UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

Fabio Augusto Ito
Cirurgião-Dentista

ESTUDO HISTOPATOLÓGICO E IMUNOHISTOQUÍMICO DE TUMORES DE GLÂNDULAS SALIVARES

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de Doutor em Estomatopatologia, área de Patologia.

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PROF. DR. OSLEI PAES DE ALMEIDA

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"É preciso amor
Prá poder pulsar
É preciso paz prá poder sorrir
É preciso chuva para florir

Cada um de nós compõe
A sua própria história
E cada ser em si
Carrega o dom de ser capaz
De ser feliz"

Renato Teixeira

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RESUMO

Além de incomuns, os tumores de glândulas salivares despertam interesses por apresentarem uma grande diversidade histológica, morfológica e de comportamento biológico. Nas últimas décadas vários estudos têm mostrado que tais diversidades estão relacionadas ao acúmulo de alterações genéticas. As investigações de tais alterações são importantes para entender os mecanismos de oncogênese, avaliar o comportamento biológico e conseqüentemente aperfeiçoar terapêuticas favorecendo o prognóstico. O objetivo deste estudo foi analisar as características histopatológicas e imunohistoquímicas dos quatro tumores de glândulas salivares mais freqüentes: adenoma pleomórfico, carcinoma mucoepidermóide, tumor de Warthin e carcinoma adenóide cístico. Para isso foi realizada análise histológica do componente epitelial e mesenquimal dos adenomas pleomórficos, classificação histológica dos tumores de Warthin, carcinomas mucoepidermóides e carcinomas adenóide cístico e estudo imunohistoquímico para Ki-67, EGF, EGFR, ErbB-2, FAS, receptor de andrógeno, receptor de estrógeno e receptor de progesterona. Dos 189 casos de adenoma pleomórfico selecionados para o estudo histopatológico, 99 (52,4%) foram classificados como estroma-rico, 69 (36,5%) como celular e 21 (11,1%) como clássico. Dos 30 casos de tumor de Warthin, 17 casos (56,7%) foram classificados como típicos, 10 (33,3%) como estroma pobre e 3 (10%) como estroma-rico. Dos 30 casos de carcinoma mucoepidermóide, 15 casos (50%) foram classificados histologicamente como de baixo grau, 3 (10%) como grau intermediário e 12 (40%) como de alto grau. Dos 30 casos de carcinoma adenóide cístico, 15 casos (50%) foram classificados como cribriforme, 8 (26,7%) como tubular e 7 (23,3%) como sólido. Ki-67, EGFR e ErbB-2 foram mais freqüentemente encontrados em carcinomas mucoepidermóides, principalmente em tumores de alto grau de malignidade. EGF e FAS foram encontrados mais freqüentemente em adenomas pleomórficos e carcinomas mucoepidermóides. Todos os casos estudados foram negativos para receptor de estrógeno e receptor de progesterona. Receptor de andrógeno foi positivo em apenas 2 casos de adenoma pleomórfico, 2 de carcinoma mucoepidermóide e 2 de carcinoma adenóide cístico. Concluindo, EGFR, ErbB-2 e FAS parecem desempenhar papel na tumorigênese de

tumores de glândulas salivares, especialmente em carcinomas mucoepidermóides, e devem ser mais extensivamente estudados; receptor de estrógeno e receptor de progesterona não desempenham papel na tumorigênese de adenomas pleomórficos, tumores de Warthin, carcinomas mucoepidermóides e carcinomas adenóide cístico; e apesar de poucos casos positivos em tumores de glândulas salivares, receptor de andrógeno deve ser melhor estudado e considerado como potencial alvo em tratamentos com drogas anti-andrógenos.

ABSTRACT

Beyond uncommon, salivary gland tumors are interesting because their great diversity of histological, morphological and biological behavior. In the last decades some studies have shown that such diversities are related to the accumulation of genetic alterations. The investigation of such alterations are important to understand the mechanisms of oncogenesis, to evaluate the biological behavior and consequently to improve therapeutics favoring the prognosis. The aim of this study was to analyze the histopathological and immunohistochemical characteristics of the four more frequent salivary gland tumors: pleomorphic adenoma, mucoepidermóide carcinoma, Warthin's tumor and adenoid cystic carcinoma. For this, it was realized the histological analysis of the epithelial and mesenchymal components of pleomorphic adenomas, histological classification of Warthin's tumors, mucoepidermoid carcinomas and adenoid cystic carcinoma and immunohistochemical study for Ki-67, EGF, EGFR, ErbB-2, FAS, androgen receptor, estrogen receptor and progesterone receptor. Of the 189 cases of pleomorphic adenoma selected for the histopathological study, 99 (52.4%) were classified as stroma-rich, 69 (36.5%) as cellular and 21 (11.1%) as classic. Of the 30 cases of Warthin's tumor, 17 cases (56.7%) were classified as typical, 10 (33.3%) as stroma-poor and 3 (10%) as stroma-rich. Of the 30 cases of mucoepidermoid carcinoma, 15 cases (50%) were classified as low grade, 3 (10%) as intermediate grade and 12 (40%) as high grade. Of the 30 cases of adenoid cystic carcinoma, 15 cases (50%) were classified as cribriform, 8 (26.7%) as tubular and 7 (23.3%) as solid. Ki-67, EGFR and ErbB-2 were more frequently found in mucoepidermoid carcinomas, particularly in high grade tumors. EGF and FAS were more frequently found in pleomorphic adenomas and mucoepidermoid carcinomas. All studied cases were negative for estrogen receptor and progesterone receptor. Androgen receptor was positive in only 2 cases of pleomorphic adenoma, 2 of mucoepidermoid carcinoma and 2 of adenoid cystic carcinoma. In conclusion, EGFR, ErbB-2 and FAS seem to play a role in the tumorigenesis of salivary gland tumors, especially in MEC, and they should be more extensively studied; estrogen receptor and progesterone receptor do not play a role in the tumorigenesis of pleomorphic adenomas,

Warthin's tumors, mucoepidermoid carcinomas and adenoid cystic carcinomas; and regardless of few positive cases in salivary gland tumors, androgen receptor should be better studied and considered as potential targets for treatment with anti-androgens drugs.

1. INTRODUÇÃO

Tumores de Glândulas Salivares:

Os tumores de glândulas salivares são conhecidos por apresentarem vários tipos histológicos, variações morfológicas e comportamentos biológicos diversos. Nos Estados Unidos a incidência anual varia de 0,4 a 6,5 casos por 100.000 indivíduos, representando menos de 3% de todas as neoplasias de cabeça e pescoço (Spiro, 1986; Ellis *et al*., 1991; Ellis & Auclair, 1996).

A revisão de grandes séries de neoplasias de glândulas salivares mostra que 64% a 80% dos tumores primários de origem epitelial ocorrem em parótida, 7% a 11% em glândula submandibular, menos de 1% em glândula sublingual e de 9% a 23% em glândulas salivares menores. Quanto a sua natureza, de 54 a 79% das neoplasias de glândulas salivares são benignas e de 21 a 46% são malignas (Eneroth, 1971; Eveson & Cawson, 1985; Seifert *et al*., 1986; Spiro, 1986; Ellis *et al*., 1991).

O pico de incidência dos tumores de glândulas salivares ocorre na sexta e sétima décadas de vida (Eveson & Cawson, 1985; Ellis *et al*., 1991). O pico de incidência de adenomas pleomórficos (AP) e carcinomas mucoepidermódes (CME) é mais baixo e ocorre na quarta e quinta décadas de vida (Ellis *et al*., 1991).

Em todos os grupos etários, o gênero feminino é afetado mais freqüentemente que o masculino, mas há certa variação do gênero de acordo com o tipo do tumor (Eveson & Cawson, 1985; Spiro, 1986; Ellis *et al*., 1991). No trabalho de Satko *et al*. (2000) foram encontrados 537 tumores de glândulas salivares em mulheres (52,6%) e 484 em homens (47,4%) com uma proporção de 1,1:1. Williams *et al*. (2001) relataram que 59,4% dos tumores foram em mulheres e os outros 40,6% em homens com uma proporção de mulheres: homens de 1,46:1. A correlação da localização dos tumores e o gênero do paciente mostra que em glândulas salivares menores as mulheres são mais freqüentemente afetadas tanto nos tumores benignos quanto nos malignos (Isacsson & Shear, 1983; Waldron *et al*., 1988).

A grande maioria dos tumores de glândulas salivares é de origem epitelial e a classificação histológica desses tumores pode apresentar dificuldades devido a grande

variabilidade morfológica. A complexidade desse grupo de tumores está ilustrada na classificação dos tumores de glândulas salivares da Organização Mundial da Saúde, onde mais de 30 tipos diferentes de tumores estão incluídos (Barnes *et al.*, 2005).

Adenoma Pleomórfico:

O AP é a neoplasia de glândula salivar mais comum representando 45% a 74% de todos os tumores de glândulas salivares (Eneroth, 1971; Eveson & Cawson, 1985; Seifert *et al*.,1986; Spiro, 1986; Satko *et al*., 2000). Cerca de 60% de todos os tumores de glândulas salivares maiores e 55% dos tumores de glândulas salivares menores são adenomas pleomórficos (Foote & Frazell, 1953; Eneroth, 1969; Loyola *et al*., 1995; Ellis & Auclair, 1996).

Pode afetar indivíduos de qualquer idade, porém ocorre mais freqüentemente em pacientes na quarta e quinta décadas de vida (Euclair *et al*., 1991; Ellis & Auclair, 1996). Apesar da baixa incidência de tumores de glândulas salivares em crianças e adolescentes, o AP é o mais freqüente nessa faixa etária (Lack & Upton, 1988; Fonseca *et al*., 1991). Quanto ao gênero, as mulheres são geralmente mais afetadas que os homens (Eveson & Cawson, 1985; Ellis & Auclair, 1996).

Clinicamente, apresenta-se como uma massa de crescimento lento, assintomático e que pode se tornar grande quando não tratado (Ellis & Auclair, 1996). Na parótida, na maioria dos casos, o AP atinge a região inferior do lóbulo superficial causando um abaulamento sobre o ângulo da mandíbula, ligeiramente abaixo e a frente da orelha. Em estágios iniciais, os adenomas pleomórficos de parótida são geralmente móveis, mas quando não tratados, os tumores se tornam mais nodulares e fixos. Raramente causam paralisia facial, mesmo em tumores maiores. Adenomas pleomórficos recorrentes de parótida são geralmente multinodulares e aparecem clinicamente como múltiplos e pequenos nódulos que à palpação são aparentemente fixos. Os adenomas pleomórficos de glândulas salivares menores aparecem como massas de crescimento lento, assintomáticos recobertos por mucosa normal e são mais freqüentes na região posterior do palato duro e no palato mole. Raramente são ulcerados, a menos que traumatizados secundariamente. No palato duro, devido à estreita relação da mucosa com o osso, os tumores são geralmente

fixos. Por outro lado, nas demais localizações a maioria dos casos é móvel (Ellis *et al*., 1991).

Esse tumor apresenta uma grande diversidade morfológica e é composto tanto de um componente epitelial como de um componente mesenquimal. O componente epitelial pode apresentar-se com células fusiformes, claras, escamosas, basalóides, cuboidais, plasmocitóides, oncocitóides, mucosas e/ou sebáceas formando diversas configurações estruturais como trabéculas, ductos, cistos e áreas sólidas. A parte mesenquimal pode ser mixóide, hialina, condróide, óssea ou uma mistura destes componentes. A proporção entre os dois componentes varia de tumor para tumor e dentro de um mesmo tumor (Ellis & Auclair, 1996). Esta proporção pode ser usada para classificar os AP em estroma-rico, celular ou típico (Seifert et a., 1976).

O tratamento do AP consiste na sua completa excisão cirúrgica. Na parótida, nos casos onde o tumor se localiza no lóbulo superficial, é realizada uma parotidectomia superficial com pequena margem de tecido normal e preservação do nervo facial. Nos casos em que o tumor está localizado no lóbulo profundo da parótida, uma parotidectomia total é recomendada (Maynard, 1988; Hardingham, 1993; Yamashita *et al*., 1993). Os tumores das glândulas submandibulares são tratados com a remoção completa da glândula (Weber, *et al*., 1990) e os de glândulas salivares menores são excisionados com margem de tecido normal (Chau & Radden 1989; Ellis & Auclair, 1996). Os casos de recorrência têm sido relacionados à incompleta excisão do tumor, principalmente aqueles que estão localizados na parótida (Buchman *et al*., 1994; Patel & Poole, 1998).

