# PLOIDY STUDIES OF PLEOMORPHIC ADENOMAS AND MINOR SALIVARY GLAND CARCINOMAS OF THE PALATE

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***ABSTRACT***

*Ploidy studies of tumors are a diagnostic and prognostic aid. The aim of the present study was to evaluate the DNA content of palate aggressive pleomorphic adenomas (PA) and adenocarcinomas of the salivary glands.*

*Twelve cases of salivary gland tumors of the palate were selected from the archives of the Oral Pathology Department, Faculty of Dentistry, University of Buenos Aires (1966-2001). Six cases corresponded to aggressive pleomorphic adenomas (PA) and the remaining six to adenocarcinomas (AD). Myxoid and epithelial areas of PA were evaluated.*

*The epithelial areas of the most aggressive cases of PA exhibited a high DNA content. The myxoid areas of same cases of PA had a 2C ploidy level. The difference in ploidy values between the myxoid and epithelial areas of PA would suggest the presence of different cell populations. DNA content and the detection of aneuploidy would be prognostic aids in palate salivary gland carcinomas.*

***Key words:*** *ploidy, tumors, minor salivary gland.*

## ESTUDIO DE PLOIDÍA EN ADENOMAS PLEOMORFOS Y CARCINOMAS DE GLÁNDULAS SALIVALES DE PALADAR

***RESUMEN***

*Los estudios de ploidía son usados como herramienta en el diagnóstico y pronóstico de tumores. El objetivo del presente estudio fue evaluar el contenido de ADN de adenomas pleomorfos (PA) y carcinomas de glándulas salivales de paladar. Se seleccionaron 12 casos de tumores de glándulas salivales de paladar de los archivos de la Cátedra de Anatomía Patológica de la Facultad de Odontología de la Universidad de Buenos Aires (1966-2001). Seis casos correspondieron a Adenomas pleomorfos y seis a adenocarcinomas. Se evaluaron las areas mixoides y epiteliales de los PA. En todos los casos de PA obtuvimos en los sectores epiteliales, un contenido elevado de DNA del orden de 4C. En tanto las areas mixoides de los PA revelaron ploidas de 2C. Los resultados mostraron tres rangos diferentes de contenidos de ADN. Laos sectores mixoides de los PA evidenciaron valores en el rango 2C, Llos sectores epiteliales de los PA fueron en un rango 4C y los adenocarcinomas mostraron valores anueploides. Se han encontrado diferencias en el contenido de DNA en los AP entre las áreas epiteliales y mixoides. Esto permitiría suponer la existencia de diferentes poblaciones celulares, con grado de agresividad diferente. El contenido de ADN y su carácter aneuploide podrían ser de utilidad pronóstica en los carcinomas de glándulas salivales menores.*

***Palabras clave:*** *ploidia, tumor, glándulas salivales menores.*

## INTRODUCTION

The salivary gland pleomorphic adenoma (PA) is undoubtedly an attractive and interesting tumor in terms of its morphology and biological behavior.

The PA is of epithelial origin but induces, during the course of its growth and evolution, a variable process of myxoid, chondroid and even osseous differentiation around epithelial islands that facilitates the infiltration process[1].

Batsakis et al.[2] studied the morphological heterogeneity of PA and supported the notion that this tumor originates in an ectodermic precursor cell[3].

Several ultrastructural, immunohistochemical and histochemical studies have examined the structural differences and similarities between the different cell types present in these tumors that share a common origin in glandular cells[3-5].

Ploidy studies are employed as a diagnostic and prognostic aid for some tumors[6-9].

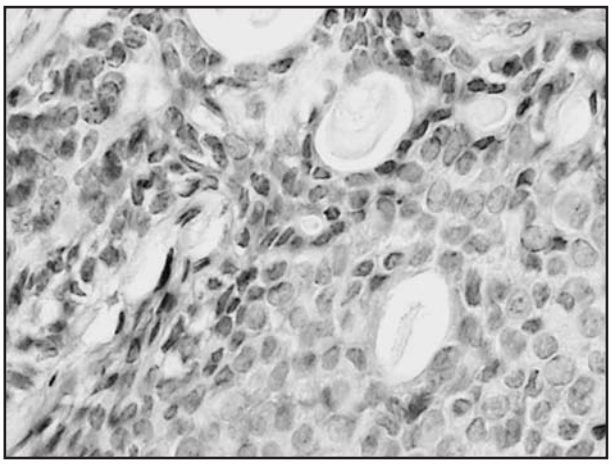

*Fig. 1: Epithelial area of a pleomorphic adenoma. Note the presence of epithelial cells exhibiting glandular differentiation and intraluminal secretion. (H&E X 40)*

