Clinical Research

Evaluation of anal cytology and human papillomavirus infection in high-risk women: a cross-sectional study

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ABSTRACT

BACKGROUND Anal cancer incidence has been on the rise over the past few decades. This study aimed to assess anal Papanicolaou (Pap) smear changes in women with high risk for dysplasia and human papillomavirus (HPV) infection.

METHODS This cross-sectional study was conducted in 121 patients referred to the Gynecology Oncology Clinic of Imam Hossein Medical Center between 2020 and 2021 in Tehran, Iran, who had cervical and vulvar dysplasia, cervical HPV infection, and abnormal cervical cytology results and were over 21 years old. Data analysis was performed using SPSS software version 21 (IBM Corp., USA) at a significance level of 0.05.

RESULTS 121 women, with a mean age of 39.69 years, were included in this study. Overall, 23.1% of women had positive anal HPV results, and 35.5% were over 40 years old. Younger age was associated with an increased risk of anal HPV ($p = 0.045$). 33.9% of women were single and had a higher risk of anal HPV. Multiple sexual partnerships and anal sex were the significant risk factors for anal cancer ($p<0.001$). Women with positive anal HPV results had significantly more genital warts ($p<0.001$). No significant difference was observed in smoking, educational level, and cervical Pap smear results between women with negative and positive rectal HPV results.

CONCLUSIONS Younger age at diagnosis, being single, multiple sexual partnerships, anal sex, and genital warts were associated with anal HPV infection in women. Abnormal anal cytology was only associated with being single and having multiple sexual partners.

KEYWORDS anal neoplasms, cytology, human papillomavirus, Papanicolaou test, uterine cervical neoplasms

Anal neoplasms are rare malignancies, accounting for only 4% of lower gastrointestinal tract neoplasms.[1] However, their incidence has doubled in the United States in the past 20–30 years, with 9,000 new cases diagnosed in 2021.[2,3] Anal Papanicolaou (Pap) smear and cytology are commonly applied for screening due to their high sensitivity, while high-resolution anoscopy is the main diagnostic tool due to its high specificity.[4–6]

Although anal cancer is rare, it is more prevalent in high-risk groups such as men who have sex with men (MSM), HIV-positive women, immunosuppressed patients, and women with a history of cervical cancer.[6,7]

Studies have identified several risk factors for anal neoplasms, including human papillomavirus (HPV) infection, anal sex, and immunosuppression.[8] However, most of these studies were conducted on the MSM population, with limited studies on women. Previous studies have suggested HPV as the primary cause of anal cancer in up to 90% of cases, with sexual transmission as the primary route of infection. Moreover, anal HPV infection was similar to cervical infection; therefore, the cervix may be a reservoir for anal infection and *vice versa*.[9,10] Moreover, recent evidence has demonstrated that the prevalence of HPV 16 in anal cancer was higher than other types of high-risk HPV (HR-HPV). Although in low-grade and high-grade lesions, other types of HR-HPV also accounted for a high proportion.[11]

Anal intraepithelial neoplasia (AIN), which can progress to squamous cell carcinoma (SCC) or invasive anal carcinoma, is a precursor of dysplasia, with HR-HPV responsible for up to 90% of cases, including HPV16 and HPV18. AIN is categorized into grades 1 (AIN 1), 2 (AIN 2), and 3 (AIN 3), with AIN 3 posing the greatest risk of progression to cancer. Anal cytology and biopsies are crucial in detecting anal dysplasia and neoplasms, as the presentation can range from no signs to genital warts or acetowhite lesions.[1,6] Hence, this study aimed to assess anal cellular cytology and HPV infection in women with cervical and vulvar dysplasia, cervical HPV infection, and abnormal cervical cytology results.

METHODS

Study design and population

This cross-sectional study was conducted in 121 patients over the age of 21 years referred to the Gynecology Oncology Clinic of Imam Hossein Medical Center between 2020 and 2021 in Tehran, Iran, who were under surveillance for abnormal Pap smear results (including atypical squamous cells suspicious for high-grade squamous intraepithelial lesion [ASC-H], low-grade squamous intraepithelial lesion [LSIL], and high-grade squamous intraepithelial lesion [HSIL]), cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia, and HR-HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, and 82). Patients with a history of colorectal cancer, cognitive disorder, or pregnancy were excluded from the study. This study was conducted following the World Medical Association's Declaration of Helsinki. Patients provided formal consent after obtaining approval from the Research Ethics Committees of the School of Medicine – Shahid Beheshti University of Medical Sciences (ID: IR.SBMU.MSP.REC.1400.577).

