

Citologia esfoliativa convencional *versus* citologia em meio líquido para prevenção e diagnóstico precoce do carcinoma escamo celular (CEC) oral.

Conventional Cytology *versus* liquid-based cytology for prevention and early diagnosis of oral squamous cell carcinoma (OSCC)

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RESUMO

Objetivando avaliar a concordância, sensibilidade e especificidade diagnóstica dos métodos citológicos: convencional e em meio líquido, foi realizado um estudo de caso-controle, no Ambulatório de Cabeça e Pescoço do Hospital de Câncer de Pernambuco, sendo selecionados 182 indivíduos doentes e 179 sãos, no período de setembro de 2002 a janeiro de 2004, os quais, permitiram através de assinatura de termo de consentimento livre e esclarecido a realização de exames (clínico/ citológico esfoliativo - cytobrush) na área carcinomatosa e em área sã, constituindo, respectivamente, o grupo de casos e controles. Os dados obtidos foram analisados através dos softwares estatísticos SPSS (Statistical Package for Social Science) e Epi-Info versão 6.4. Os resultados evidenciaram que os métodos diagnósticos citológicos apresentaram entre os casos: sensibilidade= 96,9% (IC= 95%: 92,5% a 98,8%); especificidade= 75,0% (IC= 95%: 21,9% a 98,7%) e acurácia= 96,3% (IC=95%: 92,5% a 98,5%); e entre os controles: sensibilidade= 91,0% (IC=95%: 84,1% a 95,2%); especificidade = 57,5% (IC=95%: 41,0% a 72,6%) e acurácia= 82,7% (IC=95%: 76,3% a 88,0%). A citologia convencional e em base-líquida apresentaram concordância com o diagnóstico histopatológico em mais de 90%. Conclui-se, portanto, que, se bem indicado e executado, o exame citológico pode ser utilizado rotineiramente como exame complementar, pois ambos apresentaram alta sensibilidade e razoável especificidade, cabendo à citologia convencional maior especificidade para o diagnóstico das lesões orais.

Keywords:

Oral Squamous Cell Carcinoma (OSCC), Prevention, Diagnostic Methods, Conventional Cytology, Liquid-based Cytology.

ABSTRACT

The purpose of this study was to evaluate the sensitivity, specificity, and concordance between conventional cytology and liquid-based cytology. A case-control study was developed at the Head and Neck ambulatory service of Hospital do Cancer de Pernambuco. A hundred and eighty-two patients with primary Oral Squamous Cellular Carcinoma (OSCC) (case group) and 179 individuals with normal buccal mucosa (control group) were selected, from September 2002 to January 2004. They agreed to be submitted to clinical examination and exfoliative cytology of the oral cavity, by signing a document of consent. The data were analysed by Statistical Package for Social Science (SPSS) and Epi-Info 6.4 program. Among the cases, the cytologic methods demonstrated a sensitivity= 96.9% (IC= 95%: 92.5% to 98.8%), specificity= 75.0% (IC=95%: 21.9% to 98.7%), and accuracy= 96.3% (IC=95%: 92.5% to 98.5%). Among the controls the results were: sensitivity= 91.0% (IC=95%: 84.1% to 95.2%); specificity= 57.5% (IC=95%: 41.0% to 72.6%), and accuracy= 82.7% (IC=95%: 76.3% to 88.0%). Conventional cytology and liquid-based cytology demonstrated a diagnostic concordance with histopathology of more than 90%. In conclusion if properly indicated and executed, they can be routinely used as complementary diagnostic methods. When compared to each other, conventional cytology and liquid-based cytology showed a high sensitivity and reasonable specificity, conventional cytology having higher specificity.

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INTRODUCTION

Approximately 5% of all human cancers are localized in the mouth. More than 90% of them are squamous cell carcinomas that could be identified without the need for special techniques, since it is easily accessible by direct examination

of the oral cavity. They are responsible for 99% of the deaths from oral cancer^{1,2,3,4,5}.

