Original Article

Value of human papillomavirus typing for detection of anal cytological abnormalities

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Abstract

Purpose: The objective of this study was to evaluate anal cytology and human papillomavirus (HPV) typing in patients with human immunodeficiency virus infection. **Materials and Methods:** Anal samples were collected from 61 patients (44 men and 17 women) and analyzed by PapilloCheck test and conventional cytology. **Results:** Of all anal samples, 37.7% had cytological abnormalities, 47.54% were negative and 14.75% were unsatisfactory. High-risk HPV, multiple high-risk HPV and HPV 16 infection was detected in 91.13%, 78.26% and 47.82% of the samples with cytological abnormalities and in 47.54%, 6.89% and 3.44% of the negative samples, respectively. High-risk HPV infection was significantly more frequent in anal samples with cytological abnormalities than in negative samples ($P = 0.0005$, Fisher's test), particularly multiple high-risk HPV infection ($P < 0.0001$) and HPV 16 infection ($P = 0.0002$). **Conclusions:** High-risk HPV, multiple high-risk HPV and HPV 16 infections are significantly associated with anal cytological abnormalities. Furthermore, the frequency of HPV infection in anal cytological samples suggests that high-risk HPV detection has high sensitivity, but low specificity for detection of anal cytological abnormalities, but multiple high-risk HPV typing and HPV 16 typing have a lower sensitivity and high specificity. Results suggest that HPV typing may be useful as an adjunct to cytology to screen patients for high-resolution anoscopy and biopsy.

Key words: Anal, cytology, human immunodeficiency virus, human papillomavirus, human papillomavirus typing

INTRODUCTION

Anal squamous cell carcinoma (SCC) is an uncommon cancer in the general population, but is recognized as an important source of morbidity and mortality in human immunodeficiency virus (HIV)-infected patients after the antiretroviral therapy era. The risk of anal cancer is about 30 times higher among HIV-infected patients as compared with non-HIV-infected persons.[1] Men who have sex with men (MSM) appear to be at highest risk, but other HIV-infected men and women are still at higher risk compared with HIV-uninfected individuals.[2] The development of anal intraepithelial neoplasia (AIN) and invasive SCC is associated mainly with high-risk or oncogenic human papillomavirus (HPV) infection.[3] There are many similarities between anal and cervical cancer, but no guidelines exist for anal cancer screening.[4] The commonly proposed screening methods for detecting AIN include digital rectal examination, anal visual inspection and cytological testing after the diagnosis of HIV infection.[4] Abnormal cytological results should be investigated using high-resolution anoscopy (HRA) and biopsy of suspicious lesions.[5]

Access this article online

Quick Response Code:

Website: www.ijstd.org

DOI: 10.4103/0253-7184.120540

How to cite this article:
Maia LB, Marinho LC, Barbosa TW, Velasco LF, Costa PG, Carneiro FP, *et al*. Value of human papillomavirus typing for detection anal cytological abnormalities. Indian J Sex Transm Dis 2013;34:102-6.

Cytology is an easily performed diagnostic test with a low operational cost and may be very useful to identify HPV-related cytopathic changes as well as inflammatory and neoplastic microscopic lesions.[6] The sensitivity of anal cytology for high-grade AIN, using atypical squamous cells of undetermined significance (ASC-US) as the threshold for triage to HRA, is considered as high, but its specificity is low.[7] Unlike in cervical cancer, the role of HPV testing in screening of anal cancer is not clear.[4] In this context, the objective of this study was to evaluate anal cytology, HPV detection and typing in patients with HIV infection with no visible lesions detected during external visual examinations.

MATERIALS AND METHODS

This study enrolled 61 adult (older than 18 years) HIV-positive men and women, with no visible lesions on external examination, who were treated at the Infectious Diseases Service of the University Hospital of Brasília, Brazil. Patients were invited to participate in this study and those who agreed were asked to sign an informed consent form. This study was approved by the University of Brasilia Research Ethics Committee. Patients provided the following data: Age, sex, latest CD4 count and duration of HIV infection, antiretroviral treatment and practice of anal sex, number of partners per year, drinking and smoking status.

Cytology

Samples were obtained first for conventional cytology and then for HPV typing. Endocervical brushes were used to collect samples for conventional cytology. The technique used for collection consisted of introducing the brush 4 cm into the anal canal and rotating it.[8] The slides were fixed in alcohol and stained using the Papanicolaou method. Cytological abnormalities were classified according to the 2001 Bethesda criteria: Negative for malignancy; ASC-US; low grade squamous intraepithelial lesion; ASC - cannot exclude a high-grade lesion; and high-grade squamous intraepithelial lesion and invasive carcinoma.[9]

HPV typing using microarrays

A PapilloCheck® kit (Greiner Bio-One) was used for molecular analysis using the microarrays.[10] Microarrays for deoxyribonucleic acid (DNA) genotyping may also be called DNA chips, biological chips or biochips. Samples for HPV genotyping were stored in specific fixing agents provided by the manufacturer. The collection technique was the same used for conventional cytology. The test detects 24 HPV subtypes, 6 of which are low-risk (6, 11, 40, 42, 43, 44/55) and 18, high-risk (16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 51, 39, 68, 73, 82, 53, 66 and 70). Positive results were those with a viral load (signal-to-noise ratio [SNR]) greater than 20. Results were expressed qualitative and semiquantitatively (SNR) for the 24 low- and high-risk HPV types simultaneously. SNR values lower than 100 indicate a small number of viral copies per cell, which might be a sign of initial infection or spontaneous remission. High SNR values suggest a greater probability of recurrence or persistent infection.