Anal cytology was performed using the liquid-based method by inserting a brush into the anal hole and rotating it three to five times to obtain sufficient cells for cytology. Samples were then sent to a laboratory for analysis. Data including anal intercourse, number of sexual partners, genital warts, education, marital status, and smoking history were extracted from the patient's medical records. Patients with abnormal anal cytology results were referred to the Colorectal Oncology Research Center for anoscopy and biopsy.

Statistical analysis

The normality of continuous variables was determined using the Kolmogorov–Smirnov or Shapiro–Wilk tests, and means (standard deviations) and medians (interquartile ranges) were reported accordingly. Qualitative values were reported as frequency (percentage). Comparison of variables between groups with abnormal anal HPV and Pap smear results was made using analysis of variance (ANOVA) and chi-square tests. The ANOVA test determined significant differences between two or more categorical data sets with normal distributions. The significance level was set at $p<0.05$ for all tests, and data analysis was done using SPSS software version 21 (IBM Corp., USA).

RESULTS

The study enrolled 121 high-risk women aged between 21–80 years (mean age: 39.69 years). Of these, 28 women (23.1%) tested positive for anal HPV and were compared with the negative HPV population regarding their demographic information and risk factors (Table 1). The majority of women were over 40 years of age, and a younger age was associated with a higher risk of anal HPV ($p = 0.045$). Single women had a higher risk of anal HPV ($p = 0.006$). This study also found that 13 (10.7%) women had multiple sexual partners, with 10 of these had positive anal HPV test results. Therefore, multiple sexual partnerships were identified as an important risk factor for anal cancer ($p<0.001$).

Anal sex and genital warts were the significant risk factors for positive anal HPV results. However, smoking status, educational level, and Pap smear results had

Table 1. Demographic characteristics of the patients and risk factors for abnormal anal HPV and cytology results

Variables	Total, n (%)	Anal HPV, n (%)		*p*	Anal Pap smear, n (%)		*p*
		Positive	Negative		Positive*	Negative†	
Age (years)				**0.045**			0.420
≤40	78 (64.5)	23 (29.5)	55 (70.5)		5 (6.4)	73 (93.6)	
>40	43 (35.5)	5 (11.6)	38 (88.4)		1 (2.3)	42 (97.7)	
Marital status				**0.006**			0.406
Single	41 (33.9)	16 (39.0)	25 (61.0)		3 (7.3)	38 (92.7)	
Married	80 (66.1)	12 (15.0)	68 (85.0)		3 (3.8)	77 (96.2)	
Multiple sexual partnerships				**<0.001**			**0.016**
Yes	13 (10.7)	10 (76.9)	3 (23.1)		3 (23.1)	10 (76.9)	
No	108 (89.3)	18 (16.7)	90 (83.3)		3 (2.8)	105 (97.2)	
Anal sex				**<0.001**			**0.001**
Yes	41 (33.9)	26 (63.4)	15 (36.6)		6 (14.6)	35 (85.4)	
No	80 (66.1)	2 (2.5)	78 (97.5)		0 (0)	80 (100.0)	
Smoking status				0.364			0.070
Yes	37 (30.6)	11 (29.7)	26 (70.3)		4 (10.8)	33 (89.2)	
No	84 (69.4)	17 (20.2)	67 (79.8)		2 (2.4)	82 (97.6)	
Genital warts				**<0.001**			0.184
Yes	43 (35.5)	20 (46.5)	23 (53.5)		4 (9.3)	39 (90.7)	
No	78 (64.5)	8 (10.3)	70 (89.7)		2 (2.6)	76 (97.4)	
Education				0.245			0.682
≤Diploma	57 (47.1)	10 (17.5)	47 (82.5)		2 (3.5)	55 (96.5)	
>Diploma	64 (52.9)	18 (28.1)	46 (71.9)		4 (6.3)	60 (93.7)	
Cervical Pap smear				0.643			0.839
ASC-H	2 (1.6)	0 (0)	2 (100.0)		0 (0)	2 (100.0)	
ASCUS	13 (10.7)	2 (15.4)	11 (84.6)		1 (7.7)	12 (92.3)	
HSIL	4 (3.3)	1 (25.0)	3 (75.0)		0 (0)	4 (100.0)	
LSIL	6 (4.9)	0 (0)	6 (100.0)		0 (0)	6 (100.0)	

ASC-H=atypical squamous cells suspicious for high-grade squamous intraepithelial lesion; ASCUS=atypical squamous cells of undetermined significance; HPV=human papillomavirus; HSIL=high-grade squamous intraepithelial lesion; LSIL=low-grade squamous intraepithelial lesion; Pap=Papanicolaou

*Changes in squamous cells that line the anus; †no cell changes were found in the cervix

no significant association with anal HPV infection. In addition, abnormal anal HPV and abnormal anal cytology were not significantly associated with cervical abnormalities (p = 0.2237 and p = 1, respectively). Similarly, no significant difference was found between the positive and negative anal HPV groups regarding cervical Pap smear results (p = 0.643). Among the 28 women tested positive for anal HPV, 25 (89%) were also positive for cervical HPV, with 14 (56%) of them having HPV16, as detected by the cervical HPV test. The details of anal HPV-positive patients are presented in Table 1.