The need to increase the number of cases diagnosed at early stages has to be recognized as essential in order to provide real benefit for the patients. In this manner, the attention should be directed toward asymptomatic patients, so that an early diagnosis can be made^{6,7,8}.

Conventional cytology, whose criteria were established by Papanicolaou⁹, is still widely employed. Many authors^{7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25} defend it as a very useful diagnostic method in stomatology and in the study of material found on the surface of lesions. The latter helps clarify lesions found in pemphigus, herpes simplex, paracoccidioidomycosis, actinomycosis, and candidiasis.

Exfoliative cytology is a simple method that could be added to the routine of all dental surgeons. This idea was defended by Christian²⁶, who developed a preventive program with participation of dental surgeons and dental hygiene technicians. Vidal et al⁸ also included dentist assistants and health agents in programs aimed at making early diagnosis, identifying risk factors, and teaching how to perform self-examination.

Diagnostic cytology has contributed to tracking precursor as well as malignant lesions, with the identification of potentially fatal cases and the decrease in the impact of potentially irreversible lesions. It has shown to be efficient in detecting atypia also in ulcerated and non-keratinizing lesions.

In countries (like Brazil) where preventive programs based on the Papanicolaou test have been largely implemented and continuously administered, the prevalence of cervical cancer decreased dramatically. As it has proved to be successful in the control of cervical cancer, we believe it can be employed in oral cancer for the same purpose.

Cytology in liquid medium (DNA-CITOLIQ), also referred to as "monolayer cytology", "thin-layer cytology", or preferentially "liquid sample cytology" or "liquid-based cytology", was idealized, developed and released in March 2002 by Digene do Brasil. The tests were conducted at UNIFESP and in eight Brazilian private laboratories. This method represents an improvement of Papanicolaou, and began a new era in cytology, connecting morphologic and biomolecular pathology. The studies performed on cervical samples demonstrated that the DNA-CITOLIQ System is sensitive for detecting epithelial lesions as well as microorganisms, with excellent results²⁷.

The purpose of this study was to evaluate and compare the sensitivity and specificity of conventional cytology and liquid-based cytology, as diagnostic tools employed in buccal malignant lesions and clinically normal mucosa.

MATERIALS AND METHODS

A case-control study was performed at the head and neck ambulatory service of Hospital do Câncer de Pernambuco, which is a reference center for oral cancer in the state of Pernambuco, serving rural, urban areas, and neighboring states.

The case group included 182 patients with squamous cell carcinoma in the oral cavity (CID-O 140-145)^{28,29}. They were diagnosed according to the WHO classification of tumors, which describes it as a malignant epithelial tumor with squamous cell differentiation, presenting microscopically with cells that resemble keratinocytes, intercellular bridges and/or keratinization^{30,31} (Figure 1). None of the selected patients had started treatment and none presented with other neoplasia elsewhere. The patients spontaneously agreed to participate in this study and signed a document of consent.

The control group included 179 clinically healthy individuals that were either relatives or neighbors of the case group. They spontaneously agreed to participate in this study and signed a document of consent.

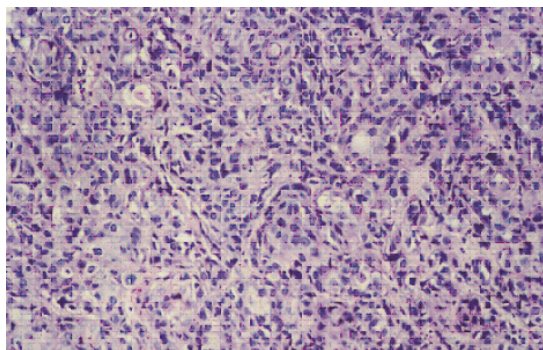


Figure 1. Photomicrography of histopathologic material from the left lateral border of the tongue, shown to be a well differentiated, keratinizing SCC (H.E. 100X).