Statistical analysis

The Prism 4 software (GraphPad Software, San Diego, CA) and the Fisher test were used for statistical analyses. The level of statistical significance was $P < 0.05$.

RESULTS

Samples were collected from 61 patients (44 men, 72.13%; 17 women, 27.86%). Clinical data on age, duration of HIV infection, antiretroviral treatment, practice of anal sex, number of partners per year, drinking, smoking and CD4 counts are shown in Table 1.

HPV (high- and/or low-risk) was found in 65.57% (40/61) of the samples, at a frequency of 63.63% (28/44) for men and 70.58% (12/17) for women. Of all samples, 60.65% (37/61) had at least one high-risk HPV. In patients who engaged in anal sex, 67.56% (25/37) of the samples had at least one high-risk HPV, versus 50% (12/24) among those who did not engage in this practice. Multiple high-risk HPV infection (more than one high-risk HPV type) was found in 20/61 (45.2%) samples. The most frequent high-risk types were HPV 16 (12/61, 19.67%), HPV 56 (9/61, 14.75%), HPV 68 (9/61, 14.75%) and the most frequent low-risk, HPV 44/55 (15/61, 24.59%).

On cytology, 37.7% (23/61) of anal samples had cytological abnormalities, 47.54% (29/61) were negative and 14.75% (9/61) were unsatisfactory. Samples with cytological abnormalities were observed in 29.41% (5/17) of women and in 40.90% (18/44) of men. High-risk HPV infection was observed in 91.13% (21/23) of the samples with cytological abnormalities and in 47.54% (13/29) of the negative samples. The frequency of high-risk HPV according to cytology results is shown in Table 2. Multiple high-risk HPV infection was observed in 78.26% (18/23) of the samples with cytological abnormalities and in only 6.89% (2/29) of the negative samples. High-risk HPV infection with SNR values > 100

was observed in 82.6% (19/23) of the samples with cytological abnormalities and in 20.68% (6/29) of the negative samples. There was a significant association between the presence of cytological abnormalities and high-risk HPV infection ($P = 0.0005$, Fisher test), particularly in the samples with multiple high-risk HPV infection ($P < 0.0001$, Fisher test) and with SNR values > 100 ($P < 0.0001$, Fisher test). Low-risk HPV was found in 47.82% (11/23) of the samples with cytological abnormalities and in 31.03% (9/29) of the negative samples, but this difference was not significant. There was no significant association between cytological abnormalities and low-risk HPV infection. The most frequent high-risk type observed in samples with cytological abnormalities was HPV 16 (11/23, 47.82%) and almost all (91.66%, 11/12) of the samples with HPV 16 had cytological abnormalities. HPV 16 was detected in only 3.44% (1/29) of the negative samples. A significant association was observed between the presence of cytological abnormalities and HPV 16 infection ($P = 0.0002$, Fisher test). The frequency of HPV types according to cytological findings is shown in Table 3.

Table 1: Clinical patient data

Clinical data	*n* (%)
Sex	
Men	44 (72.13)
Women	17 (27.86)
Patient age in years	
18-20	1 (1.63)
20-29	2 (3.27)
30-39	22 (36.06)
40-49	29 (47.54)
≥50	7 (11.47)
Time of HIV infection in years	
≤1	4 (6.55)
2-5	17 (27.86)
6-10	23 (37.70)
>10	17 (27.86)
Number of partners per year	
≤1	33 (54.09)
2-5	12 (19.67)
6-10	9 (14.75)
>10	7 (11.9)
Practice of anal sex	37 (60.65)
Use of antiretroviral therapy	57 (93.44)
Smoking	20 (32.78)
Drinking	21 (34.42)
Number of CD4+/mm^3	
<200	8 (13.11)
200-500	24 (39.34)
>500	29 (47.54)

HIV=Human immunodeficiency virus; CD=Cluster of differentiation

Table 2: Frequency of high-risk HPV according to results of cytology

High-risk HPV	I *n*=9	N *n*=29	ASC-US *n*=9	ASC-H *n*=0	LSIL *n*=10	HSIL *n*=4
Positive	3	13	8	0	9	4
Negative	6	16	1	0	1	0

