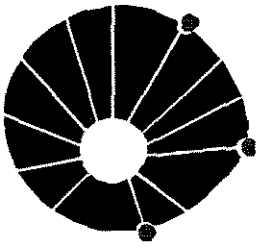
FACULDADE DE ODONTOLOGIA DE PIRACICABA



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FÁBIO DE ABREU ALVES
cirurgião-dentista

ESTUDO IMUNOHISTOQUÍMICO E ANÁLISE UNIVARIADA DOS FATORES PROGNÓSTICOS DE TUMORES DE GLÂNDULA SUBMANDIBULAR

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, para obtenção do Título de Doutor em Biologia e Patologia Buco-dental.

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Orientador: Prof. Dr. Luiz Paulo Kowalski

Banca examinadora: Prof. Dr. Fernando Augusto Soares
Prof. Dr. José Magrin
Prof. Dr. Osvaldo Di Hipólito Júnior
Prof. Dr. Márcio Ajudarte Lopes

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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, em sessão pública realizada em 15 de Março de 2002, considerou o candidato FABIO DE ABREU ALVES aprovado.

1. Prof. Dr. LUIZ PAULO KOWALSKI_____

2. Prof. Dr. JOSÉ MAGRIM_____

3. Prof. Dr. FERNANDO AUGUSTO SOARES_____

4. Prof. Dr. MARCIO AJUDARTE LOPES_____

5. Prof. Dr. OSVALDO DI HIPOLITO JUNIOR_____

"As vezes a gente quer que o fruto amadureça antes do tempo. Mas, somente o tempo sabe dar as coisas para gente, na hora certa. O tempo sabe o momento exato de dar o presente que a gente merece".

Dedico este trabalho,

Aos meus pais **HELY** e **MARIA**, pelo amor, ensinamentos e oportunidades que sempre me deram.

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SUMÁRIO

RESUMO

ESTUDO IMUNOHISTOQUÍMICO E ANÁLISE UNIVARIADA DOS FATORES PROGNÓSTICOS DE TUMORES DE GLÂNDULA SUBMANDIBULAR

O objetivo deste trabalho foi analisar as características clínicas, histopatológicas e a expressão de marcadores imunohistoquímicos em tumores de glândula submandibular. Foram avaliados 102 tumores, sendo 61 benignos e 51 malignos. Os tumores benignos consistiram de 60 adenomas pleomorfos e 1 adenoma de células basais. Entre os malignos, 23 foram carcinomas adenóide císticos, 16 carcinomas mucoepidermóides, 4 carcinomas espinocelulares, 4 adenocarcinomas sem outra especificação, 1 adenocarcinoma de células basais, 1 carcinoma de ducto salivar, 1 mioepitelioma maligno e 1 carcinoma indiferenciado. A idade média dos pacientes com tumores benignos foi de 36,3 anos e 61,7% dos pacientes eram do sexo feminino. Microscopicamente, a maioria dos adenomas pleomorfos apresentava áreas mixocondróides (Subtipo II). Todos os casos foram negativos para Ki-67 e p53. Com relação aos tumores malignos, a idade média foi 55,4 anos e 64,7% dos pacientes eram do sexo masculino. No momento do diagnóstico, 64,7% dos pacientes apresentaram tumores em avançado estádio clínico, 15,7% apresentaram metástases em linfonodos regionais e 7,84% metástases à distância. As sobrevidas global e livre de doença após 10 anos foram de 26,8% e 44,9%, respectivamente. A expressão de PCNA, Ki-67, c-erbB-2, CEA e bcl-2 não apresentou associação com as variáveis clínicas e histológicas ($p<0,05$). Entretanto, p53 correlacionou com os estádios T e N, morte e sobrevida global ($p<0,05$). Nossos resultados indicam que adenomas pleomorfos de glândula submandibular apresentam crescimento lento e têm bom prognóstico. Carcinoma adenóide cístico foi o tumor maligno mais freqüente e o prognóstico de tumores malignos em glândula

submandibular é desfavorável, principalmente em tumores com avançado estadiamento clínico e que expressaram p53.

