

## Exfoliative cytology as a tool for monitoring pre-malignant and malignant lesions based on combined stains and morphometry techniques

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**BACKGROUND:** Prevention and early diagnosis have the greatest potential for public health and are the most effective method in the long-term to control oral cancer. The aim was to apply PAP staining together with AgNOR staining and morphometric analysis in oral exfoliative cytology, to determine the sensitivity and specificity of these methods in the detection of malignant changes for the purposes of both initial population monitoring and follow-up.

**METHODS:** AgNOR, Papanicolau, and morphometric tests were conducted in samples of patients with oral cancer, oral potentially malignant disorders and controls (opposite side of lesions). Specificity and sensitivity values for each stain method and the curve under ROC area were estimated.

**RESULTS:** The diagnostic variables which allowed greatest accuracy in identifying malignancy relative to the healthy control were cluster (76.92%), satellite (75.64%), and total (90%). The diagnosis was seen to be associated with PAP and total AgNOR, total AgNOR and PAP, total AgNOR and satellites and clusters, and total AgNOR nuclear area/cytoplasmic area ratio.

**CONCLUSIONS:** The total number of AgNOR is a reliable marker for detecting neoplastic cells; this method increases sensitivity and specificity by decreasing the likelihood of false negatives or positives, as the accuracy obtained was 90%. It is also a low-cost, non-invasive, simple methodology that can be recommended to help the early detection of oral cancer and monitoring of patients with a first diagnosis of cancer.

**Keywords:** AgNOR; cancer; cytology; oral; papanicolau

### Introduction

Cancer is considered a public health problem worldwide (1). According to the World Health Organization (WHO), there are 10 million new cases and more than 6 million deaths each year from this disease worldwide (2). Cancers located in the oral cavity, defined as cancers of the lip, tongue and mouth (WHO, ICD 10 classification: C 01–06) account for 48% of all head and neck cancers (HNC), and 90% of these are epithelial cell carcinomas (3). The survival rate of patients with these cancers is 80% at 5 years, when detected at early stages, 40% when regional spread and less than 20% when there is metastasis (4).

Squamous cell carcinomas are formed in the surface epithelium of the oral cavity and produce early changes that are easy to detect on clinical examination. However, survival rates and early detection rates have not changed in the last 30 years (5), so efforts must continue to decide the best methodologies and biomarkers for population monitoring and early detection of these cancers.

As prevention and early diagnosis have the greatest potential for public health and are most effective long-term for cancer control, action must be taken in two ways: (i) training of health professionals, mainly dentists and doctors; and (ii) identifying biomarkers and methods of population monitoring and tracking for early detection of pre-cancerous lesions in the process of malignization or early signs of malignancy in incipient tumorigenic lesions or prevention of recurrence in patients after surgery for a malignant lesion. A simple, non-invasive, inexpensive technique for identifying biomarkers feasible to implement in a common laboratory and well accepted by patients, is exfoliative cytology (EC), which, applied routinely in the dental and/or doctor's office, may increase early detection of malignization in clinical examination of the oral cavity (6).

Different types of stains have been studied for use in EC. The Papanicolau (PAP) stain is considered the universal stain for EC, particularly as a method for monitoring cervical (7) cancer, enabling normal and

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