Tumor de Warthin:

Também conhecido como cistadenoma papilar linfomatoso, o tumor de Warthin (TW) é um adenoma onde um epitélio oncocítico forma vários cistos e projeções papilares sobre um estroma de tecido linfóide.

O TW é o segundo tumor benigno de glândulas salivares mais comum representando de 4% a 11% de todos os tumores de glândulas salivares (Eneroth, 1971; Spiro, 1986; Eveson & Cawson, 1989). É considerado quase exclusivo de parótida

correspondendo cerca de 95% dos casos. Quando ocorrem em qualquer outro local é denominado ectópico (Ellis *et al*., 1991).

Acometem principalmente pacientes idosos, com pico de incidência entre a 6ª e a 8ª décadas de vida tanto em pacientes do gênero masculino como feminino. Por outro lado, raramente ocorrem em indivíduos com menos de 30 anos (Eveson & Cawson, 1985; Ellis & Auclair, 1996; Maiorano et al., 2002).

O TW mostra clara predominância nos pacientes do gênero masculino. Estudos mostram proporções entre os pacientes do gênero masculino e feminino variando de 5:1, 10:1 e até 26:1 (Foote & Frazell, 1953; Chaudhry & Gorlin, 1958; Li & Liu, 1987). Entretanto, trabalhos mais recentes mostram uma diminuição dessa proporção para 1,1:1 a 1,6:1 (Eveson & Cawson, 1985; Ebbs & Webb, 1986; Lamelas *et al*., 1987; Monk & Church, 1992; Webb & Eveson, 2002). Esta alteração na prevalência dos tumores de Warthin pode estar relacionada a possíveis fatores etiológicos como o fumo. Alguns trabalhos vêm mostrando o crescimento do uso do tabaco entre as mulheres e o aumento na incidência de tumores de Warthin e carcinomas pulmonares nesse grupo de pacientes (Lamelas *et al*., 1987; Monk & Church, 1992; Yoo *et al*., 1994; Chung *et al*., 1999).

A maioria dos tumores de Warthin apresenta-se clinicamente como um crescimento indolor e às vezes flutuante na porção inferior da glândula parótida. Os tumores geralmente apresentam-se medindo cerca de 2 cm a 4 cm, mas podem alcançar tamanhos consideráveis (White *et al*., 1978; Ellis & Auclair, 1996). Cerca de 10% dos pacientes apresentam dor, pressão ou rápido crescimento do tumor (Seifert *et al*., 1986).

O termo cistadenoma papilar linfomatoso descreve muito bem os aspectos microscópicos desse tumor, os quais são caracterizados por espaços císticos delimitados por proliferações papilares de tecido epitelial formado por duas camadas de células oncocíticas em um estroma de tecido linfóide (Ellis & Auclair, 1996). Além dessas características histopatológicas clássicas, presença de metaplasia escamosa, metaplasia mucosa, inflamação aguda, cristais de colesterol, tumores múltiplos, fibras nervosas relacionadas ao tumor e alterações císticas e fibrosas têm sido descritos (Webb & Eveson, 2002).

O tratamento consiste na parotidectomia superficial, todavia, com exploração cirúrgica do leito cirúrgico de toda a parótida após remoção do tumor para localizar

possíveis tumores multifocais (Leverstein *et al*., 1997). Os índices de recorrência são de difícil avaliação devido à natureza multifocal do TW. No entanto, as taxas de recorrência relatadas variam de 2% a 25% (Frazell, 1954; Gant *et al*., 1981; Ebbs & Webb, 1986; Heller & Attie, 1988).

Carcinoma Mucoepidermóide:

O carcinoma mucoepidermóide (CME) é um tumor maligno de origem epitelial composto por variada proporção de células mucosas, epidermóides, intermediárias, colunares e claras. Pode também demonstrar um proeminente crescimento cístico, particularmente nos tumores de baixo grau de malignidade (Ellis & Auclair, 1996). É o tumor maligno de glândula salivar mais comum, representando cerca de 15% a 30% dos tumores e o segundo tumor de glândula salivar mais freqüente, ficando atrás somente do AP (Spiro, 1986; Ellis & Auclair, 1996; Spiro, 1986; Ellis & Auclair, 1996; Wahlberg et al., 2002).

O CME provavelmente origina-se do epitélio ductal, que pode sofrer metaplasia escamosa, mucosa ou ambas. O estudo ultraestrutural realizado por Chaudhry *et al*. (1989) em 15 carcinomas mucoepidermóides suporta a teoria que células pluripotenciais de reserva estão relacionadas com a histogênese deste tumor. Estas células estão presentes no ducto intercalar-acinar, como também nos ductos de médio e grande calibre.

Nas glândulas parótida e submandibular, o CME comumente apresenta-se como um tumor solitário, bem circunscrito e móvel, podendo ser clinicamente semelhante a um tumor benigno. Dor, paralisia facial e fixação a tecidos adjacentes não são comuns. Nas glândulas salivares menores a aparência clínica pode variar admiravelmente, podendo simular um tumor benigno ou uma condição inflamatória. Muitas lesões, principalmente no palato, são flutuantes, azuladas e de superfície lisa que lembram mucoceles e raramente possuem superfície granular ou papilar. Os sintomas incluem disfagia, dor, parestesia, deslocamento de dentes, ulceração ou hemorragia. Radiografias de lesões no palato ocasionalmente mostram erosão do osso subjacente (Ellis & Auclair, 1996).

Microscopicamente, o CME é caracterizado pela presença de uma variedade de tipos celulares e padrões de crescimento, os quais formam a base para o diagnóstico e sua

gradação histológica. O termo carcinoma mucoepidermóide enfatiza a presença de células mucosas e epidermóides, entretanto, o reconhecimento desse tumor pode envolver a identificação de células mucosas, epidermóides, intermediárias, colunares ou claras, cada uma proliferando sozinha ou em muitas outras combinações tanto em padrões sólidos como císticos (Ellis *et al*., 1991).

Na maioria dos tumores, as células intermediárias estão em maior número que outros tipos celulares. O termo intermediário era usado para definir as células cujo tamanho e aparências estavam entre as células basais e as células epidermóides poligonais (Stewart *et al*., 1945). No entanto, atualmente a maioria dos autores inclui as pequenas células basais nesse termo (Ellis & Auclair, 1996).

Células mucosas são as células neoplásicas no CME que contêm mucina. Morfologicamente, as células mucosas lembram células intermediárias, epidermóides, claras e colunares e geralmente estão em grupos ou dispersas ao acaso entre outros tipos celulares. Ocasionalmente são grandes, ovóides, em forma de taça, com citoplasma abundante e lembram células acinares mucosas (Ellis & Auclair, 1996).

As células epidermóides podem formar pequenos ninhos sólidos ou circunscreverem espaços císticos. Quando formam ilhas, estão comumente circundadas por pequenas células basalóides intermediárias. Pérolas de queratina e queratinização individual de células são raramente vistas no CME e geralmente estão associados a tumores inflamados (Ellis & Auclair, 1996).

A apresentação característica de muitos carcinomas mucoepidermóides é um componente cístico ou cístico-papilar proeminente e pequenas estruturas semelhantes a ductos. Os cistos são geralmente revestidos por células epidermóides, mucosas ou intermediárias que também podem ter uma proliferação extramural (Ellis & Auclair, 1996).

O potencial biológico do CME é considerado controverso, diferentes autores têm proposto várias classificações e gradações histológicas desde tumor (Foote & Frazell, 1953; Sikorowa, 1964; Jakobsson *et al*., 1968; Eversole, 1970; Healey *et al*., 1970; Thorvaldsson *et al*., 1970; Spiro *et al*., 1978; Accetta *et al*., 1984; Evans, 1984; Nascimento *et al*., 1986; Batsakis & Luna, 1990; Hicks *et al*., 1995; Goode *et al*., 1998).

Healey *et al*. (1970), Thorvaldsson *et al*. (1970), Batsakis & Luna (1990), Clode *et al*., (1991) e Auclair *et al*. (1992) sugerem a utilização da gradação histológica do CME em três grupos (baixo grau, grau intermediário e alto grau) baseado principalmente na arquitetura do tumor, padrão de invasão, proporção dos tipos celulares, pleomorfismo histológico e freqüência de mitoses.

O grau histológico é um importante fator prognóstico, visto que a taxa de mortalidade aumenta de acordo com o grau histológico de malignidade. A maioria dos tumores é considerada de baixo grau de malignidade e os pacientes que desenvolvem este tipo de tumor geralmente apresentam um bom prognóstico após remoção cirúrgica. Entretanto, alguns pacientes com tumores de baixo grau de malignidade, pequenos e sem sintomatologia, que aparentemente recebem tratamento adequado, morrem da doença (Goode *et al*., 1998; Pires *et al.*, 2004).

O tratamento dos carcinomas mucoepidermóides depende do estadiamento do tumor. É recomendado que tumores nos estádios I e II localizados na parótida sejam tratados por excisão conservadora com preservação do nervo facial (Spiro *et al*., 1978). As glândulas submandibulares afetadas devem ser totalmente extirpadas e segundo alguns autores, podem receber radioterapia coadjuvante nos casos de alto grau de malignidade ou naqueles em estádios avançados devido ao pior prognóstico dos tumores nessa localização (North *et al*., 1990; Ellis & Auclair, 1996; Hosokawa *et al*., 1999). Os tumores de glândulas salivares menores devem ser excisados com margens de tecido normal podendo incluir parte do tecido ósseo adjacente (Olsen *et al*., 1981). Os tumores de alto grau de malignidade ou em estádios avançados necessitam de um tratamento agressivo, podendo ser adicionado, além da ressecção cirúrgica, esvaziamento cervical e radioterapia (Ellis & Auclair, 1996; Hosokawa *et al*., 1999).

Carcinoma Adenóide Cístico:

Carcinoma adenóide cístico (CAC) é um tumor maligno de origem epitelial caracterizado por demonstrar crescimento persistente e por apresentar metástases, principalmente à distância, após longos períodos. O tumor é predominantemente composto de células mioepiteliais, mas pode apresentar células com diferenciação ductal. O padrão

microscópico clássico é conhecido como cribriforme. No entanto, outros dois padrões, chamados de tubular e sólido, também podem ser observados (Ellis & Auclair, 1996).

Ocorrem principalmente em glândulas salivares menores ou na parótida (Matsuba *et al*., 1984; Spiro, 1986; Pacheco-ojeda *et al*., 2000, Ito *et al*., 2005). Pode afetar pacientes em qualquer idade, porém é mais freqüente em pacientes entre a quarta e sexta décadas de vida (Ellis & Auclair, 1996) e a literatura mostra uma pequena predominância do gênero feminino, assim como encontrada nos tumores de glândulas salivares como um todo, isto é, três mulheres para cada dois homens (Matsuba *et al*., 1984; Ellis & Auclair, 1996).

Como muitos outros tumores de glândulas salivares, o CAC tem sido descrito como tendo muitas apresentações microscópicas apesar das células que formam esses padrões terem tamanho, forma e coloração uniforme. Desse modo, o tumor é composto por células isomórficas arranjadas em vários padrões morfológicos (Ellis *et al*., 1991).

O padrão cribriforme é considerado o mais importante e de mais fácil identificação microscópica. Esse padrão clássico do CAC é comumente reconhecido pelo arranjo em "queijo suíço" das células tumorais. Essas células são caracterizadas por terem núcleos hipercromáticos e angulados, citoplasma escasso, nucléolos evidentes e raras figuras de mitose, arranjadas em grupos de tamanho e forma variados contendo vários espaços circulares ou ovais preenchidos por substância mucinosa (Ellis *et al*., 1991).

O padrão tubular é o segundo maior subtipo do CAC. As células tumorais são iguais àquelas presentes no tipo cribriforme, entretanto, o arranjo dessas células é diferente. Estruturas ductais isoladas são formadas por camadas de células isomórficas. Em cortes longitudinais são vistas estruturas ductais ou tubulares que são responsáveis pela designação desse tipo histológico (Ellis *et al*., 1991).