Clinical and laboratorial procedures performed on both groups

History and physical examination were performed. The latter included careful examination of the oral cavity and was followed by exfoliative cytology (cytobrush) of the areas previously diagnosed as squamous cell carcinoma, in the case group, and of the correspondent clinically normal areas, in the control group. The smears were prepared on properly identified slides and immersed in absolute alcohol. The processing (Papanicolaou staining, preparation of slides) and conventional microscopic interpretation took place at Centro Integrado de Anatomia Patológica (CIAP) – HUOC/UPE. Right after submitting the first sample for conventional cytology evaluation, a second sample was collected for liquid-based[®] cytology and accommodated in the specific Digene kit (with a solution that conserves DNA), known as Universal Collection Medium – UCM[®]. The second samples were sent to Laboratório Digene do Brasil, São Paulo, for processing.

The material was collected in loco by a technician who was previously evaluated by the Kappa test, described in Andrade and Zicker³². She demonstrated an excellent intra (Kappa= 0.854) and inter-examiner (Kappa= 0.869) reliability. The conventional cytology and liquid-based[®] cytology interpretations were carried out by a cytopathologist (examiner 1) who received training in DNA-Citoliq at Centro de Treinamento Digene (CTD). She was also evaluated by the Kappa test for intra and inter-examiner (with 2 other cytopathologists) reliability in conventional cytology. A specific cytogram was used.

Each interpretation, both in conventional and liquid-based cytology, were performed independently, with no sharing of results.

In conventional cytology, the intra-examiner (examiner 1: gold-standard) concordance was perfect (100%) in the case group, and excellent in the control group (Kappa= 0.908). In liquid-based[®] cytology, the concordance was excellent both in the case (kappa= 0,869) and control group (Kappa= 0.901).

In the case group, the conventional cytology inter-examiners agreement between examiners 1 and 2 was excellent (kappa= 0,805); good between 1 and 3 (Kappa= 0.726), and excellent between 2 and 3 (Kappa= 0.909). In the control group, examiner 1 showed good concordance with examiners 2 and 3 (Kappa= 0.713 for both); the concordance between examiners 2 and 3 was also good (Kappa= 0.608).

Laboratorial methods

Liquid-based cytology has not been proposed as a substitute for conventional cytology, but rather as an improvement

of the Papanicolaou test. It is a product that aggregates the basic principles of liquid-based[®] cytology, with a reduced operational cost and a fast and reproducible protocol. Similarly to the cytology applied in the uterine cervix, the goal is to detect precursor lesions and cancer in the oral cavity.

The technician and cytopathologist (examiner 1) who participated in this study were trained at Centro de Treinamento da Digene (CTD) do Brasil, in São Paulo. The training included every step of processing, from using the pipette and preparing slides, to the final diagnostic evaluation. The criteria utilized are internationally recognized and are the ones applied in conventional cytology (The Bethesda System –TBS)³³.

Cytologic interpretation

1. Type of sample:

- 1.1. Conventional Cytology
- 1.2. Liquid medium[®] cytology

2. Pre-analytical evaluation

Causes of rejection of the sample:

- 2.1. Lack of identification or misidentification of slides and/or slide recipients
- 2.2. Damaged or absent slides
- 2.3. Causes unrelated to the laboratory (specify)
- 2.4. Other causes (specify)

3. Adequacy of the sample:

- 3.1. Satisfactory
- 3.2. Unsatisfactory (specify)

4. Descriptive diagnosis

- 4.1. Within normal limits
- 4.2. Benign cellular alterations
 - 4.2.1. Inflammation
 - 4.2.2. Repair
 - 4.2.2.3. Immature squamous metaplasia
 - 4.2.4. Atrophy due to inflammation
 - 4.2.5. Radiation
 - 4.2.6. Others (specify)
- 4.3. Atypia
 - 4.3.1.1 Of squamous cells
 - 4.3.1.2. ASCUS: characterized by morphologic alterations of undetermined significance (Bethesda 2001)
 - 4.3.1.3. LSIL: Low grade squamous intraepithelial lesion (HPV and NIC I)
 - 4.3.1.4. HSIL: High grade squamous intraepithelial lesion (NIC II and III)
 - 4.3.1.5. High grade squamous intraepithelial lesion, unable to exclude invasion (in situ)
 - 4.3.1.6. Invasive squamous cell carcinoma

