraepithelial neoplasia. In this research, all patients meeting the inclusion criteria were admitted into the study. According to the results, the "1 - β " value calculated based on the occurrence rates determined in the power analysis was found to be 86%. This calculation indicates that the study has sufficient power. However, since this study was planned using a retrospective cohort design, prospective studies are needed for long-term follow-up results of these patients.

Conclusion

In conclusion, active anal HPV infection is common in patients with cervical intraepithelial neoplasia, even if they do not have a history of anal intercourse. Current smoking is the only identifiable risk factor for anal HPV infection in this group of women. Therefore, testing for anal high-risk HPV infection in women with cervical dysplasia may be valuable for defining the future anal dysplasia risk. Current smoking status should also be asked of these patients, since it is major risk factor for co-existing anal HPV infection. The co-existence of anal HPV infection has the potential to lead to a paradigm shift in the future, where anal cancer screening may be included in cancer screening programs for high-risk women, similar to HPV screening for cervical cancer. Prospective studies are needed to define the clinical value of anal HPV screening in women with cervical intraepithelial neoplasia.

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