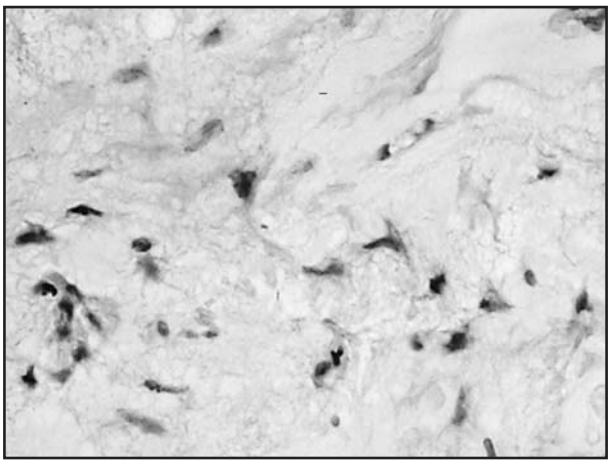

*Fig. 2: Myxoid area of the same pleomorphic adenoma shown in Fig. 2 featuring stellar cells in a myxoid matrix and no blood vessels. (H&E X 40)*

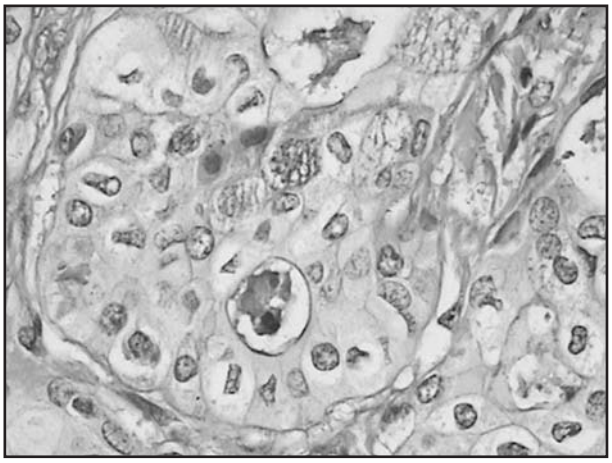

*Fig. 3: Adenocarcinoma in an accessory salivary gland. The neoplastic cells adopt an alveolar pattern. Note the marked anisocariosis in some of the nuclei. (H&E X 40).*

The aim of the present study was to evaluate the DNA content of pleomorphic adenomas of the palate and of accessory salivary gland carcinomas of the palate, and establish differences and similarities between epithelial and myxoid areas of PA.

## MATERIALS AND METHODS

Twelve cases were selected from a total of 112 salivary gland tumors from the files of the Oral Pathology Department of the Faculty of Dentistry, University of Buenos Aires (1966-2001). Six cases corresponded to aggressive pleomorphic adenomas (PA) and the remaining six to adenocarcinomas (AD) (four adenoid cystic carcinomas, one adenocarcinoma, one mucoepidermoid carcinoma). The selected cases of PA exhibited clearly defined patterns of epithelial and myxoid differentiation.

The paraffin blocks corresponding to each of the selected cases were sectioned alongside a celloidin cylinder stained with the Schiff reagent to measure section thickness accurately in keeping with a method previously described by our laboratory[10]. Serial eight-micron sections were obtained and stained alternately with hematoxylin-eosin or the Feulgen reaction.

The Feulgen DNA staining reaction [11] involved hydrolysis in 5N HCl for 10 minutes at room temperature (20°C) followed by staining with Schiff's reagent. Hydrolysis time was previously adjusted with a hydrolysis curve employing ten sections obtained from one of the selected cases. Staining with Schiff's reagent was carried out at room temperature for 90 minutes. The sections were then washed in a sulfurous rinse solution and mounted in balsam.

The areas for measurement of DNA content were selected by light microscopy examination of hematoxylin-eosin stained histological sections. In the case of PA, separate areas characterized by epithelial and myxoid patterns were chosen (Fig. 1, 2, 3). In the epithelial areas of PA, the most variabilidadactive cells were evaluated, whereas in the case of carcinomas, the histologically most aggressive sites were selected in keeping with the diagnostic criteria of the pathologist.

