Conventional and liquid-based cytology (Liqui-PREP™) anal findings in male HIV anoreceptive patients in a referral University hospital in Rio de Janeiro, Brazil

Achados de citologia convencional e de citologia em base líquida (Liqui-PREP®) anal em pacientes masculinos HIV soropositivos anorreceptivos, em um hospital universitário de referência do Rio de Janeiro, Brasil

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ABSTRACT

Introduction: The anal lesions seem to have a natural history that closely resembles cervical lesions, with signs that precede the invasion. Cytological changes of anal epithelium induced by HPV can be detected through cytology, as it is considered an effective screening method. **Objective:** To identify the frequency of atypical epithelial conventional cytology results by comparing anal samples through Liqui-PREP™ technology in HIV-positive men. **Methods:** Cross-sectional descriptive and analytical study of 33 men who have sex with men (MSM), HIV-positive and anoreceptive attended at the Gaffrèe and Guinle University Hospital (HUGG), Rio de Janeiro, from June to July, 2016. Collection of anal samples for the conventional cytology and Liqui-PREP™ cytology was carried out. For significance of findings, Fisher exact test with 95% confidence interval was used and cytological Kappa index was employed for concordance between the two cytological methods. **Results:** The age ranged from 23 to 60 years (mean=39.06). The CD4 cell count was between 200 to 500/mm^3 on 16 (48.5%) and 13 (39.4%), and 50% was diagnosed with HIV for more than 6 years. In conventional cytology one case was considered unsatisfactory (3%). Among the cases considered satisfactory, 9 (28.1%) were diagnosed with ASC-US; 4 (12.5%) LSIL; 2 (6.3%) ASC-H, and 2 (6.3%) HSIL. Through Liqui-PREP™ method, 7 cases were considered unsatisfactory (21.2%). Among the satisfactory cases, 7 showed ASC-US (26.9%); 4 (15.4%) ASC-H; 2 (7.7%) LSIL; and 2 (7.7%) HSIL. The difference of unsatisfactory cases between both methods, although higher for Liqui-PREP™, was not statistically significant (p=0.054). The correlation was moderate (0503; p<0.006 [0.1765–0.8298]). **Conclusion:** The cytologic atypia is common among MSM HIV (+), and the anal conventional cytology and liquid by Liqui-PREP™ cytology are equivalent, although they are more unsatisfactory in the latter technique.

Keywords: anal neoplasm; cytological techniques; HIV; male homosexuality.

RESUMO

Introdução: As lesões anais parecem ter uma história natural, que se assemelha às de lesões de colo uterino, com sinais que precedem a invasão. As alterações citológicas do epitélio anal induzidas pelo HPV podem ser detectadas por citologia, um método de rastreio considerado efetivo. **Objetivo:** Identificar a frequência de atipias epiteliais nos resultados da citologia convencional comparando amostras anais pela tecnologia Liqui-PREP® em homens HIV positivos. **Métodos:** Estudo transversal, descritivo e analítico de 33 homens que fazem sexo com homens (HSH), HIV positivos e anorreceptivos atendidos no Hospital Universitário Gaffrèe e Guinle (HUGG), Rio de Janeiro, no período de junho a julho de 2016. Os pacientes foram submetidos à coleta de amostras anais para citologia convencional e citologia Liqui-PREP®. Para significância de achados, foi usado o teste exato de Fisher com intervalo de confiança de 95%, e para concordância entre os dois métodos citológicos, foi utilizado o índice de Kappa. **Resultados:** A idade variou de 23 a 60 anos (média=39,06). A contagem de células CD4 foi entre 200 e 500/mm^3 para 16 (48,5%) e 13 (39,4%) dos casos analisados, e 50% tinham o diagnóstico de HIV há mais de seis anos. Na citologia convencional, um caso foi considerado insatisfatório (3%). Entre os casos considerados satisfatórios, 9 (28,1%) foram diagnosticados como células escamosas atípicas de significado indeterminado possivelmente não neoplásicas (ASC-US); 4 (12,5%) como lesão intraepitelial de baixo grau (LSIL); 2 (6,3%) como células escamosas atípicas não sendo possível excluir lesão intraepitelial de alto grau (ASC-H) e 2 (6,3%) como lesão intraepitelial de alto grau (HSIL). Pelo método Liqui-PREP®, 7 casos foram considerados insatisfatórios (21,2%). Entre os casos satisfatórios, 7 como ASC-US (26,9%); 4 (15,4%) como ASC-H; 2 (7,7%) como LSIL e 2 (7,7%) como HSIL. A diferença de insatisfatório entre os métodos, embora maior para Liqui-PREP®, não foi estatisticamente significativa (p=0,054). A concordância foi moderada (0,503; p<0,006 [0,1765–0,8298]). **Conclusão:** É frequente a atipia citológica entre HSH HIV (+), e as citologias anal convencional e em meio líquido pela técnica Liqui-PREP™ se equivalem, embora sejam mais insatisfatórias na técnica citológica Liqui-PREP®.