Six patients with abnormal anal cytology were referred to the colorectal clinic for anoscopy and biopsy. One of these patients with Pap smear cytology of LSIL and ASC-H in anoscopy and biopsy had a small high-grade anal lesion treated locally with trichloroacetic acid.

In this study, multiple sexual partnerships (p = 0.016) and anal sex (p = 0.001) were two risk factors for positive anal smear results. However, no significant difference was found between women with negative and positive anal smear results regarding cervical Pap smear.

DISCUSSION

Of 121 high-risk women who were screened for anal cancer, 23.1% had abnormal anal HPV results. Younger women who had anal sex and genital warts were at higher risk. Abnormal anal HPV results were also more common in single women and those with multiple sexual partners.

Although anal cancer is rare, its prevalence has increased in the past three decades, with a higher incidence rate in women.[10] Anal cancers mostly include SCC, followed by adenocarcinomas and melanomas. Despite the success of cervical cancer screening and vaccination programs, the rising incidence of anal cancer requires preventive measures.[3,12] Screening programs for high-risk populations have been proposed, as anal cancer is preventable with a 5-year survival rate of 67% and a risk of metastasis.[2,4,6] Anal cancers have been associated with a history of cervical dysplasia, cervical cancer, and vulvar cancer. An interval of 4–16 years was proposed between the diagnosis of cervical cancer and the occurrence of anal cancer.[1] There was a persistent risk of anal cancer development for 25 years, with a hazard ratio of 2.9 in patients with CIN II and 4.2 in patients with CIN III.[3] The increasing incidence of anal cancer can be partly explained by the increased incidence rate and the absolute number of HIV-positive MSM, and renal transplant recipients are also likely to contribute to this trend. Future research is needed to determine whether screening programs benefit these risk groups.[13]

Although no significant association between anal cancer and fissures or hemorrhoids has been found, a prolonged viral infection of anal mucosa is a risk factor for anal dysplasia and cancer.[4,8] HPV infection in the anal mucosa might serve as a reservoir for HPV in cervical reinfection, although no history of anal sex was found in women with a history of cervical infection.[4] The most common viral etiologies in the anal mucosa are HPV16 and HPV18, followed by HPV33 and HPV31. Although sexual intercourse is the primary route of transmission, other routes, such as skin-to-skin contact, have also been proposed.[6] Several studies have investigated the association between anal sex and anal dysplasia, with varying results. In line with Tatti et al's[7] and Hosseini et al's [8] findings, this study found anal sex as a significant risk factor for anal cancer, with a significant association with anal dysplasia (p = 0.001) and positive anal HPV test results (p<0.001). However, Inthasorn et al[10] and Calore et al[14] found no significant association between anal intercourse and anal dysplasia.

Inthasorn et al[10] found that anal dysplasia was not associated with marital status or multiple sexual partnerships, although multiparity was associated with a higher rate of abnormal anal cytology. However, multiple sexual partnerships could increase the risk of anal cancer.[6,8,14] In this study, having multiple sexual partners was associated with abnormal anal cytology results (p = 0.016) and abnormal anal HPV results (p<0.001). Moreover, marital status was associated with a higher rate of anal HPV infection and considered a risk factor for anal cancer. Married participants had lower abnormal anal HPV results (p = 0.006) but not lower abnormal anal cytology results (p = 0.406). The risk of anal dysplasia may be increased due to genital dysplasia or a history of genital malignancy.

Hosseini et al[8] and Calore et al[14] found that patients with cervical HSIL had a higher rate of anal dysplasia. However, in this study, no significant association was found between abnormal cervical test results and abnormal anal cytology results. There was also no significant association between HSIL and a higher rate of anal dysplasia, although this might be due to the limited sample size.

Regarding age, Hosseini et al[8] and Inthasorn et al[10] found no association with anal dysplasia, while Moscicki et al[12] found a higher risk of anal cancer in younger CIN III patients at the time of diagnosis and a higher risk of 5 years progression after initial diagnosis. This study found that women under 40 had a higher risk of anal HPV infection (p = 0.045) but no significant association with abnormal anal cytology (p = 0.420).

Smoking has also been associated with abnormal anal cytology results and abnormal anal HPV results with prolongation of viral clearance.[8,14] Although smoking is a risk factor for anal cancer, Tatti et al[7] found that it was not associated with abnormal anal cytology (p = 0.070) or anal HPV (p = 0.364).