O tipo sólido é caracterizado pela formação de ninhos ou lençóis de células de tamanho e forma variadas. Quase não há formação de espaços circulares ou ovais e não se observam estruturas tubulares e ductais. Necrose central, pleomorfismo e figuras de mitose podem ser encontrados ocasionalmente, fatos que não ocorrem em outros padrões do CAC (Ellis *et al*., 1991).

O tipo sólido é definido como de alto grau de malignidade na gradação histológica do CAC (Chilla *et al*., 1980; Batsakis *et al*., 1990). Por outro lado, o padrão tubular é

considerado de baixo grau e o tipo clássico ou cribriforme está situado entre os dois (Batsakis *et al*., 1990). Além disso, o modo de invasão, invasão perineural, atividade mitótica, necrose e pleomorfismo celular e nuclear são achados adicionais que muitas vezes são incorporados à gradação histológica desse grupo de tumores (Santucci & Bondi, 1986).

O CAC é reconhecido pelo grande potencial de recorrências e metástases, principalmente tardias e à distância. Matsuba *et al*. (1986) reportaram taxa de recorrência de 18% em uma série de 71 pacientes seguidos por um período de 15 anos. Spiro *et al*. (1973) relataram uma taxa de recorrência de 39,7% e uma taxa de metástase de 34,9%, sendo que 92% dessas eram à distância. O trabalho de Spiro (1997) mostra uma taxa de insucesso no tratamento de 62%, sendo que 38% desses ocorreram devido às metástases à distância.

No CAC, as metástases à distância, principalmente no pulmão e ossos, são mais freqüentes que as metástases em linfonodos regionais, apesar deste não ser comum em qualquer outro tumor de glândula salivar (Ellis & Auclair, 1996).

O tratamento do CAC consiste na excisão cirúrgica ampla (Dal Maso & Lippi, 1985; Casler & Conley, 1992). A radioterapia isolada parece ser inadequada, entretanto, alguns trabalhos mostram que quando a radioterapia é usada em conjunto com a cirurgia em tumores em estádios avançados, ela tem mostrado ser eficiente no controle local do tumor, especialmente quando há evidências microscópicas de tumor residual (Nascimento *et al*., 1986; Koka *et al*., 1989; Teshima *et al*., 1993).

Ki-67:

Ki-67 é um antígeno nuclear presente somente em células em proliferação e ausente em células quiescentes (Barrett *et al*., 2002). Uma correlação significativamente alta entre o ki-67 e características histopatológicas de malignidade têm sido relatadas em tumores de glândulas salivares (Zhu *et al*., 1999; Lazzaro & Cleveland, 2000; Alves *et al.*, 2004). Em carcinomas mucoepidermóides, a expressão de Ki-67 tem sido correlacionada à agressividade, comportamento maligno, diminuição da sobrevida e pior prognóstico. Além disso, o aumento de sua expressão parece estar relacionado ao grau histológico e pode eventualmente ser útil para determinar se um tumor de grau intermediário pode se

comportar como um tumor de baixo ou alto grau de malignidade (Skalova *et al*., 1994; Hicks & Flaitz, 2000; Okabe *et al*., 2001).

EGF:

O fator de crescimento epidérmico (EGF), que foi primeiramente isolado de glândulas submandibulares de ratos, é um potente mitogênico que inicia a síntese de DNA e a replicação celular em diversos tipos celulares originados do ectoderma ou endoderma. Seus efeitos são mediados através de seu receptor de superfície específico, que é uma glicoproteína transmembrânica que tem atividade tirosina-quinase (Hubler *et al*., 1992).

O EGF em glândulas salivares está envolvido na diferenciação acinar e ductal (Yamahara *et al*., 1988; Kosky *et al*., 1997) e tem sido detectado em glândulas salivares de fetos humanos, contudo sua distribuição e intensidade decrescem com a idade. Em adultos, só é encontrado no sistema ductal e em pequenas quantidades nos ácinos (Tsukitani *et al*., 1987). Há poucos estudos sobre o EGF em tumores de glândulas salivares.

EGFR:

O receptor de fator de crescimento epidérmico (EGFR) é uma glicoproteína transmembrânica de 170 kD pertencente à família ErbB de receptores tirosina-quinase que media os efeitos de vários fatores como o EGF e o fator de crescimento transformante alfa. É considerado um importante mediador do crescimento, diferenciação e sobrevida celular (Piludu *et al*, 2003).

Aumento na atividade ou superexpressão de EGFR tem sido associado à progressão tumoral de diversos tumores epiteliais malignos em diversas localizações como cabeça e pescoço, trato gastrointestinal, pulmão, mama e cérebro (Salomon *et al*., 1995; Woodburn, 1999). Recentemente, anticorpos monoclonais terapêuticos contra EGFR têm sido desenvolvidos para diminuir sua expressão e conseqüentemente interromper a progressão do tumor ou, até mesmo, causar sua regressão (Shelton *et al*., 2005).

Em glândulas salivares normais, o EGFR tem sido descrito em células epiteliais ductais e mucosas e sua ativação relacionada à produção de mucina (Katopodi *et al*., 2003, Piludu *et al*., 2003). Sua presença também tem sido descrita em tumores de glândulas

salivares (Yamada *et al*., 1989; Shintani *et al*, 1995; Gibbons *et al*., 2001; Vered *et al*., 2002).

ErbB-2:

ErbB-2 é um proto-oncogene localizado no cromossomo 17q que codifica uma glicoproteína transmembrânica de 185 kD pertencente à família ErbB de receptores tirosina-quinase e que desempenha um papel importante no desenvolvimento, diferenciação e sinalização mitogênica em células normais (Slamom *et al*., 1987). Vários cânceres humanos, particularmente carcinomas de mama, mostram amplificação do gene ErbB-2 e superexpressão de seus produtos gênicos (De Potter, 1994). Além disso, ErbB-2 tem sido descrito como fator prognóstico e alvo terapêutico no tratamento de um grupo de câncer de mama (Shak *et al*., 1999).

Em glândulas salivares normais, epitélio ductal apresenta fraca imunopositividade para ErbB-2, nível de expressão semelhante ao encontrado em tumores de mama que não expressam ou não amplificam esse proto-oncogene (Press *et al*., 1990). Entretanto, em tumores de glândulas salivares, expressão de ErbB-2 tem sido sugerida como um papel importante na tumorigênese e progressão dos tumores e possuir significado clínico em determinados subtipos (Karja et al., 1994; Press et al., 1994; Giannoni et al., 1995; Cho et al.,1997; Kyung-Já et al., 1997; Dori et al., 2002; Glisson et al., 2004).

FAS:

A ácido graxo sintetase (Fatty Acid Synthase - FAS) é uma enzima metabólica chave que participa da síntese endógena de ácidos graxos saturados de cadeia longa a partir dos pequenos substratos de carbono acetil-CoA e malonil-CoA (Kuhajda, 2000).

FAS está presente em altos níveis em diversos tipos de cânceres humanos. Alta expressão de FAS tem sido relatada em vários cânceres epiteliais humanos como os de mama, ovário, próstata, pulmão, cólon esôfago, bexiga, estômago, melanoma e carcinoma espinocelular oral, como em sarcomas de tecidos moles (Rashid et al., 1997, Alo et al., 2000; Oskouian, 2000; Piyathilake et al., 2000; Nemoto et al., 2001; Kusakabe et al., 2002;

Swinnen et al., 2002; Takahiro et al., 2003; Innocenzi et al., 2003, Visca et al., 2003; Silva et al., 2004). Entretanto, não há estudos desta enzima em tumores de glândulas salivares.

A expressão da proteína FAS tem sido ainda considerada como um marcador para o prognóstico de alguns destes tumores, como o câncer de próstata (Epstein *et al*., 1995), de mama (Alo *et al*., 1996), de ovário (Alo *et al*., 2000), de colon (Visca *et al*., 1999), melanoma (Innocenzi *et al*., 2003) e também sarcomas de tecidos moles (Takahiro *et al*., 2003). De acordo com Baron *et al*. (2004), tumores que expressam grandes quantidades de FAS apresentam um comportamento biológico mais agressivo comparado com aqueles que apresentam níveis normais, pois a atividade desta enzima proporciona uma vantagem seletiva para o crescimento celular. Além disso, trabalhos experimentais têm demonstrado que FAS é essencial na sobrevida de células malignas já que sua inibição é capaz de reduzir a proliferação celular através do bloqueio da replicação de DNA, consequentemente, causando apoptose nessas células (Pizer *et al*., 1998; Li *et al*., 2001).

Receptores de Estrógeno, Andrógeno e Progesterona:

Receptores de estrógeno (ER), andrógeno (AR) e progesterona (PgR) são receptores de hormônios sexuais que tem sido descritos como fatores importantes na patogênese e terapia de alguns tumores, principalmente cânceres de mama e próstata (Glas *et al*., 2002; Nasser *et al*., 2003). Além disso, o estado desses receptores hormonais é considerado como um dos mais importantes parâmetros para o prognóstico desses cânceres e é simultaneamente um critério para o planejamento de terapias hormonais para estas doenças. Terapias com antagonistas de hormônios sexuais têm sido utilizadas com sucesso em cânceres de mama positivo para ER e/ou PgR e em cânceres de próstata positivo para AR (Hortobagyi, 1998; Teymoortash et al., 2001; Berthelet et al., 2005).

Apesar de descritos em alguns trabalhos, pouco se sabe sobre a expressão desses receptores hormonais em tumores de glândulas salivares (Dimery *et al*., 1987; Barnes *et al*., 1994; Shick *et al*., 1995; Gaffney *et al*., 1995; Kapadia *et al*., 1998; Fan *et al*., 2001; Nasser *et al*., 2003).

2. PROPOSIÇÃO

O presente estudo teve como objetivo analisar e correlacionar às características histopatológicas e a expressão imunohistoquímica de alguns anticorpos em tumores de glândulas salivares oriundos de uma série de 496 casos analisados previamente.

O primeiro estudo teve como objetivo analisar as características histopatológicas e a expressão imunohistoquímica de EGF, EGFR, ErbB-2. FAS e Ki-67 em 41 adenomas pleomórficos, 30 carcinomas mucoepidermóides e 30 carcinomas adenóide cístico.

O segundo estudo teve como objetivo analisar as características histopatológicas e a expressão imunohistoquímica de receptor de andrógeno, receptor de estrógeno e receptor de progesterona em 41 adenomas pleomórficos, 30 tumores de Warthin, 30 carcinomas mucoepidermóides e 30 carcinomas adenóide cístico.

O terceriro estudo teve como objetivo descrever os achados histopatológicos de 189 adenomas pleomórficos de glândulas salivares.

3. CAPÍTULO 1

Salivary gland tumors: Immunohistochemical study of EGF, EGFR, ErbB-2, Fatty Acid Synthase and Ki-67.

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Abstract:

Epidermal growth factor (EGF) and its receptors have been implicated in the tumorigenesis of several types of cancer. Epidermal growth factor receptor (EGFR) and ErbB-2 are known to be overexpressed in a variety of tumors, have been related with poor prognosis and can stimulate the expression of fatty acid synthase (FAS), enzyme responsible for endogenous synthesis of saturated fatty acids that has been associated to proliferation and prognosis of some cancers. Expression of EGF, EGFR, ErbB-2, FAS and Ki-67 were accessed by immunohistochemistry in formalin-fixed, paraffin-embedded specimens. EGF was positive in 70% of MEC and PA and in only 23.3% of ACC. Ki-67, EGFR and ErbB-2 were more frequent in MEC, especially in high grade tumors. FAS was found in more than 50% of PA, MEC and ACC. EGF, EGFR, ErbB-2 and FAS seem to be important in the tumorigenesis of salivary gland tumors, particularly in MEC.

Keywords: Salivary gland neoplasia, EGF, EGFr, ErbB-2, Fatty acid sinthase, Ki-67.

Introduction:

Growth factors and their receptors play an important role in the growth of normal tissue and in the development and progression of human cancers[1]. Epidermal growth factor (EGF) was first isolated from the mouse submandibular gland and it is a potent mitogen that initiates DNA synthesis and cell replication in a diversity of cell types[2]. EGF in the salivary glands is involved in acinar and ductal differentiation[3,4]. It has been detected in human fetal salivary glands, however, its distribution and intensity decreases with age. In adults it is primarily found in the ductal system whereas in the acini is less frequent[1,5].