HPV=Human papillomavirus; ASC-US=Atypical squamous cells of undetermined significance; ASC-H=Atypical squamous cells, cannot rule out high-grade squamous intra-epithelial lesion; LSIL=Low grade squamous intraepithelial lesion; HSIL=High grade squamous intraepithelial lesion

DISCUSSION

In the present study, we used HPV DNA testing and cytology to diagnose HPV infection and cytological abnormalities in anal samples of HIV-infected patients without lesions on external anal examination. As in previous studies, most men and women were infected with high-risk HPV types and multiple high-risk HPV infection (more than one high-risk HPV type) was common in anal cytology samples.[11] High-risk HPV was detected mainly in samples of those patients who engaged in anal sex, but high-risk HPV was frequently detected even in those without this practice. The association between anal sex and HPV infection has not been clearly defined in the literature as some authors found relative prevalence of anal HPV regardless of the practice of anal sex.[12]

Table 3: Frequency of HPV types according to cytological findings

HPV types	Cytological abnormalities *n*=23	Negative *n*=29
High-risk		
16	11	1
56	7	2
68	5	4
39	5	1
70	5	1
59	4	1
51	4	0
53	3	1
18	3	0
31	3	0
66	2	1
45	2	0
33	2	0
52	2	0
73	2	0
58	1	2
35	1	1
82	0	1
Low-risk		
44\55	8	7
11	6	1
06	5	4
42	2	2
40	2	1
43	1	0

HPV=Human papillomavirus

Regarding HPV typing, the most frequent high-risk subtype detected in the present study was HPV 16, which is the most common oncogenic type in anal intraepithelial lesions and SCC, but other high-risk HPV subtypes such as HPV 56 and HPV 68 were also frequently detected.[13] Different high-risk types, such as HPV 58, HPV 51, HPV 52 and HPV 53, HPV 39, have also been detected frequently in other studies.[11] The frequency of low-risk HPV types on anal cytological samples has been varied and similar to the results reported herein, HPV 44/55, HPV 6 and HPV 11 have been frequently detected.[11]

Different tests for detection of high-risk HPV DNA have been used in anal biopsy and scraping specimens, with the Hybrid Capture II test and the PapilloCheck test being commercially available for routine diagnosis. The PapilloCheck assay may be considered a reliable screening test for HPV detection and typing.[14] The advantage of the PapilloCheck test is its ability to distinguish HPV types and to provide SNR values for each type. High SNR values suggest a greater probability of recurrence or persistent infection. Furthermore, the PapilloCheck test allows identification of multiple HPV infection.

The frequency of cytological abnormalities observed in the present study was consistent with previous investigations in which cytological abnormalities were observed in 38.7% and 46% of patients with no visible anal lesions.[15,16] Cytological interpretations do not always correlate with lesion severity and according to previous studies, patients with ASC-US or worse should be referred for anoscopy.[17]

The frequency of high-risk HPV infection on cytological samples (negative and with cytological abnormalities) observed in the present study suggests that high-risk HPV detection has high sensitivity, but low specificity for detection of anal cytological abnormalities. In agreement with these results, studies in MSM have shown high-risk HPV testing for triage of ASC-US or greater on anal cytology to be very sensitive, with a high negative predictive value (NPV); however, the specificity and the positive predictive value are low, because of the high prevalence of high-risk HPV in HIV-positive patients.[7]

In cervical cancer screening, high-risk HPV testing is combined with the Pap test to screen women aged 30 years and older and to screen patients with ASC-US cytology for colposcopic examination.[18] High-risk HPV may also be used post-colposcopy and post-treatment.[19] Unlike in cervical cancer, the role of HPV test as an adjunct test to cytology in screening of anal cancer has yet to be defined. Nevertheless, recent studies have shown that HPV DNA tests can be very useful to improve the sensitivity of cytology to detect AIN and in post-treatment and post-HRA follow-up, because of their excellent NPV.[20] Furthermore, according to the present results, the frequency of HPV 16 and multiple high-risk HPV infection on anal cytological samples (negative and with cytological abnormalities) suggests that HPV 16 and multiple high-risk HPV typing is useful because of its high specificity for detection of anal cytological abnormalities.

The limitations of this study included its small sample size and the fact that the authors have not yet completed follow-up of all patients.

CONCLUSION

This study demonstrated a high frequency of high-risk HPV infection in anal samples of HIV-positive men and women without visible lesions and a significant association between high-risk HPV infection and abnormal cytology. Furthermore, the results suggest that high-risk HPV detection has high sensitivity, but low specificity for detection of anal cytological abnormalities. Conversely, multiple high-risk HPV typing and HPV 16 typing have a lower sensitivity, but a high specificity for detection of anal cytological abnormalities. The results of the present study suggest that typing may be useful as an adjunct to cytology to screen patients for referral to HRA and biopsy. Further studies with a larger sample size should be conducted to validate screening methods for patients with HIV infection.

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Source of Support: Fundação de Apoio à Pesquisa do Distrito Federal (FAP-DF) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **Conflict of Interest:** None declared.