Palavras-chave: neoplasias do ânus; técnicas citológicas; HIV; homossexualidade masculina.

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INTRODUCTION

Anal cancer represents 1 to 2% of all colon tumors, and 2 to 4% of all cancers affecting the large intestine[1]. It is considered the fourth most common type of cancer in the USA, and its incidence is steadily increasing over the last decades[2,3]. It is specially worrying among some population groups at risk, such as: transplanted, non-HIV chronic

immunodeficients, those with autoimmune diseases using medication, anoreceptive individuals of both sexes with sexually transmitted diseases, mainly Human Immunodeficiency Virus infection (HIV) and Human Papillomavirus (HPV), and chronic anal lesions carriers[4-6].

Anal lesions seem to have a natural history that resembles those of the cervix, with signs preceding the invasion. HPV-induced cytologic alterations of the anal epithelium can be detected by cytology, which is considered an effective screening method[7]. There are currently two cytological screening methods: the conventional cytology (CC) and the liquid-based cytology (LBC). Disadvantages with the conventional method include inadequate sample by desiccation of the material, cells obscured by multiple layers of cellular material, by blood, by inflammatory cells or by fecal contamination. LBC would have as characteristic the collected samples stored in a collector with preservative liquid allowing better preservation of the cytomorphological properties, less agglomeration and less obscuration of the cells. An additional advantage is the possible use of the residual material for molecular biology tests[8].

Specifically, the liquid-based cytology can be carried out by automated and non-automated methods, and in Brazil, ThinPrep (Hologic, Inc., Marlborough, MA) and SurePath (Becton, Dickinson and Company, Franklin Lakes, NJ) are available. Liqui-Prep™ is among the non-automated techniques[9].

The liquid-based cytology has been reported as a preferred method for the evaluation of anal swabs as it eliminates air-drying artifacts, fecal material, and bacteria commonly found in conventional anal smears, as well as inflammatory debris that may obscure cellular details, and offers cell performance increase and better preservation of the sample[10,11].

OBJECTIVE

To identify the frequency of epithelial atypia in the results of conventional cytology comparing with the Liqui-PREP™ cytology technology of anal samples in HIV-positive men who have sex with men (MSM) at a referral hospital in Rio de Janeiro, Brazil.

METHODS

A cross-sectional, descriptive and analytical study was carried out with 33 HIV-positive and anoreceptive men who have sex with men (MSM), aged 23–60 years, attended at the Immunology Outpatient Clinic of the Gaffrèe and Guinle University Hospital (HUGG) of UNIRIO in Rio de Janeiro, Brazil, from June to July, 2016.

The study was submitted and approved by the Research Ethics Committee of HUGG / UNIRIO.

Data collection

The medical records were analyzed for the collection of information, such as: age, CD4 lymphocyte count and time of HIV infection diagnosis.

Sample collection

After signing the informed consent form (ICF), the patients were placed in the left lateral decubitus position (Sims position) for the collection of material for conventional oncotic cytology (CC) and liquid base cytology (LBC). Endocervical brush was used.

1. The brush was introduced about 2 to 4 cm from the anal border, 3 to 4 clockwise rotating movements, and removed in spiral motion[12];
2. Shortly thereafter, for CC, the bristles tip of the brush was scrubbed over the entire surface of the previously identified clean opaque edge glass sheet. The slides were put down in 70% plastic bottles;
3. Then the brush bristles tip with the residual material was placed into the bottle (previously identified with patient name and number) with Liqui-PREP™ preservative liquid for the preparation of LBC following the instructions of the product.

The collected material was sent to the Cytopathology Laboratory of the HUGG / UNIRIO for preparation and analysis.

Reading the smears

The reading of the material was performed by four analyzers, under a conventional optical microscope, in increments of 100x and 400x. The Bethesda System (TBS)[13] was used to analyze the sample (anal transformation zone and squamous epithelium), adequacy (satisfactory and unsatisfactory) and interpretation of the material. The results were classified as follows: unsatisfactory (INS) or satisfactory (SAT) in the initial analysis. Following as a negative for intraepithelial lesion or malignancy (NLM), atypical squamous cells of undetermined significance, possibly non-neoplastic (ASC-US), atypical squamous cells of undetermined significance, it is not possible to rule out high-grade lesions (ASC-H), atypical glandular cells (AGC), low-grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (CCE), adenocarcinoma *in situ* (ADCIS) and invasive adenocarcinoma (ADCI)[13].