The EGF receptor family, which mediates EGF biological activity, is composed by four different transmembrane tyrosine kinase receptors: ErbB-1 (Her-1 or EGFR), ErbB-2 (Her-2 or neu), ErbB-3 (Her-3) and ErbB-4 (Her-4)[6]. Overexpression of EGFR and ErbB-2 has been shown in breast tumors[7], prostate cancer[8], oral squamous cell carcinoma[9] and salivary gland tumors[10-13]. In addition, the presence of EGFR and ErbB-2 has also been

associated with accelerated tumor progression and resistance to therapy for multiple types of malignancies[14,15]. ErbB-2, which is considered the favorite heterodimerization partner for other members of the EGFR family, plays a central role in signaling network[6]. It represents a successful therapeutic target as exemplified by the drug Herceptin (Trastuzumab), which is used for the treatment of a subset of breast cancer patients[16]. It was recently demonstrated that overexpression of ErbB-2 in breast epithelial cells stimulates Fatty Acid Synthase (FAS) mRNA synthesis[17]. In salivary gland tumors, overexpression of EGFR and ErbB-2 assayed by immunostaining techniques has been reported in mucoepidermoid carcinoma, adenoid cystic carcinoma and salivary duct carcinoma[10,12,18,19,20].

FAS is the enzyme responsible for the endogenous synthesis of saturated long-chain fatty acids from the carbon substrates acetyl-CoA and malonyl-CoA[21,22]. In normal cells, with the exception of lipogenic tissues, endogenous fatty acid synthesis is minimal since most of the fatty acids are supplied by the dietary fat[22,23]. On the other hand, several studies have demonstrated an upregulation of FAS in cancers of the prostate[24], breast[25], bladder[26], ovary[27], lung[28], melanoma[29], stomach[30], mouth[9,21] and soft tissue sarcomas[31]. In some of these tumors, particularly of the ovary, breast and melanoma, FAS overexpression has been suggested as a potential prognostic factor[25,27,29]. Additionally, experimental data have demonstrated that FAS enzymatic activity is essential for the cancer cell survival, since its specific inhibition is able to reduce cell proliferation by blocking DNA replication and causing apoptosis in cancer cell lines[32,33]. To the best of our knowledge, there is no other study analyzing FAS expression in salivary gland tumors in the English language literature.

This work describes the expression and correlation of EGF, EGFR, ErbB-2, FAS and Ki-67 in pleomorphic adenomas, mucoepidermoid carcinomas and adenoid cystic carcinomas of the salivary glands

Materials and Methods:

Files of the Department of Pathology, Londrina Cancer Institute, Paraná State, Brazil, from 1972 to 2001 were retrieved and 496 cases of salivary gland tumors with

epithelial or mesenchymal origin were analyzed[34]. The last 41 cases of pleomorphic adenoma and the last 30 cases of mucoepidermoid carcinoma and adenoid cystic carcinoma were used for immunohistochemical evaluation (Table 1).

Hematoxylin and eosin stained slides were retrieved and revised. PA were classified as stroma-rich, cell-rich and classic (balanced amount of epithelial and stromal component)[35], MEC were graded according to Ellis and Auclair[36] as low, intermediate and high grade, and ACC were classified as cribriform, tubular and solid types.

Sections from formalin-fixed and paraffin-embedded tissue were cut at 3 μm thickness and mounted on glass slides. For EGF, ErbB-2, FAS and Ki-67 staining, slides were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water, transferred to sodium citrate buffer (pH 6.0), and heated twice for 12 min in a 750 W microwave oven. Slides were cooled in room temperature for 20 min and endogenous peroxidade was blocked by incubation in a 0.05% solution of hydrogen peroxide for 30 min. For EGFR, slides were deparaffinized, rehydrated and incubated with proteinase K for 5 min and hydrogen peroxidade for 30 min. All sections were incubated overnight at 4 °C with the following primary antibodies: anti-EGF (clone Ab-3, 1/50 dilution, Oncogene, Boston, MA, USA), anti-EGFR (clone H11, 1/200 dilution, Dako, Carpinteria, CA, USA), anti-ErbB-2 (1/200 dilution, Dako, Carpinteria, CA, USA), anti-FAS (1/3000 dilution, Transduction Laboratories, Lexington, KY, USA) and anti-Ki-67 (clone MIB-1, 1/200 dilution, Dako, Carpinteria, CA, USA). The slides for EGFR, ErbB-2 and FAS were washed in phosphate-buffered saline (PBS) and incubated with a dextran polymer reagent conjugated with peroxidase and secondary antibody (Envision+, Dako, Carpinteria, CA, USA) for 1h and subsequently reacted with 3,3′-diaminobenzidine and counterstained with hematoxylin. For Ki-67 and EGF, LSAB+ (Dako, Carpinteria, CA, USA) visualization system was used following manufacturer orientations. Positive and negative controls were performed in all reactions.

Immunoreactivity for EGF, EGFR, cytoplasmic ErbB-2 and FAS was evaluated semiquantitatively and scored as negative (0–5% of positive tumor cells), weak (6-50% of positive tumor cells) and strong (>50% positive tumor cells). Membrane ErbB-2 expression were scored on a 0-3+ scale, as follows: 0, staining of <10% of tumor cells; 1+, faint and

partial membrane staining in ≥10% of tumor cells; 2+, weak to moderate complete membrane staining in ≥10% of tumor cells; or 3+, moderate to strong complete membrane staining in ≥10% of tumor cells. Scores of either 2+ or 3+ were defined as overexpression. Ki-67 positivity was counted using an image computer analyzer (Kontron 400, Carl Zeiss, Germany). A minimum of 1,000 tumor cells was counted and the results were expressed as the percentage of Ki-67 positive tumor cell nuclei.

For frequency analysis in contingency tables, statistical analyses of associations between variables were performed by the chi-square test, Fisher's exact test or Kruskal-Wallis test (with significance set for $p < 0.05$).

Results:

From 41 cases of PA, 25 (61%) were females and 16 (39%) males, with a mean age of 46.4 years, ranging from 16 to 75 years. Thirty cases were located in the parotid, 6 in the submandibular and 5 in minor salivary glands. Sixteen cases (53.3%) of MEC were in males and 14 (46.7%) in females, and the mean age was of 51.3 years, ranging from 5 to 81 years. Nineteen cases were located in the parotid 3 in the submandibular and 8 in minor salivary glands. About the 30 cases of ACC, 15 (50%) were in males with a mean age of 52.4 years, ranging from 28 to 88 years. Eight cases were located in the parotid, 2 in the submandibular and 20 in minor salivary glands (Table 1).

According to the histopathological classification, 22 cases (55%) of PA were stroma-rich, 13 (30%) cell-rich and 6 (15%) classic. Fifteen cases (50%) of MEC were classified as low grade, 3 (10%) as intermediate and 12 (40%) as high grade tumor. Cribriform pattern was the most common subtype of ACC with 15 cases (50%) followed by solid and tubular with 8 and 7 cases, respectively. (Table 2).

The immunohistochemical study showed variable positivity for EGF in the cytoplasm of striated and intercalary ductal cells and weak citoplasmic staining in acinic cells of the normal salivary gland presented in the analyzed specimens. PA and MEC showed the highest percentage of EGF positive cases with around 70% each. In both tumors, positivity was mostly found in the cytoplasm of luminal ductal cells. Although PA

and MEC showed similar percentage of positive cases, MEC demonstrated higher percentage of strong positive cases (p=0.049). In ACC, EGF was positive in only 7 cases (23.3%) and was statistically different when compared to PA (p<0.001) and MEC (p<0.001) (Table 2).

Two different patterns of EGFR positivity were identified, a sharply demarcated membrane staining (EGFRm) and a cytoplasmic staining (EGFRc). MEC and ACC showed similar percentage of EGFRm positive cases with 66.7% and 63.3%, respectively. PA demonstrated the lowest percentage with only 29.3%. When the EGFRc was accessed, MEC and ACC demonstrated both 73.3% of positive cases and PA were positive in 46.4% of the cases (Table 2). There were not statistically significant differences in the expression of EGFRc among the three tumors. When PA, MEC and ACC were compared by their immunohistochemical expression of EGFRm, there were statistically significant differences between PA and both MEC (p=0.005) and ACC (p=0.013). In ACC, the cribriform pattern demonstrated the highest values of EGFRm and EGFRc positivity when compared with the other two subtypes (p=0.006 and p=0.024, respectively) (Table 2).

ErbB-2 also demonstrated two different patterns of staining, a sharply demarcated membrane (ErbB-2m) and a cytoplasmic staining (ErbB-2c). ErbB-2c staining was noted in 78% of the cases of PA, 83.3% of MEC and 50% of ACC (Table 2). Overexpression of ErbB-2m was present in only 4.9% of PA, 36.7% of MEC and 13.3% of ACC (Table 2). Again, in PA and MEC, positivity was mainly found in the ductal luminal cell. In MEC, ErbB-2c showed a positive association with ErbB-2m (p=0.016) and both were more commonly expressed in high grade tumors (Table 2). ErbB-2c was statistically different between PA and both MEC (p=0.023) and ACC (p=0.029) and also between MEC and ACC (p=0.011). ErbB-2m was statistically different only between PA (4.9% +2 or +3) and MEC (36.7% +2 or +3).

Cytoplasmic positivity for FAS was most commonly found in both PA and MEC (68.3% and 66.7%, respectively). Moreover, MEC showed the highest percentage of strong positive cases, 60% against 32.1% of PA. Even though ACC demonstrated the lowest percentage of positive cases, 53.3% of the cases were positive for FAS. Once more, in MEC and particularly in PA, FAS was mostly found in ductal luminal cells. There was

statistically significant difference in the expression of FAS between MEC and ACC (p=0.027). In addition, there was a positive association between FAS and EGF in ACC (p=0.015) and in MEC positivity for FAS was more frequent in high grade tumors (p=0.031, Table 2). Importantly, there was not statistically significant relation between FAS and ErbB-2 in PA, MEC and ACC.

Ki-67 was positive in few cells disperse in cases of PA. In ACC, the mean Ki-67 index was 9.5 and in MEC 10.1, statistically not different from PA. There was no significant relation between Ki-67 and other studied markers. However, in MEC, Ki-67 demonstrated higher indexes in high grade tumors (mean index: 15.1, ranging from 3.1 to 38.6, p=0.042).

Discussion:

EGF in human salivary glands has been detected in serous acini and ducts. In the acini, EGF has been found in secretory granules, indicating that the growth factor is released into the saliva through granule exocytosis. In the ductal system, it is most commonly found in the principal cells of striated ducts. In these cells, abundant small cytoplasmic vesicles are localized both apically and basally, suggesting that ductal cells can release their products not only into the saliva but also into the interstitium[37]. Yamahara et al.[4] studied 48 PA and related 86% of these cases positive for EGF. Katopodi et al.[2] related 68.8% of MEC and 50% of ACC positive for EGF. We found EGF immunoreactivity in 70% of PA, 70.7% of MEC and only 23.3% of ACC.

The complex ErbB receptor family (EGFR, ErbB-2, ErbB-3 and ErbB-4) plays a critical event in human tumorigenesis. Overexpression of EGFR and ErbB-2 has been associated with several malignancies, like, breast[37], prostate[8], oral squamous cell carcinoma[9,39] as well as salivary gland tumors[10-13] and is correlated with a poor prognosis and a relative resistance to chemotherapy[36,40,41]. Recently, several studies on EGFR[42] and ErbB-2[43] expressions have demonstrated therapeutic implications as well. Herceptin, a therapeutic method based on targeting cell-surface ErbB-2 oncoprotein by a humanized monoclonal antibody, has been successfully used in ErbB-2 overexpressing breast cancer[43].

In salivary gland tumors, little is known about the expression of EGFR. Yamada et al.[44] reported 33.8% of PA and 25% of MEC with positive immunostaining for EGFR, Shintani et al.[12] did not find positivity for EGFR in 19 ACC, Gibbons et al.[45] reported 100% of MEC and 100% of ACC positive for EGFR and Vered et al.[1] described 85% of positivity for EGFR in 27 cases of ACC. This discrepancy among several studies could be explained by the used technique, which includes fixation of the tissue, antigen retrieval, scoring criteria and/or by the type of the antibody used to identify EGFR. In the present study, we assessed the membrane and cytoplasmic immunopositivity for EGFR. Membrane positivity was found in 66.7% of MEC, 63.3% of ACC and 29.3% of PA. Cytoplasmic positivity was present in 73.3% of MEC and ACC and in 46.4% of PA. In addition, both membrane and cytoplasmic EGFR were differently expressed when PA was compared with the malignant tumors and in ACC, both were most commonly found in the cribriform pattern.