Results and discussion

Among the 182 cases, ten (5.5%) and eight (4.4%) samples were considered unsatisfactory on conventional cytology and liquid-based cytology, respectively. Out of the 179 controls, seven (3.9%) and ten (5.6%) samples were considered unsatisfactory on conventional cytology and liquid-based cytology, respectively. In this manner, in conventional cytology 17 (5.2%) samples were disregarded due to excessive hemorrhage, scarcity of cells, or inappropriate staining. In liquid-based[®] cytology, 18 (5.0%) samples were rejected for presenting scarcity of cells - perhaps caused by delay in pipetting, or conditions related to the patient himself or collect - and inappropriate staining (see tables 1, 2 and 3). However, this was not shown to be a statistically significant loss.

In the case group, conventional cytology and liquid-based

cytology showed diagnostic agreement with histopathology in 131 (76.2%), and 117 (67.3%) SCC cases, respectively. However, if HSIL diagnoses - which corresponds to carcinoma in situ - were considered positive for disease, those numbers would increase to 167 (97.1%) and 166 (95.5%), respectively. Thus, in conventional cytology 5 cases did not correspond to the histopathologic result; in liquid-based[®] cytology 8 cases did not agree with the histopathologic diagnoses (Table 1).

Table 1 – Distribution of cases according to the diagnoses obtained by conventional and liquid-based[®] cytology.

Diagnosis	Conventional cytology n (%)	Liquid-based [®] cytology N (%)
SCC	131 (72.0)	117 (64.3)
HSIL	36 (19.8)	49 (27.0)
LSIL	3 (1.6)	5 (2.8)
ASCUS	2 (1.1)	3 (1.5)
Unsatisfactory	10 (5.5)	8 (4.4)
Total	182 (100.0)	182 (100.0)

Abbreviations: SCC= Squamous cell carcinoma; HSIL= High grade squamous intraepithelial lesion; LSIL= Low grade squamous intraepithelial lesion; ASCUS = Atypical squamous cells of undetermined significance.

Among the cases, the agreement between the two cytologic methods was weak (Kappa= 0.178), with sensitivity= 96.9% (IC= 95%: 92.5% to 98.8%); specificity= 75.0% (IC= 95%: 21.9% to 98.7%); positive predictive value= 99.4%; negative predictive value= 37.5%, and accuracy= 96.3% (IC=95%: 92.5% to 98.5%). The results classified as SCC and HSIL were considered positive; the remainder were considered negative, and the unsatisfactory were excluded (Table 2).

Table 2 – Distribution of cases according to the diagnoses obtained by conventional cytology versus liquid-based[®] cytology.

Diagnosis	Conventional cytology					Total n (%)	p* value
	SCC (%) n (%)	HSIL n (%)	LSIL n (%)	ASCUS n (%)	Unsatisfactory n (%)		
SCC	91(69.5)	18(50.0)	1(33.3)	-	7(70.0)	117(64.3)	< 0.001
HSIL	30(22.9)	16(44.4)	-	-	3(30.0)	49(27.0)	
LSIL	1(0.8)	2(5.6)	2(66.7)	-	-	5(2.7)	
ASCUS	2(1.5)	-	-	1(50.0)	-	3(1.6)	
Unsatisfactory	7(5.3)	-	-	1(50.0)	-	8(4.4)	
Total	131 (100.0)	36 (100.0)	3 (100.0)	2 (100.0)	10 (100.0)	182 (100.0)	

Abbreviations: SCC= Squamous cell carcinoma; HSIL= High grade squamous intraepithelial lesion; LSIL= Low grade squamous intraepithelial lesion; ASCUS = Atypical squamous cells of undetermined significance. * Qui-square test.