ABSTRACT

IMMUNOHISTOCHEMICAL STUDY AND UNIVARIATE ANALYZIS OF THE PROGNOSTIC FACTORS OF SUBMANDIBULAR SALIVARY GLAND TUMORS

The aim of this work was to analyze clinocopathological and immunohistochemical expression in submandibular salivary gland tumors. A total of 112 tumors was evaluated, being 61 benign and 51 malignant. The benign tumors were compose of 60 pleomorphic adenomas and 1 basal cell adenoma. Among the malignancies, 23 were adenoid cystic carcinoma, 16 mucoepidermoid carcinomas, 4 squamous cell carcinomas, 4 adenocarcinomas NOS, 1 basal cell adenocarcinoma, 1 salivary duct carcinoma, 1 malignant myoepithelioma and 1 undifferentiated carcinoma. The patients with benign tumors presented a mean age of 36.3 years and 61.7% of them were females. Microscopically, most of the pleomorphic adenomas were rich in myxocondroid areas (subtype II) and all cases were negatives for p53 and Ki-67. Considering malignant tumors, the mean age was 55.4 years and 64.7% of the patients were males. At the time of diagnosis, 64.7% of the patients presented tumors in advanced clinical stage, 15.7% presented regional lymph nodes metastases and 7.84% distant metastases. The expression of PCNA, Ki-67, c-erbB-2, CEA e bcl-2 was not correlated with clinicopathological features ($p<0.05$). However, p53 expression was associated with T and N stages, death and overall survival ($p<0.05$). Our results showed that pleomorphic adenomas of submandibular glands presented low growth rate and good prognosis. Adenoid cystic carcinoma was the most common malignant tumor, and submandibular malignant tumors presented poor prognosis, mainly in tumors with advanced clinical stage and with p53 expression.

INTRODUÇÃO

Os tumores de glândulas salivares são relativamente raros, compreendendo de 1 a 4% de todas as neoplasias do organismo. A grande maioria desses tumores está localizada na parótida, correspondendo a cerca de 70% dos casos. Com relação à glândula submandibular, a proporção é de 5 a 10%, na sublingual de 1%, nas glândulas salivares menores de 5 a 15% e 5% outros locais (GATES 1982, EVESON & CAWSON 1985, SPIRO 1986, PYPER et al. 1987).

A incidência de tumores malignos nas glândulas salivares varia consideravelmente de acordo com o local. Em torno de 25% dos tumores localizados na parótida são malignos, na submandibular varia de 35 a 55%, e nas glândulas salivares menores de 40 a 85%. A maioria dos tumores localizados nas glândulas sublinguais é maligno, representando 80 a 90% dos casos (ENEROTH 1970, ENEROTH 1971). Avaliando uma série de 2.410 casos de tumores de glândula salivar, EVESON & CAWSON (1985) encontraram freqüência de tumores malignos semelhante à de ENEROTH (1970, 1971) em relação as glândulas menores, submandibular e sublingual. Entretanto, na parótida somente 14,7% dos tumores foram malignos. No estudo realizado por LOPES et al. (1999) em 196 tumores de glândulas salivares menores, apenas 68 casos (34,7%) foram benignos. Adenoma pleomorfo foi o tipo histológico predominante com 65 casos (95,4%), e os outros 3 casos eram adenomas de células basais (4,4%). Esta grande diferença com relação à maioria dos casos ser maligno, está relacionada provavelmente ao levantamento ter sido realizado no Hospital AC Camargo (Hospital do Câncer-SP), centro de referência para tratamento de câncer. BARDWIL et al. (1966) e SPIRO (1986) também encontraram alta incidência de tumores malignos em glândulas salivares menores, 87% e 80% respectivamente. Com relação à

glândula submandibular, alguns autores têm descrito uma incidência maior de tumores benignos (EVENSON & CAWSON 1985, PYPER et al. 1987), enquanto que outros observaram incidência igual entre neoplasias benignas e malignas (BATSAKIS 1986, FRIEDMAN et al. 1986).