Differently of EGFR, ErbB-2 is extensively studied in salivary gland tumors. Analyzing benign and malignant salivary gland tumors, Karja et al.[11] related positivity for ErbB-2 in 33.3% of the PA. Positivity for ErbB-2 varies from 30 to 38% in MEC[10,19,20,41] and 0 to 100% in ACC[11,12,19,20,45-47]. Higher ErbB-2 overexpression in MEC compared to ACC was described in the study by Glisson et al.[19], who concluded that tumors of excretory duct origin, including MEC, salivary duct carcinoma and squamous carcinoma, show higher frequency of ErbB-2 positivity than those of intercalated duct origin like ACC, acinic cell carcinoma, adenocarcinoma, malignant mixed tumor, and myoepithelial carcinoma. We found membrane positivity for ErbB-2 in 4.9% of PA, 36.7% of MEC and 13.3% of ACC. Besides being more frequent in MEC, ErbB-2 expression showed positive association with tumor grading. Whereas 63.2% of the ErbB-2m scored 0 or +1 were low grade MEC, 72.7% of +2 and +3 MEC were high grade tumors. Similar results were described in other works[10,18,41], including an association of ErbB-2 expression with an increased risk of death and poor prognosis[10,18].

In the present study, PA, MEC and ACC showed cytoplasmic positivity for EGFR and ErbB-2. Similar findings have been related in the literature[10,11,20,45,48]. This cytoplasmic reactivity may account for the internalization process of the ligand receptor complex by an

endocytotic process and for the activation of a transductional signal cascade that may lead to changes in the cellular metabolism[48]. Since ErbB receptor family could be related with the pathogenesis of salivary gland tumors, ErbB-2 has been demonstrated to activate FAS expression in breast cancer[17] and EGF can up regulate FAS expression in prostate cancer cell lines[49], we considered that FAS might play a role in the pathogenesis of salivary gland tumors. There is no other paper describing the expression of FAS in salivary gland tumors. Association of FAS and ErbB-2 has been shown in breast cancer and also associated with a poor prognosis in some tumors[17]. Nevertheless, the results presented here didn't show an evident association between them in PA, MEC and ACC. On the other hand, FAS was co-expressed with EGF in ACC and was more frequent in high grade MEC.

Cell proliferation has been considered one of the most important biological mechanisms in tumorigenesis[50]. Ki-67 antibodies are useful to evaluate the proliferative activity of tumors identifying an antigen expressed in G1, S and G2 phases of cycling cells[51]. In salivary gland tumors, proliferative activity has been used to differentiate benign from malignant tumors[52] and has been associated with poor overall survival, disease-free survival and tumor grade[53,54]. Comparison of our results with other Ki-67 studies is difficult because there are marked differences in the used technique, grading criteria of the tumors and methods of results analysis. In the present study, Ki-67 was considered an occasional finding in PA, whereas in ACC and MEC the mean index was 9.5 and 10.1, respectively. In addition, there was not correlation between the expression of Ki-67 with other studied markers. In general salivary gland tumors do not show high proliferative index. Nevertheless, in MEC ki-67 expression is related with the histological grade, and as in previous reports[55-57], we observed higher values in high grade tumors.

In summary, our results demonstrated that EGF, EGFR, ErbB-2, FAS and Ki-67 are expressed in different patterns and intensity in PA, MEC and ACC. MEC showed the highest percentage of membrane positive cases for EGFR and ErbB-2. Membrane and cytoplasmic ErbB-2 and FAS positivity were more frequent in high grade than in low grade MEC. FAS positivity was not related with EGFR, ErbB-2 or Ki-67 expression. In conclusion, EGFR, ErbB-2 and FAS seem to play a role in the tumorigenesis of salivary gland tumors, especially in MEC, and they should be more extensively studied.

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Table 1. Demographic data and localization of 41 cases of pleomorphic adenomas, 30 of mucoepidermoid carcinomas and 30 of adenoid cystic carcinomas of a Brazilian population.

	Age		Gender		Localization		
	Mean age	Age range	Male	Female	Parotid	Submandibular	Minor
Pleomorphic Adenoma	46.4	16-75	16	25	30	6	5
Mucoepidermoid Carcinoma	51.3	5-81	16	14	19	3	8
Adenoid Cystic Carcinoma	52.4	28-88	15	15	8	2	20

Table 2. Immunohistochemical profile of pleomorphic adenomas (n=41), mucoepidermoid carcinomas (n=30) and adenoid cystic carcinomas (n=30).

	EGF			EGFRc			EGFRm			ErbB-2c			ErbB-2m		FAS		
	-	+	++	-	+	++	-	+	++	-	+	++	0, +1	+2, +3	-	+	++
PA																	
Stroma-rich (n=22)	8	13	1	11	9	2	16	4	2	7	2	13	21	1	6	12	4
Cell-rich (n=13)	2	10	1	8	4	1	9	4	0	2	4	7	12	1	6	3	4
Classic (n=6)	2	4	0	3	2	1	4	1	1	0	2	4	6	0	1	4	1
Total *n*	12	27	2	22	15	4	29	9	3	9	8	24	39	2	13	19	9
(%)	(29.3)	(65.8)	(4.9)	(53.6)	(36.6)	(9.8)	(70.7)	(22)	(7.3)	(22)	(19.5)	(58.5)	(95.1)	(4.9)	(31.7)	(46.3)	(22)
MEC																	
Low grade (n=15)	5	7	3	4	9	2	5	7	3	3	10	2	12	3	8	1	6
Intermediate grade (n=3)	1	1	1	0	2	1	1	2	0	1	2	0	3	0	1	1	1
High grade (n=12)	3	6	3	4	6	2	4	3	5	1*	3*	8*	4*	8*	1*	6*	5*
Total *n*	9	14	7	8	17	5	10	12	8	5	15	10	19	11	10	8	12
(%)	(30)	(46.7)	(23.3)	(26.7)	(56.6)	(16.7)	(33.3)	(40)	(26.7)	(16.7)	(50)	(33.3)	(63.3)	(36.7)	(33.3)	(26.7)	(40)
ACC																	
Cribriform (n=15)	10	4	1	1*	10*	4*	1*	8*	6*	7	5	3	12	3	7	8	0
Tubular (n=7)	6	1	0	2	2	3	4	2	1	3	1	3	6	1	3	3	1
Solid (n=8)	8	0	0	5	3	0	6	2	0	5	0	3	8	0	4	2	2
Total *n*	23	6	1	8	15	7	11	12	7	15	6	9	26	4	14	13	3
(%)	(76.6)	(20)	(3.3)	(26.7)	(50)	(23.3)	(36.7)	(40)	(23.3)	(50)	(20)	(30)	(86.7)	(13.3)	(46.7)	(43.3)	(10)

*$p<0.05$

4. CAPÍTULO 2

Immunohistochemical study of androgen, estrogen and progesterone receptors in salivary gland tumors.

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Abstract:

Background: Estrogen, progesterone and androgen receptors have been related to growth and development of some tumors and have been successfully used as a prognostic factor, particularly in breast and prostate cancer. Studies with sex hormone receptors in salivary gland tumors are controversial.

Methods: The immunohistochemical expression of these hormone receptors in 41 pleomorphic adenomas, 30 Warthin's tumors, 30 mucoepidermoid carcinomas and 20 adenoid cystic carcinomas were accessed using a dextran polymer reagent conjugated with peroxidase and secondary antibody.

Results: All cases were negative for estrogen and progesterone receptors. Androgen receptor was positive in 2 cases each of pleomorphic adenoma, mucoepidermd carcinoma and adenoid cystic carcinoma.

Conclusions: These results do not support a role for estrogen and progesterone in the tumorigenesis of pleomorphic adenomas, Warthin's tumors, mucoepidermd carcinomas and adenoid cystic carcinomas. However, androgen receptors can play a role in a small set of salivary gland tumors, and this deserves further studies.

Keywords: Androgen receptor, Estrogen receptor, Progesterone Receptor, Salivary gland neoplasms

Introduction:

Estrogen, progesterone and androgen, acting by specific receptors, play an important role in the growth and development of several tumors, including breast, endometrium, and prostate carcinomas (1,2). Additionally, therapy with sex hormones antagonists in estrogen receptor (ER) and progesterone receptor (PgR) positive breast carcinomas and in androgen receptor (AR) positive prostate cancer have decreased recurrences and improved survival (3,4). Contrary to breast and prostate cancer, the role of sex hormones in other tumors is limited. In salivary gland tumors, ER, PgR and AR expression have been described in several studies (2,5,6,7,8,9,10,11). However, there are great disparities in the literature data, possibly due variations in the used technique, which includes fixation of the tissue, antigen retrieval, scoring criteria and/or type of antibodies used. We sought to investigate the immunohistochemical expression of ER, PgR and AR in 41 pleomorphic adenomas (PA), 30 Warthin's tumors (WT), 30 mucoepidermoid carcinomas (MEC) and 30 adenoid cystic carcinomas (ACC) of salivary glands origin.

Patients and Methods:

Files of the Department of Pathology, Londrina Cancer Institute, Paraná State, Brazil, from 1972 to 2001 were retrieved and 496 cases of salivary gland tumors were analyzed (12). The last 41 cases of PA and the last 30 cases of WT, MEC and ACC were selected for immunohistochemical evaluation. PA were classified as stroma-rich, cell-rich and classic (balanced amount of epithelial and stromal components) (13), WT as stroma-rich, stroma-poor and classic, MEC as low grade, intermediate grade and high grade according to Ellis and Auclair (14) and ACC as cribriform, tubular or solid.

Sections from formalin-fixed and paraffin-embedded tissue were cut at 3 μm thickness and mounted on glass slides. Tissues were deparaffinized, rehydrated through graded concentrations of alcohol to distilled water. For antigen retrieval of progesterone receptor, the slides were transferred to sodium citrate buffer (pH 6.0), and heated twice for 12 min in a 750W microwave oven. For estrogen receptor and progesterone receptor, Tris-

EDTA pH 9 solution in pressure cooker was used as antigen retrieval. Slides were cooled at room temperature for 20 min. Endogenous peroxidade was blocked by incubation in a 0.05% solution of hydrogen peroxide for 30 min. The sections were incubated overnight at 4 °C with the following primary antibodies: anti-Androgen receptor (clone AR441, 1/50 dilution, Dako, Carpinteria, CA, USA), anti-Estrogen receptor (clone 1D5, 1/50 dilution, Dako, Carpinteria, CA, USA) and anti-Progesterone receptor (clone PgR 636, 1/50 dilution, Dako, Carpinteria, CA, USA). The slides were washed in phosphate-buffered saline (PBS) and incubated with a dextran polymer reagent conjugated with peroxidase and secondary antibody (Envision+, Dako, Carpinteria, CA, USA) for 1h and subsequently reacted with 3,3′-diaminobenzidine and counterstained with hematoxylin. Positive and negative controls were included in all reactions.

Immunoreactivity was evaluated semiquantitatively under a light microscope and scored as negative (0–5% of positive cells), weak (6-50% of positive cells) and strong (>50% positive cells). Statistical comparisons between variables were made with Fisher's exact test and the chi-square test.

Results:

From 41 cases of PA, 25 (61%) were females and 16 (39%) males, with a mean age of 46.4 years, ranging from 16 to 75 years. Thirty cases were located in the parotid gland, 6 in the submandibular and 5 in minor salivary glands. The majority of WT were in males (24 cases, 80%), all cases were located in the parotid gland and the mean age was 56 years, ranging from 42 to 78 years. Sixteen cases (53.3%) of MEC were in males and 14 (46.7%) were in females, the mean age was of 51.3 years, ranging from 5 to 81 years. Nineteen cases of WT were located in the parotid 3 in the submandibular and 8 in minor salivary glands. Regarding the 30 cases of ACC, 15 (50%) were in males, the mean age was of 52.4 years, ranging from 28 to 88 years. Eight ACC cases were located in the parotid, 2 in the submandibular and 20 in minor salivary glands (Table 1).