Statistics

For significance of findings, Fisher exact test with 95% confidence interval was used and cytological Kappa index was employed for concordance between the two cytological methods.

RESULTS

Age varied from 23 to 60 years (mean=39.1). Concerning CD4 cell counts, 4 (12.1%) cases had a count lower than 200/mm^3, 16 (48.5%) had a count between 200 and 500/mm^3 and 13 (39.4%) patients had a count greater than 500 cells/μL. Regarding the time of HIV infection among the patients studied, 50% had been diagnosed with HIV for more than 6 years (**Table 1**).

Through the conventional cytology method, 1 case was considered unsatisfactory (3%). Among the 32 (97%) cases considered satisfactory, 15 (46.9%) were NML; 9 (28.1%) were diagnosed as ASC-US; 4 (12.5%) as LSIL; 2 (6.3%) as ASC-H and 2 (6.3%) as HSIL. Through the Liqui-PREP™ method, 7 cases were considered unsatisfactory (21.2%). Among the 26 (78.8%) satisfactory cases, 11 (42.3%) were diagnosed as NML; 7 as

ASC-US (26.9%); 4 (15.4%) as ASC-H; 2 (7.7%) as LSIL, and 2 (7.7%) as HSIL (**Table 2**). The difference of unsatisfactory diagnosis among methods, although higher in Liqui-PREP™, was not statistically significant (p=0.054). However, by evaluating the agreement between the two methods using the Kappa test, it was possible to observe only a moderate concordance (0.503; p<0.006 [0.1765–0.8298]).

Evaluation of the results according to the age shows that patients over 35 years presented around 60% atypia in the anal cytology independently of the technique. Regarding CD4 lymphocyte counts, there was no significant difference between the diagnoses and time of HIV; those with more than 6 years of infection had a discrete and non-significant higher frequency of cytologic atypia (**Table 3**).

Table 1 – Profile of HIV (Human Immunodeficiency Virus) seropositive men who have sex with men (MSM), who underwent conventional anal cytology, and Liqui-PREP™ liquid medium at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

Data	n (%)
Age (years)	
23–35	11 (33.3)
>35	22 (66.7)
CD4 T cell count	
<200	4 (12.1)
200–500	16 (48.5)
>500	13 (39.4)
Time of infection with HIV* (years)	
<1	12 (37.5)
1–6	4 (12.5)
>6	16 (50)

*No information on HIV infection time was found in one patient.

DISCUSSION

Regarding the suitability of the sample, the liquid-based cytology presented 21.2% of unsatisfactory material, while the conventional cytology presented only 3%, and in both methods the cellular shortage was the reason. One possible justification is the fact that conventional cytology was the first to be made, and the remaining material stayed in the brush for cytology in liquid medium. A previous study[12] used the same methodology and the same liquid medium and also found greater unsatisfactory material in the liquid-based cytology compared to the conventional cytology. In another study using only the automated liquid-base technique, the material

Table 2 – Conventional anal cytology and Liqui-PREP™ liquid medium findings in seropositive for Human Immunodeficiency Virus (HIV) men, who have sex with men (MSM), who underwent anal cytology at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

Diagnosis	Conventional cytology n (%)	Liqui-PREP™ n (%)	p-value
Unsatisfactory	1 (3)	7 (21.2)	0.0539
Satisfactory	32 (97)	26 (78.8)	
NML	15 (46.9)*	11 (42.3)*	0.7945
Atypical	17 (53.1)*	15 (57.7)*	
ASC-US	9 (28.1)*	7 (26.9)*	
ASC-H	2 (6.3)*	4 (15.4)*	
LSIL	4 (12.5)*	2 (7.7)*	
HSIL	2 (6.3)*	2 (7.7)*	

*Percentage among the satisfactory cases considered for analysis; NML: Negative for malignancy and squamous intraepithelial lesion; ASC-US: atypical squamous cells of undetermined significance; ASC-H: atypical squamous cells, and cannot exclude high-grade intraepithelial lesion; LSIL: low-grade intraepithelial lesion; HSIL: high-grade intraepithelial lesion.

Table 3 – Conventional anal cytology and Liqui-PREP™ liquid medium findings according to age, CD4 lymphocyte count and time of infection among HIV-positive men, who have sex with men, who underwent anal cytology at the Gaffrèe and Guinle University Hospital, Rio de Janeiro, Brazil.

