*Original Article*

# How deep must the brush be introduced in the anal canal for a more effective cytological evaluation?

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**ABSTRACT**

**Objective.** Anal cytology sensitivity varies widely in the literature, between 45% and 98%, which may occur due to the lack of standardization in terms of the distance the brush must be introduced into the anal canal. Therefore, our objective was to investigate if the site of collection influences the result of this exam.

**Methods.** We collected samples with the brush being introduced 4 cm in the anal canal of 114 patients (Group A) and 2 cm in other 94 patients (Group B) before the proctological exam. We performed five rotations with the brush before retrieving it and smearing it over the slide, subsequently submitting it to standard cytopathological examination. All the patients are HIV-infected. We submitted the results to statistical evaluation.

**Results.** In Group A, 39 patients had condylomata in the anal canal, and cytology was positive in 29 of them (74.3%). We also observed cytological alterations in 30 of 75 patients (40%) without clinical lesions in the anal canal. In Group B, there were 54 patients with condylomata in the anal canal and in 13 (24.1%) there was cytological confirmation. In other 40 patients, with no HPV-generated clinical lesions, we found that in nine (22.5%) there were cytological abnormalities. Statistical tests revealed that the exams done in the patients of Group A were more effective.

**Conclusion.** The collected specimens with more deeply inserted brushes in the anal canal had a more effective examination.

Key words: Papillomavirus infections. Carcinomas in situ. Cytology. Squamous cells. Anal canal.

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## Introduction

The incidence of infection with Human Papillomavirus (HPV) among patients with anal squamous-cell carcinoma varies between 67% and 95% in the literature.[1-4] Nevertheless, other factors appear to be involved in the genesis of anal carcinoma, such as practice of receptive anal sex, immunodepression, and tabagism.[5] The importance of immunological depression and human immunodeficiency virus (HIV) infection results from the observation that this anal tumor, which is more common in women over 50 years old,[6,7] has been showing a progressive increase in incidence among men over 30 and 40 years old.[8]

The anal canal tumors are more frequent than those of the anal margin[2,9] and its preferential localization is in the anal transition zone (ATZ).[10] This site involves the pectineal line and can be identified from 0.6 cm under up to 2 cm over this anatomical parameter.[11] Histologically, the ATZ presents areas of normal rectal mucosa and squamous epithelium, besides a typical picture with cells of various sizes, with palisade arrangements, with microvilli that tend to form columns.[12] It is suggested that it is a metaplastic squamous epithelium and that it contains endocrine cells in its deepest portion.[12]

High grade squamous intraepithelial lesions (HSIL), which precede anal squamous-cell carcinoma and have a clear association with oncogenic HPV, can be identified in the ATZ.[13] The risk of evolution to invasive carcinoma can be associated with higher grades of dysplasia[14] and, despite the lack of evidence,[15] it is believed that the treatment for these lesions may prevent the malignant transformation.[13,16,17]

Due to the possibility of detection of these precedent lesions, programs of standardized screening and treatment protocols for

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SIL should be created.[15] Thus, anal samples have been used in the Papanicolaou test (Pap test).[18-22] However, the sensitivity of the Pap test varied in the literature from 45% to 98%.[20,23,24] Such differences occurred, perhaps, due to the lack of standardization in the collection and in the choice of the best site in the anal canal to retrieve the samples. In various studies, the brushes were introduced two,[25] three,[23,26] or four[27,28] centimeters from the anal margin.

## Objective

We decided to conduct this study to assess if the collection site was influential in the accuracy of the anal Pap test among the HIV-positive patients.

## Method

This is a retrospective study approved by the Research Ethics Committee and by the Institute's Scientific Commission. We included HIV-positive adult patients with reported or present induced anal HPV lesions. The analysis of the medical records revealed that we collected the anal samples using brushes (cytobrush) introduced 4 cm in the anal canal of 114 of them (Group A) and 2 cm in other 94 patients (Group B) before performing the proctological exam. We divided each group according to the presence or not of HPV-induced clinical lesions in the anal canal.