Kappa test= 0.178

In the control group, the agreement between the two cytologic methods was very weak (Kappa= 0.375), with sensitivity= 91.0% (IC= 95%: 84.1% to 95.2%); specificity= 57.5% (IC= 95%: 41.0% to 72.6%); positive predictive value= 86.7% (IC= 95%: 79.3% to 91.8%); negative predictive value= 67.6% (IC= 95%: 49.4% to 82.0%) and accuracy= 82.7% (IC= 95%: 76.3% to 88.0%). The results classified as normal were considered positive; the remainder were considered negative, and the unsatisfactory were excluded (Table 3).

Conventional cytology and liquid-based cytology proved to be able to identify and classify cellular alterations characteristic of malignancy. Therefore, they are valuable as auxiliary diagnostic methods. When compared to each other, they showed a high sensitivity and reasonable specificity, conventional cytology being more reliable. The latter was defended and largely used by Mao³⁴, and according to Vidal and Silva⁷, if used appropriately, it has an excellent sensitivity and specificity when compared to histopathologic diagnoses.

Some authors^{7,8,26}, defend the use of cytology as a screening method for prevention and early diagnosis of oral cancer. Sciubba⁵

further³⁵ mentions the computer-assisted exfoliative cytology as an accurate method for detecting premalignant and malignant buccal lesions.

Tabela 3 – Distribution of controls according to the diagnoses obtained by conventional cytology versus liquid-based® cytology.

Liquid-based cytology Diagnosis	Conventional cytology					p* value
	Normal n (%)	Inflammatio n n (%)	ASCUS n (%)	Unsatisfacto ry n (%)	Total n (%)	
Normal	111 (84.7)	17 (44.7)	-	7 (100.0)	135 (75.4)	< 0.001
Inflammation	10 (7.6)	19 (50.0)	-	-	29 (16.2)	
ASCUS	1 (0.8)	1 (2.6)	3 (100.0)	-	5 (2.8)	
Unsatisfactory	9 (6.9)	1 (2.6)	-	-	10 (5.6)	
Total	131 (100.0)	38 (100.0)	3 (100.0)	7 (100.0)	179 (100.0)	

Abbreviations: ASCUS = Atypical squamous cells of undetermined significance. * Chi-square test
Kappa test= 0.375

Gynther, Rozell and Heimdahl³⁶ cite direct buccal microscopy as a method for selecting areas to be submitted to cytology and biopsy. Moreover, Sciubba³⁵ mentions the need to carry out studies to evaluate the sensitivity and specificity of oral scan laboratories inc. (Oral CDX). This is a computer-assisted method for the analysis of exfoliative cytology material, which adds to clinical examination in the differentiation of premalignant and malignant alterations found in “benign” lesions, the purpose being to find carcinomas of innocuous appearance, at the most curable and early stages.

The advantages of using conventional cytology in the oral mucosa, similarly to the uterine cervix, are its low cost, good patient tolerability, and easy applicability to large populations. The disadvantages are the number of unsatisfactory cases on account of technical reasons which depend on training in collect, fixation and sample preparation. In spite of its merits, one can not ignore the false-negative results. Furthermore, there are cases with cytomorphological alterations of innacurate diagnostic resolution. Liquid-based cytology has been considered an important alternative for improving cytology sensitivity, as it decreases cell loss during sample preparation, and provides a better cell distribution. In addition, it allows the use of molecular cytopathology techniques such as in situ hibridization, immunohistochemical tests, and determination of nucleic acids in molecular biology²⁷. The best performance of Liquid-based cytology is achieved with a high quality presentation: clear bottom, good distribution of cells, ease in distinguishing individualized cells, and good cell preservation. However, it lacks the specificity of conventional cytology (Figures 2,3, 4 and 5).