	NML		Atypical		Total		p-value (IC95%)
	CC n (%)	LBC n (%)	CC n (%)	LBC n (%)	CC* n (%)	LBC# n (%)	
Age							
≤35	7 (63.6)	3 (37.5)	4 (36.3)	5 (62.5)	11 (100)	8 (100)	
>35	8 (38)	8 (44.4)	13 (62)	10 (56.6)	21 (100)	18 (100)	ns
Total	15 (46.9)	11 (42.3)	27 (53.1)	15 (57.7)	32 (100)	26 (100)	
CD4 cell count							
<200	0	0	4 (100)	4 (100)	4 (100)	4 (100)	
200–500	9 (60)	7 (58.3)	6 (40)	5 (41.7)	15 (100)	12 (100)	ns
>500	6 (46.1)	4 (40)	7 (53.9)	6 (60)	13 (100)	10 (100)	
Total	15 (46.9)	11 (42.3)	17 (53.1)	15 (57.7)	32 (100)	26 (100)	
Time of infection with HIV (years)							
<1	5 (41.7)	2 (25)	7 (58.3)	6 (75)	12 (100)	8 (100)	
1–6	3 (75)	2 (66.7)	1 (25)	1 (33.3)	4 (100)	3 (100)	ns
>6	7 (43.7)	8 (53.3)	9 (56.3)	7 (46.7)	16 (100)	15 (100)	
Total	15 (46.9)	12 (46.1)	17 (53.1)	14 (53.9)	32 (100)	26 (100)	

*One case was considered unsatisfactory; #seven cases were considered unsatisfactory; CC: Conventional Cytology; LBC: Liquid-Based Cytology; ns: not significant.
The following cytologic results were considered atypical: ASC-US, ASC-H, LSIL and HSIL.

was considered unsatisfactory in 10.7% of cases due to a shortage of cells[14]. Eleutério et al.[11], studying immunocompetent women, did not report unsatisfactory cases using the SurePath™ technique.

About 60% of patients of this study have shown abnormal cytology (ASC-US or more) considering both methods. Several studies have shown that infected with HIV men who have sex with men presented a high prevalence of abnormal cytology. Salit et al.[15] have investigated 401 HIV-seropositive MSM, 67% of which showed abnormal cytology. In a Brazilian study, Silva et al.[16] observed that the highest prevalence (49.5%) was in HIV-seropositive MSM. Selvaggi[14] made a 10-year follow-up on studies that used anal cytology to track anal lesions in HIV-seropositive MSM, and cytology revealed 41 to 93% abnormality, most of which histologically confirmed[14-16].

Concerning the association between clinical data and positive results, patients over 35 years of age often presented more cytological atypia than those younger than 35 years. Studies have shown that anal cancer is much rarer at younger age, and that in the progression of high-grade anal lesions to carcinoma, the median age at diagnosis of cancer is around 51 years[17,18].

In the association with CD4 lymphocyte count, no significant difference between CC and LBC was found. The highest prevalence of altered samples was in patients with more than 500 cells/μL, 41.1 and 40%, with a little difference between the negative ones, 40 and 36.3%. In patients between 200–500 cells/μL count, the percentage of altered cytology was of 35.2 (CC) and 33.3% (LBC), the percentage of altered cytology was of 35.2 (CC) and 33,3% (LBC), and negative cytology was twice high, 60 (CC) and 63.6% (LBC). In patients below 200 cells/μL count, all cytologies were altered in both CC and LBC. Several studies relate the incidence of anal cancer with low CD4 count when associated with HPV infection[19]. Sendagorta et al. [20] reports that the risk of HIV-seropositive presenting anal lesions increases as CD4 decreases. Leeds and Fang[21] consider that the risk of progression of anal lesions is directly correlated with the degree of immunosuppression and with CD4 T cell counts.

Patients HIV infected for more than 6 years showed the highest positivity rates, 52.9 (CC) and 50% (LBC), and with one year or less: 41.1 (CC) and 42.8% (LBC) were nearly ten times more than the ones infected between 1 and 6 years: 5.8 (CC) and 7.8% (LBC). Crum-Cianflone et al.[22], in a cohort with 4,901 HIV-seropositive patients, noted that people infected for more than 15 years had 12 times more chances to develop anal cancer compared to those infected for less than 5 years, concluding that there is an association of high rates of anal cancer with the time of HIV infection, and emphasizing the urgent need to establish screening and prevention strategies for cancer.

CONCLUSION

The cytologic atypia is frequent among HIV (+) MSM, and the anal conventional cytology and liquid-based by Liqui-PREP™ cytology technique are equivalent, although with a small and not significant superiority of conventional cytology.

Conflict of interests

The authors declare no conflict of interests.

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