According to the histopathological examination, 22 cases (55%) of PA were classified as stroma-rich, 13 (30%) as cell-rich and 6 (15%) as classic. Seventeen cases

(56.7%) of WT were classified as classic, 10 (33.3%) as stroma-poor and 3 (10%) as stroma-rich. Fifteen cases (50%) of MEC were classified as low-grade, 3 (10%) as intermediate-grade and 12 (40%) as high-grade. Cribriform pattern was the most common subtype of ACC with 15 cases (50%) followed by the solid with 8 cases (26.7%) and tubular pattern with 7 cases (23.3%).

Immunohistochemistry for ER and PR were negative in all cases studied. AR was positive in only 2 of 41 cases of PA (figure 1A), negative in all WT, positive in 2 of 30 cases of MEC (figure 1B), and in 2 of 30 cases of ACC (figure 1C). Of the 2 cases positive for AR in PA, one was classified as classic and the other as cell-rich. Both MEC positive cases were high-grade tumors and both ACC were of cribriform type.

Discussion:

It is well known that hormonal therapy is very useful in the treatment of breast and prostate cancer (3,4). However, despite of the expression of sex hormones receptor in other types of cancer like endometrial carcinomas (15), carcinomas of the thyroid (16), renal cell carcinomas (17), melanoma (18), and meningiomas (19), the efficacy of hormonal therapy in these tumors has not yet been established.

Studies with ER, PgR and AR in salivary gland tumors are conflicting (Tables 2 and 3). ER expression was detected by Jeannon et al. (20) in 3 of 10 MEC, 3 of 6 ACC and in 4 of 10 PA, while Nasser et al. (2) reported 1 of 10 MEC positive for ER, and in Glas et al. (1) study, 19% of PA demonstrated immunoreactivity for ER. On the other hand, this study and several others (8,9,21,22,23,24) did not reveal ER expression in the cases studied.

PgR has been reported more frequently than ER. Shick et al. (9) and Dori et al. (22) studding ACC found 50% and 7.4% of PgR positive cases, respectively. Jeannon et al. (20) related one positive case out of 10 cases of PA, but all 10 cases of MEC and 6 of ACC were negative. Nasser et al. (2) related one positive MEC, but all ACC, PA and WT were negative. Teymoortash et al. (24) found 6 cases positive for PgR in 9 cases of WT, but all 5 cases of PA were negative. Glas et al. (1) reported 60 positive cases of PA out of 69 studied, and also suggested that PgR could be a prognostic factor in recurrent PA of the

parotid gland. In the present study, we did not find PgR expression in PA, WT, MEC and ACC.

There are few studies of AR in PA, WT, MEC and ACC. Moriki et al. (25) related all these tumors negative for AR. Nasser et al. (2) did not find AR expression in benign tumors, but related 2 of 10 MEC and 2 of 10 ACC positive for AR. In our study, AR was seen in only 2 cases each, of 41 PA, 30 MEC and 30 ACC. In contrast to the low or absent AR expression in PA, MEC, WT and ACC, it has been described to be positive in almost all salivary duct carcinomas (2,10,25) and therefore potentially useful for their diagnosis in biopsy or cytological samples (25,26). Additionally, Locati et al. (27) reported a complete remission with androgen-deprivation therapy in a recurrent AR expressing adenocarcinoma of the parotid gland. Although AR has been described in salivary gland tumors, particularly in malignancies, it is not known if this fact is due to a role for androgen in the pathogenic process or simply represents an epiphenomenon of the malignant transformation (2).

In summary, our results support that ER, PgR do not play a role in the tumorigenesis of PA, WT, MEC and ACC. On the other hand, considering the report of a remission with an androgen-deprivation therapy of an adenocarcinoma of salivary gland (27), eventual cases positive for AR should be better studied and considered as potential targets for treatment with anti-androgens drugs.

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Table 1: Demographic data from 41 cases of pleomorphic adenoma (PA), 30 cases of Warthin's tumor (WT), 30 cases of mucoepidermoid carcinoma (MEC) and 30 cases of adenoid cystic carcinoma (ACC):

	Age		Gender		Location		
	Mean age	Age range	Male	Female	Parotid	Submandibular	Minor
Pleomorphic Adenoma	46.4	16-75	16	25	30	6	5
Warthin's Tumor	56	42-78	24	6	30	0	0
Mucoepidermoid Carcinoma	51.3	5-81	16	14	19	3	8
Adenoid Cystic Carcinoma	52.4	28-88	15	15	8	2	20

Table 2: Summary of the reports of estrogen receptor (ER), progesterone receptor (PgR) and androgen receptor (AR) in pleomorphic adenoma (PA) and Warthin's tumor (WT):

Authors	Year	PA				WT			
		No. of cases	ER +	PgR +	AR +	No. of cases	ER +	PgR +	AR +
Lamey et al.	1987	4	0	0	-	-	-	-	-
Jeannon et al.	1999	10	4	1	-	-	-	-	-
Moriki et al.	2001	10	-	-	0	10	-	-	0
Teymoortash et al.	2001	5	0	0	-	9	0	6	-
Glas et al.	2002	69	13	60	-	-	-	-	-
Nasser et al.	2003	10	0	0	0	10	0	0	0
Our study	2006	41	0	0	2	30	0	0	0

Table 3: Summary of the reports of estrogen receptor (ER), progesterone receptor (PgR) and androgen receptor (AR) in mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC):

		MEC				ACC			
Authors	Year	No. of cases	ER +	PgR +	AR +	No. of cases	ER +	PgR +	AR +
Miller et al.	1994	-	-	-	-	5	0	-	-
Shick et al.	1995	-	-	-	-	12	0	6	-
Jeannon et al.	1999	10	3	0	-	6	3	0	-
Dori et al.	2000	-	-	-	-	29	0	2	-
Moriki et al.	2001	6	-	-	0	8	-	-	0
Nasser et al.	2003	10	1	1	2	10	0	0	2
Pires et al.	2004	136	0	-	-	72	0	-	-
Our study	2006	30	0	0	2	30	0	0	2

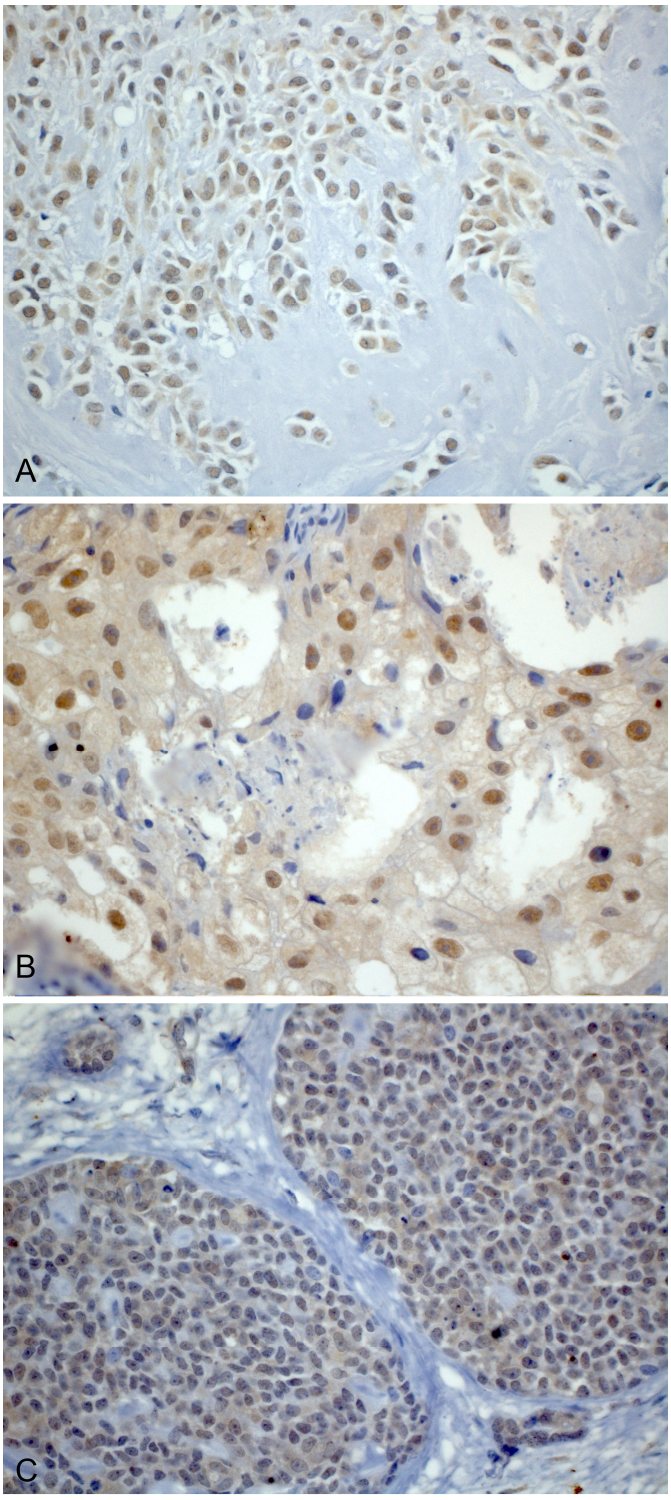

Figure 1: Androgen receptor (AR) positivity in A: pleomorphic adenoma (x400), B: mucoepidermoid carcinoma (x400) and C: adenoid cystic carcinoma (x400).

5. CAPÍTULO 3

Histopathological Findings of Pleomorphic Adenomas of the Salivary Glands.

Running title: Histopathology of Pleomorphic Adenomas

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Abstract:

Pleomorphic adenoma (PA) is the most frequent salivary gland tumor and it is known by its great variety of histopathological aspects. Despite the diverse histological patterns, the presence of both epithelial and mesenchimal-like tissue is common to all PA. In this paper, we describe the histopathological characteristics of 189 cases of PA with special reference to the epithelial and mesenchimal components. Plasmacytoid cells were the most commonly found cellular type followed by fusiform and cuboidal cells. Trabeculae and duct-like structures were the most frequent patterns formed by the epithelial cells. Myxoid and chondroid stroma were the most frequently found mesenchimal-like tissue usually forming the so called myxochondroid stroma.

Key Words: Histopathology, Pleomorphic adenoma, Salivary gland neoplasms

Introduction:

Salivary gland tumors are rare, comprising less than 3% of all neoplasms of the head and neck region[1] and are known by their complex microscopical features. Pleomorphic adenoma (PA) is the most common salivary gland tumor and represents 60% to 73% of the parotid gland tumors, 12% to 60% of the submandibular and 14% to 70% of the minor salivary glands tumors[2,3,4]. It is a benign neoplasm composed by epithelial and myoepithelial cells arranged in a great variety of morphological patterns, with areas of mesenchymal differentiation[6,7]. Epithelial cells tipically form duct-like structures associated with non-ductal cells presenting variable shapes and forms. The stromal element demonstrates varying degrees of myxoid, hyaline, cartilaginous, or osseous differentiation[6].

In this paper we describe the histopathological characteristics of 189 cases of PA with special reference to the morphology of the epithelial cells and stromal components.

Methods:

Salivary gland tumors were retrieved from the files of the Department of Pathology, Londrina Cancer Institute, Paraná State, Brazil, including the period from 1972 to 2001. PA was the most frequent tumor with 269 cases, comprising 54.2% of all tumors and 80.3% of the benigns[4]. Considering the presence of representative amount of the tumor and discarding biopsy cases, 189 out of 269 were used in this study.

The tumors were classified as myxoid or stroma-rich, cellular or cell-rich and classic (balanced amount of epithelial and stromal components) as described by Seifert et al.[5]. The epithelial component was analyzed taking into consideration the presence of plasmacytoid, spindle, clear, squamous, basaloid, cubic, oncocitoid and mucous cells and the morphological pattern (trabecular, ductal, cystic and solid). The stromal component was analyzed according to the presence of myxoid, hyaline, chondroid or calcified tissue.

Results:

The majority of cases were located in the parotid gland (70.9%) followed by minor salivary glands (18%) and submandibular gland (11.1%). No cases were found in the sublingual gland. The mean age was 42.9 (±16) years ranging from 13 to 90 years and the peak of incidence was in the fourth and fifth decades. 124 cases (65.6%) were in females while 65 (34.4%) in males. PA were classified as stroma-rich in 99 cases (52.4%), cell-rich in 69 (36.5%) and classic in 21 cases (11.1%) (Table 1).