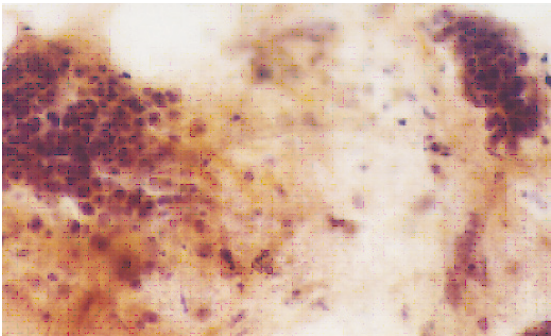


Figure 2. Photomicrography of conventional cytology material obtained from exfoliation of the left lateral border of the tongue, shown to be a SCC (Papa 40X).

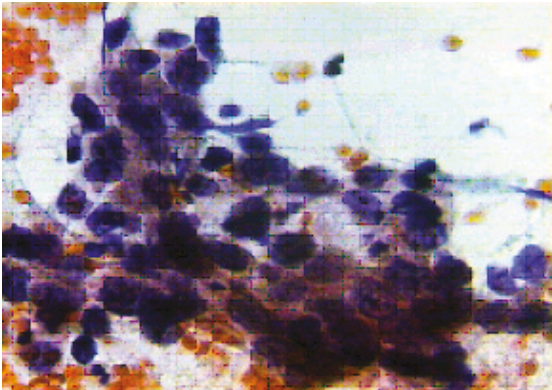


Figure 3. Photomicrography of liquid-based® cytology material obtained from exfoliation of the left lateral border of the tongue, shown to be a SCC (Papa 40X).

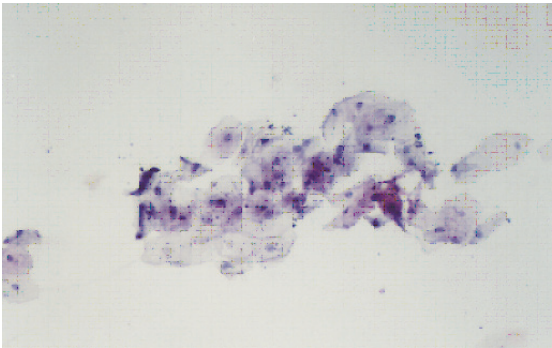


Figure 4. Photomicrography of conventional cytology material obtained from exfoliation of the left lateral border of the tongue, shown to be normal (Papa 40X).

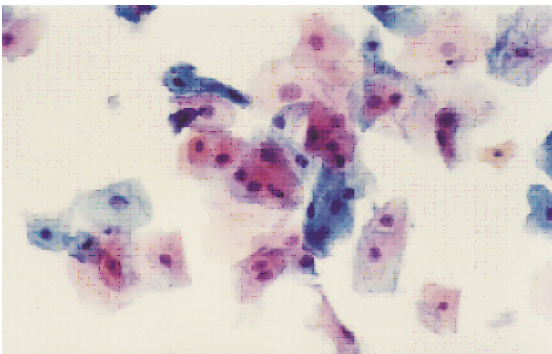


Figure 5. Photomicrography of liquid-based® cytology material obtained from exfoliation of the left lateral border of the tongue, shown to be normal (Papa 40X).

The acceptance and collaboration of the patients enrolled in this study, as well their interest (also mentioned by Vidal et al⁸) in the subject explored, demonstrate that the population wants and needs health education, in order to take better care of themselves and consequently have a higher quality of life.

CONCLUSION

Conventional cytology and liquid-based cytology demonstrated a diagnostic concordance with histopathology of more than 90%. Therefore, if properly indicated and executed, they can be routinely used as complementary diagnostic methods.

When compared to each other, conventional cytology and liquid-based cytology showed a high sensitivity and reasonable specificity, conventional cytology having higher specificity.

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