Collection and smear technique: We introduced the brushes with rotating movements and spun them five times before retrieving them and smearing them over the slides. We performed this smear technique, rubbing the brushes over the slide, with rotating movements and making sure that the glass surface was completely covered. We placed the slides in plastic recipients containing 70-degree alcohol and sent them to the cytopathologist for the standard staining. After the complete proctological exam, including anoscopy, we divided the patients.

In the laboratory, the slides were submitted to the Pap test staining. The samples were considered satisfactory whenever we could identify squamous epithelium cells and cylindrical cells of the rectal mucosa. The findings were classified as: 1) normal; 2) *atypical squamous cells of undetermined significance* (ASCUS); and 3) low (LSIL) or high grade squamous intraepithelial lesions (HSIL).

We used the chi-square statistical test with 95% confidence intervals. Values of $p < 0.05$ were considered significant.

## Results

The introduction of the brush in the anal canal was well tolerated. None of the patients complained about anal pain or bleeding during the procedure, or in the days that followed it.

In Group A, 39 patients presented condylomata acuminated in the anal canal and 29 (74.3%) of them had a positive result in the Pap test (16 LSIL, 11 HSIL and two ASCUS). In the Group B, 54 had visible lesions in the anal canal, 13 of them (24.1%) with positive Pap test (five LSIL and eight HSIL). The statistical evaluation showed that the samples obtained with the brushes introduced 4 cm from the anal margin were more efficient ($p < 0.0001$). Besides that, we identified cytological alterations in 30 (40%) of the 75 patients of Group A without clinical lesions. There were seven HSIL, 19 LSIL, and four ASCUS. In Group B, among the 40 patients without clinical lesions, we observed abnormalities in nine (22.5%), with five of them being HSIL and four LSIL. There was no statistical difference ($p = 0.093$).

## Discussion

Standardization and refinement of the techniques to reveal the precedents of anal carcinoma are essential to choose the tests that will facilitate the diagnosis of these lesions, whose treatment may prevent the progression to invasive carcinoma.[18,22] The cytological abnormalities we observed in 40% of the patients without visible lesions lead us to suggest that anal cytology could be used for this screening, selecting patients for assessment of the anorectal region using a colposcope, acetic acid, and biopsies.

It is suggested now that most anal carcinomas have a biological pattern similar to that of the cervical carcinoma.[29] Similarly, the HPV possesses a special tropism through the squamous-columnar epithelium of the ATZ, as occurs in the uterine cervix. Thus, it seems logical that the anal samples obtained from this area may help us in the early detection of cytopathic lesions caused by the HPV.[30]

A study showed that blindly collected anal cytology identified more lesions than the biopsies obtained during the anal colposcopy using acetic acid.[22] The agreement between the results of the cytology and the biopsy was observed in 32% to 50% of the reports.[14,18] Inter- and intraobservers' significant disagreements were observed in the cytological interpretations of the SIL,[14] leading to restrictions related to the method. These facts have been used to justify the differences between the published data (45% to 98%).[20,23,24]

The main localization of the lesions is the ATZ, where there were six lesions for each one identified in the anal margin.[9] Besides that, in the same study, the authors reported that basaloid, mucoepidermoid, and squamous-cell carcinomas appear in the ATZ, and this area may also be the site of origin of the malignant melanomas.[9]

Taking into consideration that the pectineal line is located 2 cm from the anal margin and that the ATZ is up to 1 or 2 cm from this anatomical point, the brush should be introduced 4 cm in the anal canal. Various studies have suggested that the extension of the anal canal varies from 3.27 to 3.4 cm in men and from 2.52 to 2.93 cm in women,[31-34] which justifies the sample collection introducing the brush more deeply. Screening of the premalignant lesions of the anal canal's tumors should include the whole ATZ, according to what was proposed in the present study, and it is important to keep in mind that the lesions can be dotted or sparse.[9] Although many authors have introduced the brush up to 6 cm inside the anal canal, we believe that 4 cm are sufficient to obtain good samples of the patients' whole ATZ. On the other hand, with shorter-distance collections, many clinical and subclinical lesions will not be diagnosed.

## Conclusion

The results obtained in the present study, comparing samples collected for anal Pap test using brushes introduced 4 cm or 2 cm, allowed for the conclusion that the efficacy of the test is higher when the brush is more deeply introduced.

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