DNA content evaluation was performed employing a Carl Zeiss MPM800 microscope on line with an image

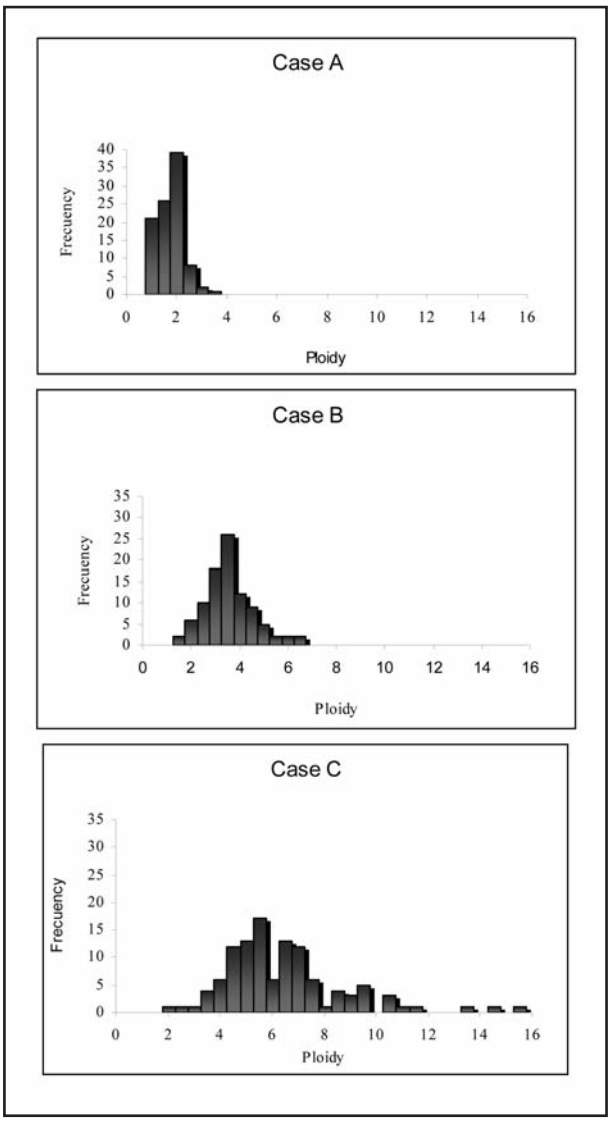


*Fig. 4: Case A: Ploidy histogram of a myxoid area of a Pleomorphic Adenoma. Case B: Ploidy histogram of an epithelial area of a Pleomorphic Adenoma. Case C: Ploidy histogram of an Adenocarcinoma.*

analyzer (IBAS, Kontron Electronic). The images were captured with a Zeiss x 40, A75 objective.

The diploid content value was determined for each case individually by assessing the DNA content of 20 lymphocytes in the tumor stroma.

One hundred nuclei were measured in each case employing the DNA IBAS-Kontron software.

Measurement criteria were established. In the case of PA, two different areas were analyzed, i.e. epithelial and myxoid areas (Fig. 1, 2). Epithelial areas exhibited a pattern of cords and nests and high cell density (Fig. 1). Myxoid areas exhibited a lower cell density and featured small groups of cells or isolated cells interspersed with a mucoid-like substance (Fig. 2). In the case of malignant tumors, the areas of greatest atypia and anaplasia were selected for measurement (Fig. 3).

**TABLE 1. Analysis of measurements of pleomorphic adenoma**

| Case | Ploidy value | | p |
|---|---|---|---|
| | Epithelial areas | Myxoid areas | |
| 1 | 2.53 ±0.66 n=142 | 1.96 ±0.74 n=104 | 1.23E-09 * |
| 2 | 4.92 ±10.33 n=104 | 2.43 ±0.93 n=50 | 0.09 |
| 3 | 3.23 ±1.16 n=130 | 2.43 ±0.92 n=105 | 2.71E-08 * |
| 4 | 4.34 ±2.11 n=122 | 2.28 ±1.43 n=121 | 1.11E-16 * |
| 5 | 3.86 ±3.73 n=104 | 2.80 ±1.03 n=94 | 0.00816 * |
| 6 | 1.94 ±3.19 n=102 | 0.75 ±0.73 n=115 | 1.43E-04 * |

ANOVA "*": p value < 0.05

The software affords values of Total Optical Density (TOD) and the area in pixels for each nucleus measured. In addition, the mean ploidy value (mean ratio TOD/lymphocyte TOD), the ploidy histogram and the standard deviation values were obtained.

The data were corrected according to previously published correction procedures[12].