Plasmacytoid cells (Figure 1a) were the most commonly found cellular type, been present in all studied tumors and they were the predominant cellular type in 32 cases (16.9%). They represented less than 30% of the tumor cells in 37.6% of the cases, 31% to 50% in 49.2% of the cases and more than 50% in 13.2% of the cases. Spindle cells (Figure 1b) were present in 180 cases (95.2%), representing the second cellular type most frequent. It was predominant in 21.7% of the cases and corresponded to less than 30% of the tumor cells in 65.1% of the cases, 31% to 50% in 21.2% of the cases and more than 50% in 9% of the cases. Cuboidal cells (Figure 1c) were found in 85.7% of the cases, in 48.1% it

represented less than 30% of the tumor cells, in 34.9% it represented 31% to 50% of the tumor cells and in only 2.6% it corresponded to more than 50% of the tumor cells. On the other hand, cuboidal cells were the predominant cell type in 58 cases (30.7%). Basaloid cells (Figure1d) were the fourth cellular type more frequent, been present in 49.2% of the cases and in all of them corresponded to less than 50% of the tumor cells. Additionally, basaloid cells were most commonly found in cell-rich PA subtype. Squamous cells (Figure 1e) were found in 40.2 % of the cases, however in only 3 cases they represented more than 35% of the tumor cells. Clear cells (Figure 1f) were present in only 69 cases (36.5%), representing less than 30% of the tumor cells in 33.3% of the cases and they were the predominant cellular type in 6 cases. Mucous and oncocitic cells were considered occasional findings. They were present in 22.2% and 9.5% of the cases, respectively, but in none they were present in more than 15% of the tumor cells (Table 2). In 54 cases (28.6%) there was not predominance of a single cellular type, showing a balanced admixture of two or more cellular types.

In general, nuclear features of all cell types were uniformly bland with small or absent nucleoli. Mitosis were rare, however, in 6 cases (3.2%) we found focal cellular atypia non related to infarcted tissue. Five cases (2.6%) demonstrated infarction with necrotic areas. In one case, disperse pigmented cells were found in the parenchyma of the tumor.

Concerning the morphological patterns of the epithelial component, trabeculae formation (Figure 2a) was found in 96.8% of the cases. It was present in all stroma-rich and classic subtypes and in 91.3% of the cell-rich subtype. Ductal, cystic and solid formations (Figure 2a-2c) were seen in 92.6%, 37.6% and 60.3% of the cases, respectively. These tree architectural configurations were most commonly found in cell-rich or classic than in stroma-rich tumors (Table 3).

Myxochondroid stroma was present in 82.5% of the cases. Myxoid areas (Figure 3a) were found in 94.2% of the cases corresponding to 44.6% of the stromal component. It was present in 92.9% of the stroma-rich tumors, 97.1% of the cell-rich subtype and in 90.5% of the classic form. The stroma-rich subtype demonstrated higher percentage of myxoid tissue when compared to other subtypes. Chondroid areas (Figure 3b) were seen in 82.5% of the

cases corresponding to 32.2% of the stromal component and they were less frequent in cell-rich tumors. Hyalinization (Figure 3c) was found in 79.9% of the cases, mainly in the cell-rich variant, corresponding to only 18.1% of the stromal component. In only 22 cases (11.6%) hyalinization predominated over myxoid or chondroid stroma. Calcifications (Figure 3d) were seen in only 4 cases (2.1%) and did not correspond to more than 5% of the stroma (Table 3).

Discussion:

PA is a slow-growing benign salivary gland tumor, most commonly arising in the parotid gland. It accounts for 60% to 73% of the parotid gland tumors, 12% to 60% of the submandibular and 14% to 70% of the minor salivary glands tumors[2,3,4]. Female patients are more affected than males[2,6,8,9] and the peak of incidence occurs in the fourth and fifth decades[2,8]. In the present study, PA was also more frequent in the parotid gland of female patients with age between 30 and 50 years.

Regardless of the great variety of histopathological aspects the main diagnostic feature is the presence of both epithelial and mesenchimal-like tissues. The proportion of these tissues has been used to subclassify PA, however, it does not have therapeutic or prognostic significance[6]. In our study, stroma-rich subtype corresponded to 52.4% of the cases, cell-rich 36.5% and classic 11.1%, results interestingly close to those reported by Stennert et al.[10] and Paris et al.[11].

In the current study, plasmacytoid cells were the most frequent cell type followed by spindle cells. Ellis and Auclair[6] related that these cells appear to be in transition from one form to the other. Additionally, in PA, plasmacytoid cells seem to originate from luminal rather than myoepithelial cells[12]. The other cellular types, with the exception of the squamous cells that are commonly abrupt and organized in islands, also seem to be closed associated with one another and correspond to transition forms from one type to another[6].

Cuboidal cells located in hypocellular areas were considered to have a pre-chondroprogenitor phenotype, they express cartilage-derived morphogenic protein (CDMP-1), that may play a role in the acceleration of the transdifferentiation from cuboidal

neoplastic myoepithelial cells to lacunar cells in an autocrine manner[13]. Acinar phenotypes such as mucous cells in PA could reflect either an abnormal line of differentiation or luminal cells with increased synthesis and/or retention of variably mature glycoproteins[14]. Development of oncocytes has been associated with acinar and striated duct cells[15] and this metaplasia probably occurs in other cell types as well[16]. In our study, cuboidal cells were the third more common cellular type. On the other hand, mucous cells and oncocytes were considered occasional, been present in few cases and in little amounts.

It is known that benign PA may, in some cases, contain focal areas of marked atypia and/or bizarre tumor cells[6,7]. In addition, atypical cytomorphology could be found in tumors previously manipulated by biopsy or fine needle aspiration, especially within infarcted areas and necrotic tissue[7]. We found 6 cases (2.6%) containing cellular atypia non-related to infarcted areas. These findings were similar to those previously reported by Takeda et al.[7]. On the other hand, Ohtake et al.[17] described 51% of the analyzed cases with cellular atypia, 6% focal, 15% sporadic and 30% singular.

Mesenchymal-like elements of PA including chondroid and myxoid tissues were shown to be related to neoplastic myoepithelial cells migrating into the stroma[18]. In the present study, the predominance of the myxochondroid stroma was clear. Hyalinization of the stroma was ccommon, although in small quantities, nevertheless in 22 cases (11.6%), it predominated over myxoid or chondroid tissue. Prominent zones of hyalinization have been related to an aggressive behavior or malignant transformation of PA[19], however we believe that hyalinization as an isolated fact is not sufficient to predict this progression.

In summary, these results emphasize the immense variety of cells, architectures and morphological characteristics present in PA of the salivary gland. Since PA is the most frequent salivary gland neoplasia and they can resemble other salivary gland tumors, the knowledge about these variations is essential for a correct diagnosis.

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Table 1: Demographic data and histological classification of 189 cases of pleomorphic adenoma.

Variables	Patients, n (%)
Location:	
Parotid	134 (70.9)
Submandibular	21 (11.1)
Minor	34 (18)
Age, y:	
<20	8 (4.2)
21-30	39 (20.6)
31-40	41 (21.7)
41-50	43 (22.8)
51-60	28 (14.8)
61-70	18 (9.5)
>70	12 (6.3)
Sex:	
Females	124 (65.6)
Males	65 (34.4)
Histological classification:	
Stroma-rich	99 (52.4)
Stroma-poor	69 (36.5)
Classic	21 (11.1)

Table 2: Cellular types present in the epithelial component of pleomorphic adenomas:

Tumor cells	Cells of the epithelial component							
	Plasmacytoid	Spindle	Cuboidal	Basaloid	Squamous	Clear	Mucous	Oncocytic
<30% *n (%)*	71 (37.6)	123 (65.1)	91 (48.1)	80 (42.3)	67 (35.4)	63 (33.3)	42 (22.2)	18 (9.5)
31-50% *n (%)*	93 (49.2)	40 (21.2)	66 (34.9)	13 (6.9)	9 (4.8)	3 (1.6)	0	0
>51% *n (%)*	25 (13.2)	17 (9)	5 (2.6)	0	0	3 (1.6)	0	0
Total *n (% of total)*	189 (100)	180 (95.2)	162 (85.7)	93 (49.2)	76 (40.2)	69 (36.5)	42 (22.2)	18 (9.5)

Table 3: Epithelial and stromal morphology of pleomorphic adenomas:

	Epithelial Component				**Stromal Component**			
	Trabeculae	Ducts	Cysts	Solid	Myxoid	Chondroid	Hyalinization	Calcification
Stroma-rich *n (%)*	99 (100)	90 (90.9)	28 (28.3)	47 (47.5)	92 (92.9)	89 (89.9)	75 (75.8)	2 (2)
Cell-rich *n (%)*	63 (91.3)	65 (94.2)	34 (49.3)	51 (73.9)	67 (97.1)	49 (71)	61 (88.4)	2 (2.9)
Classic *n (%)*	21 (100)	20 (95.2)	9 (42.9)	16 (76.2)	19 (90.5)	18 (85.7)	15 (71.4)	0 (0)
Total *(% of total)*	183 (96.8)	175 (92.6)	71 (37.6)	114 (60.3)	178 (94.2)	156 (82.5)	151 (79.9)	4 (2.1)

Figure Legends:

Figure 1: Epithelial cell types present in pleomorphic adenomas. A: plasmacytoid cells (H&E, x200); B: fusiform cells (H&E, x200); C: cuboidal cells (H&E, x400); D: basaloid cells (arrows, H&E, x200); E: squamous cells (H&E, x100); F: clear cells (H&E, x400).

Figure 2: Morphological patterns of the epithelial component. A: trabecular (left) and solid (right) (H&E, x100); B: ductal (H&E, x100); C: cystic (H&E, x50).

Figure 3: Mesenchymal-like components of pleomorphic adenomas. A: myxoid (H&E, x100); B: chondroid (H&E, x100); C: hyaline (H&E, x100); D: calcifications (H&E, x100)

Figure 1:

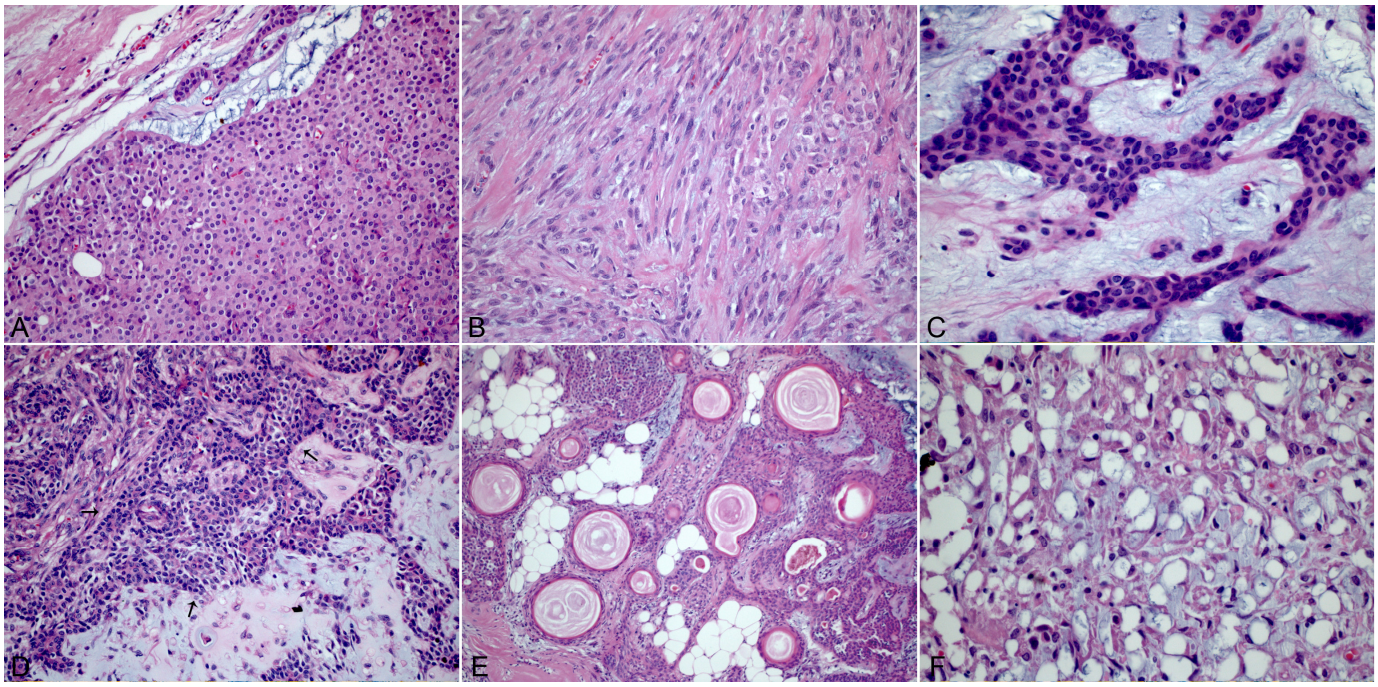


Figure 2:

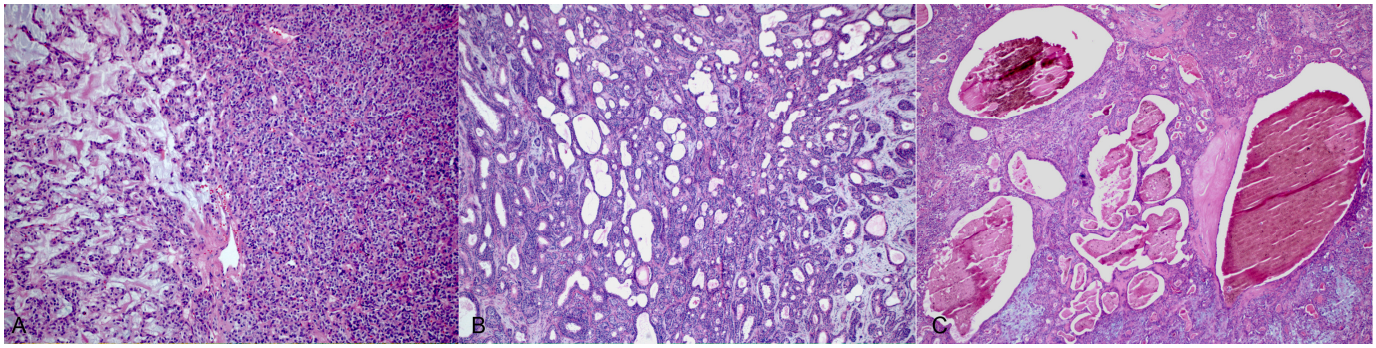
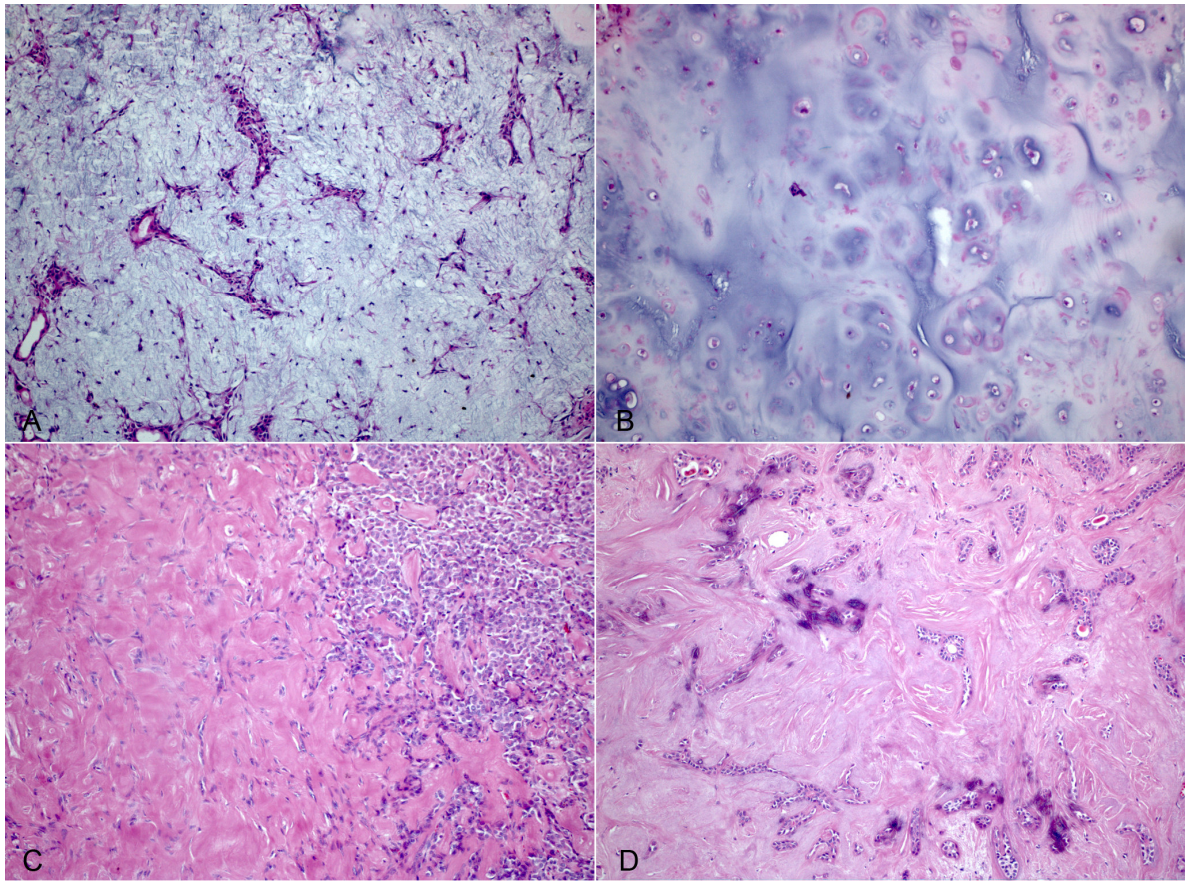


Figure 3:



6. CONCLUSÕES

1. EGF, EGFR, ErbB-2, FAS e Ki-67 expressam em padrões e intensidades distintos em adenomas pleomórficos, carcinomas mucoepidermóides e carcinomas adenóide cístico.

2. ErbB-2, FAS e Ki-67 são mais freqüentemente encontrados em carcinomas mucoepidermóides de alto grau do que em carcinomas mucoepidermóides de baixo grau e de grau intermediário.

3. Em adenomas pleomórficos, carcinomas mucoepidermóides e carcinomas adenóide cístico, positividade para FAS não está relacionada à positividade para EGFR, ErbB-2 ou Ki-67.

4. EGFR, ErbB-2 e FAS parecem desempenhar papel na tumorigênese de tumores de glândulas salivares, especialmente em carcinomas mucoepidermóides, e devem ser mais extensivamente estudados.

5. Receptor de estrógeno e receptor de progesterona não desempenham papel importante na tumorigênese de adenomas pleomórficos, tumores de Warthin, carcinomas mucoepidermóides e carcinomas adenóide cístico.

6. Em tumores de glândulas salivares, apesar de poucos casos positivos, receptor de andrógeno deve ser mais bem estudado e considerado como potencial alvo em tratamentos com drogas anti-andrógenos.

7. A maioria dos adenomas pleomórficos é estroma-rico, apresenta maior proporção de células plasmocitóides e fusiformes, forma trabéculas e ductos e possue estroma mixocondróide.

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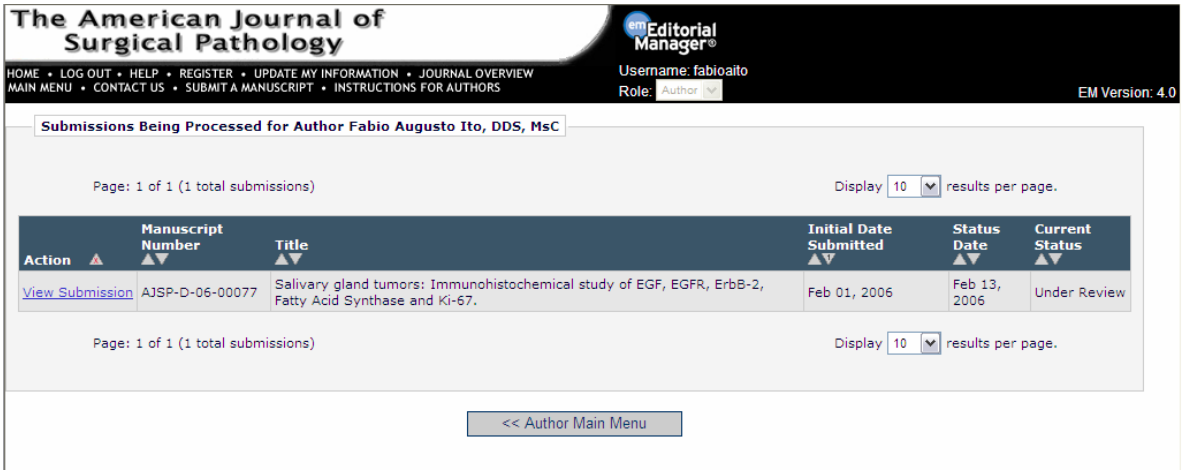

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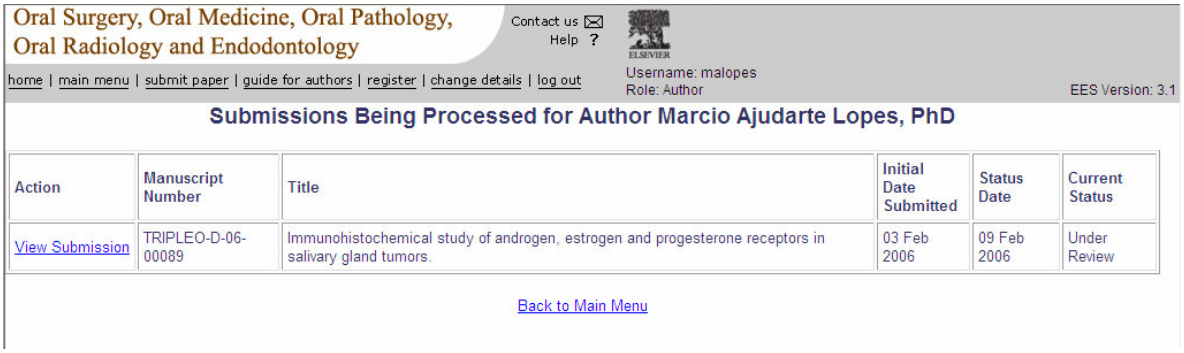

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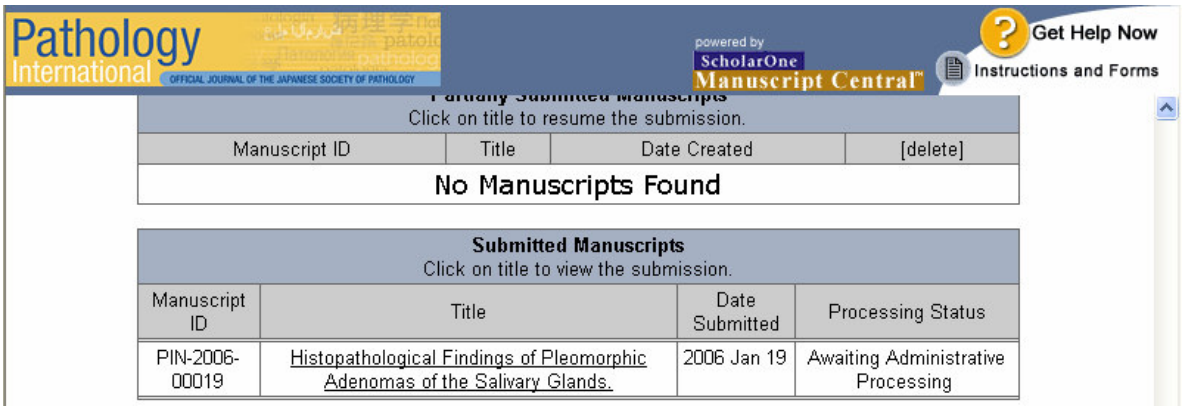
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Certificamos que o Projeto de pesquisa intitulado "Levantamento epidemiológico de lesões bucais do instituto do câncer de londrina-pr", sob o protocolo nº **110/2001**, do Pesquisador ***Fabio Augusto Ito***, sob a responsabilidade do Prof. Dr. **Márcio Ajudarte Lopes**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 21 de dezembro de 2001

We certify that the research project with title "Epidemiological survey of oral lesions of the londrina cancer institute", protocol nº **110/2001,** by Researcher ***Fabio Augusto Ito***, responsibility by Prof. Dr. **Márcio Ajudarte Lopes**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Resarch at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, December 21 2001

p/ *Prof. Dr. Pedro Luiz Rosalen*
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