Tatti et al[7] and Hosseini et al[8] reported genital warts as important risk factors for abnormal anal cytology. Among the subjects in this study, genital warts were also significantly associated with abnormal anal HPV results; however, no significant association was found between genital warts and abnormal anal cytology results. The difference in study design and statistical analysis method may cause this difference. In this study, the assessment of educational level among patients revealed no significant association between

education and anal dysplasia and anal HPV infection. Higher education was hypothesized to reduce the incidence of anal dysplasia due to greater awareness of sanitary precautions, but this was not proven.

This study's limitations included the lack of causal relationship due to the cross-sectional and single-center design and limited number of subjects with abnormal anal Pap result. In conclusion, younger age at the time of diagnosis based on abnormal HPV/Pap smear co-test results, being single, multiple sexual partnerships, anal sex, and genital warts were associated with abnormal anal HPV results in high-risk women. In contrast, abnormal anal cytology was only associated with being single and having multiple sexual partners.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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REFERENCES

1. Saleem AM, Paulus JK, Shapter AP, Baxter NN, Roberts PL, Ricciardi R. Risk of anal cancer in a cohort with human papillomavirus-related gynecologic neoplasm. Obstet Gynecol. 2011;117(3):643–9.
2. National Cancer Institute. Cancer Stat Facts: Anal Cancer [Internet]. National Cancer Institute; 2022 [cited 2022 Oct 20]. Available from: https://seer.cancer.gov/statfacts/html/anus.html.
3. Stier EA, Chiao EY. Anal cancer and anal cancer precursors in women with a history of HPV-related dysplasia and cancer. Semin Colon Rectal Surg. 2017;28(2):97–101.
4. van Eer K, Laâbi I, van Benthem BH, Steenbergen RDM, King AJ, Medical Microbiological Laboratories and the Public Health Services: Medical Microbiological Laboratories: Certe; et al. The association between viral load and concurrent human papillomavirus infection at the genital and anal sites of young women and the impact of vaccination. Tumour Virus Res. 2022;13:200233.
5. Aguiar C, García L, Boccardo MB, Vassel M, Arriola A, Jaumandreu S, et al. Anal PAP, HPV tests and magnifying chromoendoscopy with biopsies in the diagnosis of anal intraephitelial neoplasia. J Coloproctology. 2020;40(3):220–6.
6. Krzowska-Firych J, Lucas G, Lucas C, Lucas N, Pietrzyk Ł. An overview of human papillomavirus (HPV) as an etiological factor of the anal cancer. J Infect Public Health. 2019;12(1):1–6.
7. Tatti S, Suzuki V, Fleider L, Maldonado V, Caruso R, Tinnirello Mde L. Anal intraepithelial lesions in women with human papillomavirus-related disease. J Low Genit Tract Dis. 2012;16(4):454–9.
8. Hosseini MS, Khosravi D, Farzaneh F, Ebrahimi A, Arab M, Ashraf Ganjoie T, et al. Evaluation of anal cytology in women with history of abnormal pap smear, cervical intraepithelial neoplasia, cervical cancer and high risk hpv for anogenital dysplasia. Asian Pac J Cancer Prev. 2018;19(11):3071–5.
9. Lin C, Slama J, Gonzalez P, Goodman MT, Xia N, Kreimer AR, et al. Cervical determinants of anal HPV infection and high-grade anal lesions in women: a collaborative pooled analysis. Lancet Infect Dis. 2019;19(8):880–91.
10. Inthasorn P, Wetpithayakom N, Laiwejpithaya S, Lohsiriwat V, Jaishuen A, Viriyapak B. Prevalence of abnormal anal cytology in women with abnormal cervical cytology. Asian Pac J Cancer Prev. 2021;22(7):2165–9.
11. Lin C, Franceschi S, Clifford GM. Human papillomavirus types from infection to cancer in the anus, according to sex and HIV status: a systematic review and meta-analysis. Lancet Infect Dis. 2018;18(2):198–206.
12. Moscicki AB, Darragh TM, Berry-Lawhorn JM, Roberts JM, Khan MJ, Boardman LA, et al. Screening for anal cancer in women. J Low Genit Tract Dis. 2015;19(3 Suppl 1):S27–42.
13. van der Zee RP, Richel O, de Vries HJ, Prins JM. The increasing incidence of anal cancer: can it be explained by trends in risk groups? Neth J Med. 2013;71(8):401–11.
14. Calore EE, Giaccio CM, Nadal SR. Prevalence of anal cytological abnormalities in women with positive cervical cytology. Diagn Cytopathol. 2011;39(5):323–7.