## RESULTS

The values of DNA content were corrected in keeping with the corresponding procedures[12] and analyzed separately for each of the six mixed tumors. Table 1 shows the corresponding ploidy and standard deviation values for the epithelial and myxoid areas.

Fig. 4 contains histograms with different DNA distribution patterns. Case A corresponds to a myxoid area of a PA with diploid DNA values; case B corresponds to epithelial cells of a PA with tetraploid DNA values; case C corresponds to an adenocarcinoma with a characteristic aneuploid distribution pattern.

DNA content was consistently greater in the epithelial area than in the myxoid area. Five of the six tumors exhibited statistically significant differences between areas (Table 1).

In carcinomas, DNA content was measured in the areas of greatest atypia.

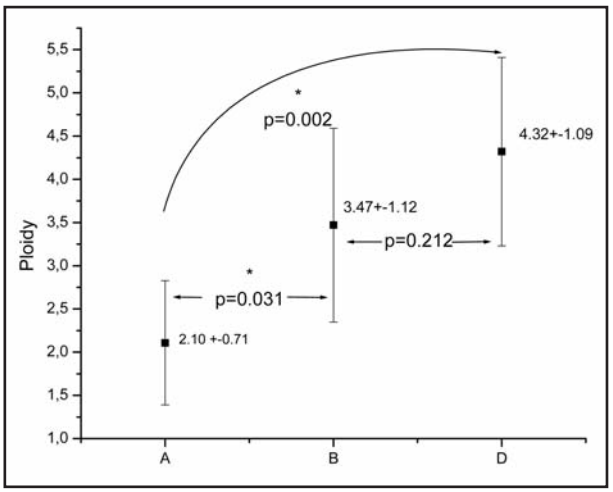


*Fig. 5: Schematic representation of the differences in ploidy values in salivary gland tumors. A: Myxoid areas of pleomorphic adenoma, B: Epithelial areas of pleomorphic adenoma, C: Salivary gland carcinomas.*

We evaluated the mean ploidy value and standard deviation for the epithelial and myxoid areas of PA and of carcinomas. Figure 5 reveals three different ranges of DNA content: a lower level in the 2C range in myxoid areas of PA, an intermediate level in the 4C range in epithelial areas of PA and a higher level with values in excess of 4C in carcinomas. Statistically significant differences were found between epithelial and myxoid areas (p=0.031) and between myxoid areas and carcinomas (p=0.002).

## DISCUSSION

Salivary gland tumors of the palate exhibit characteristic features related to clinical evolution and response to therapy that distinguish them from major salivary gland tumors. The present study addresses the case of palate tumors in particular. Previous ploidy studies have been performed by flow cytometry[13-15,20,23-25]. This technique allows for the measurement of a large number of cells but fails to distinguish between tumor and non-tumor cells. Image cytometry allowed for the measurement of a representative number of exclusively tumor cells and the pre-selection of measurement areas[12]. In the case of adenocarcinomas and PA we selected the most aggressive areas for measurement. Additionally, in the case of PA, we measured myxoid and epithelial differentiation areas separately.

Pleomorphic adenomas have low tumor aggressiveness and are predominantly diploid[18,20,24]. However, some aneuploid PA have been found. Martin AR et al. described, for the first time, 3 cases of aneuploid PA in a series of 15 cases; only one of these 3 tumors was localized in the palate[13]. Other authors described some cases of aneuploid PA, i.e. 1 in a series of 31[14], 7 in a series of 25[15] and 2 in a series of 8[23]. However, these studies fail to specify whether these cases correspond to major salivary gland or palate tumors.

In the present study, out of a total of 112 salivary gland tumors, we selected 6 adenocarcinomas and 6 pleomorphic adenomas with clinical and histological features of aggressiveness. The present data reveal unequivocal differences in ploidy levels between the glandular epithelial areas (aneuploid) and myxoid areas (normal ploidy level).

The diploid nature of myxoid tumor cells could be attributed to a process of gland tissue induction.

The high ploidy levels in carcinomas would be partly due to the pre-selection for measurement of the areas with the greatest histological atypia. These areas closely correlate with histopathological diagnosis. Within this context, ploidy analysis would be a valuable prognostic method.

The present study suggests the value of performing routine ploidy studies in palate salivary gland tumors employing microspectrophotometric techniques that allow the separate evaluation of epithelial and myxoid areas of tumors. Ploidy analysis has proved to be a reliable aid in the evaluation of tumor aggressiveness.

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