Adenoma pleomorfo é o tumor benigno mais comum envolvendo as glândulas salivares maiores e menores, acometendo em torno de 60 a 70% de todas as neoplasias da parótida, 40 a 60% de tumores na glândula submandibular, 40 a 70% de tumores em glândulas salivares menores, por sua vez a glândula sublingual é raramente afetada por esta neoplasia (EVESON & CAWSON 1985, SPIRO 1986, CHIDZONGA et al. 1995). LASKAWI et al. (1995) estudaram 38 casos de tumores benignos de glândula submandibular tratados cirurgicamente entre 1966 e 1992. Adenoma pleomorfo foi o tipo histológico predominante com 35 casos (92%). CHIDZONGA et al. (1995) estudaram clínica e histologicamente 206 casos de adenomas pleomorfos. O tumor foi mais freqüente no sexo feminino (58,3%), sendo a parótida o local mais afetado com 39,8% dos casos, em seguida as glândulas salivares menores e submandibulares com 37,9% e 22,3%, respectivamente. Considerando as glândulas salivares menores o palato foi o local mais envolvido com 55 casos (26,7%). Nenhum caso foi registrado nas glândulas sublinguais.

Adenoma pleomorfo é caracterizado por grande diversidade histológica com componentes epiteliais e estromal. O estroma neoplásico pode apresentar áreas fibrosas, hialinas, mixóides e cartilaginosas, sendo as células mioepiteliais responsáveis pela produção destas matrizes extracelulares (DARDICK et al. 1982, PALMER, 1985). Para avaliar a quantidade de estroma, SEIFERT et al. (1980) classificaram os adenomas pleomorfos em 4 subtipos histológicos: I- aproximadamente metade da massa tumoral (30-

50%) é composta de estroma; II- a maior parte (> 80%) do tumor é composta de estroma; III- < 30% do tumor é formado de estroma e IV- < 30% do tumor é estroma, mas com a predominância de células monomórficas (plasmocitóides ou hialinas). CHAU & RADDEN (1989) avaliaram a quantidade de estroma em 53 adenomas pleomorfos de glândulas salivares menores, 29% dos casos eram pobres em estroma (subtipos III e IV). Estes autores compararam os subtipos histológicos com os tumores que não eram encapsulados, mas não houve diferença significativa. CESINARO et al. (1994) encontraram alta incidência dos subtipos I e II (78,5% dos casos), todavia os autores não mencionaram a localização dos adenomas pleomorfos.

O carcinoma mucoepidermóide é considerado o tumor epitelial maligno mais comum das glândulas salivares menores e maiores (REGESI et al. 1985). O comportamento biológico do carcinoma mucoepidermóide é muito variado, e conseqüentemente várias classificações histológicas têm sido propostas na tentativa de caracterizar as variantes desse tipo de carcinoma (FOOTE & FRAZELL 1953, HEALEY et al. 1970, BATSAKIS & LUNA 1990). FOOTE & FRAZELL (1953) classificaram os carcinomas mucoepidermóides em baixo grau e alto grau. Os tumores de baixo grau eram associados clinicamente a tumores benignos, entretanto, a recorrência era freqüente e alguns casos apresentavam metástases. Os tumores de alto grau apresentavam curso clínico agressivo e freqüentemente os pacientes faleciam por recorrências locais ou metástases. Contudo, um grande número de carcinomas mucoepidermóides não podia ser classificado como baixo grau e nem como alto grau. Alguns pesquisadores (HEALEY et al. 1970, BATSAKIS & LUNA 1990) sugeriram que deveria ser criado um terceiro subtipo, que apresentava características clínicas e histológicas intermediárias entre o carcinoma mucoepidermóide de

baixo e alto grau. AUCLAIR et al. (1992) analisaram 143 carcinomas mucoepidermóides intraorais para identificar as características clínicas e histológicas mais importantes para a graduação deste tumor. Observaram que, presença de sintomatologia e localização do tumor em língua ou assoalho bucal foram os fatores clínicos que sugeriram comportamento mais agressivo. Com relação aos aspectos histológicos, presença de necrose, invasão perineural, anaplasia celular, menor quantidade de espaços císticos e mitoses atípicas foram as características que indicaram a maior agressividade do tumor. Nenhum dos 122 pacientes classificados como de baixo grau morreu devido à doença. Dos 10 pacientes com carcinoma mucoepidermóide de alto grau, 6 morreram por causa do tumor. A maioria destes pacientes teve metástases regionais e recorrência, e todos metástases à distância. O sistema proposto por ELLIS & AUCLAIR (1996) está resumido no quadro abaixo:

Característica histopatológica	Escore
Componente intra-cístico menor que 20%	+2
Presença de invasão neural	+2
Presença de necrose	+3
Mitoses (4 ou + por campo em maior aumento)	+3
Presença de anaplasia celular	+4

Escore: 0-4: baixo grau de malignidade; 5-6: grau intermediário de malignidade; ? 7: alto grau de malignidade.

Carcinoma adenóide cístico representa aproximadamente 20% dos tumores malignos de glândula salivar, sendo o tumor maligno mais freqüente da glândula submandibular (ANDERSEN et al. 1991, CAMILLERI et al. 1998, SYKES et al. 1999).

Vários padrões microscópicos são descritos: 1- Cribiforme é o tipo clássico, composto de pequenas células basofílicas arranjadas em monocamadas, formando ilhas ou cordões de variados tamanhos e contendo no interior material hialino basofílico ou eosinofílico. 2- Tubular é composto de estruturas ductais formadas por 2 ou mais camadas de células. 3- Sólido é formado por ilhas de células basalóides com escasso citoplasma. Alguns tumores sólidos podem conter áreas de necrose, pleomorfismo celular e mitoses. O carcinoma adenóide cístico raramente apresenta microscopicamente um único padrão. Usualmente dois ou mais padrões são encontrados em um mesmo tumor.

Estudos sobre tumores malignos de glândulas salivares menores realizados por WALDRON et al. (1988) e LOYOLA et al. (1995), mostram incidência de carcinoma mucoepidermóide de 35,9% e 44%, respectivamente. Um grupo de 113 pacientes com tumores malignos de glândulas salivares foi revisado por O'BRIEN et al. (1986), com objetivo de correlacionar os dados clínicos e características histológicas com a sobrevida. Entre os 113 pacientes, havia 61 do sexo masculino e 53 do feminino, a idade média foi de 55 anos. Carcinoma mucoepidermóide e carcinoma adenóide cístico foram os tipos histológicos mais freqüentes, correspondendo a 43% e 27% dos casos, respectivamente. Cerca de 2/3 dos carcinomas adenóide císticos ocorreram na glândula submandibular e nas glândulas salivares menores. Quando associados os fatores clínicos como a sobrevida, o sexo não foi considerado importante, no entanto, a idade foi significativa. Pacientes com menos de 60 anos tiveram prognóstico melhor do que os mais idosos. O local anatômico do tumor primário também foi importante, visto que, os tumores de glândulas submandibulares tiveram pior prognóstico. A baixa incidência dos diversos tipos de tumores malignos em glândulas salivares torna difícil estabelecer seu valor prognóstico. Entretanto, alguns fatores

como tipo histológico, grau de diferenciação do tumor e local de origem podem influenciar significativamente o prognóstico (SPIRO et al. 1973, EVANS 1984). Todavia, o estádio clínico é considerado o fator mais importante (SPIRO et al. 1978, SPIRO et al. 1979, BORTHNE et al. 1986, O'BRIEN et al. 1986).

ANDERSEN et al. (1991) estudaram 95 pacientes com carcinomas de glândulas submandibular, sublingual e salivares menores. Em 38 casos (40%) a glândula acometida era a submandibular, e o tumor mais comum foi carcinoma adenóide cístico (16 casos), seguido por 6 casos de adenocarcinoma, 6 de carcinoma ex-adenoma pleomorfo, 3 de carcinoma mucoepidermóide, 3 de carcinoma espinocelular, 3 de carcinoma indiferenciado e 1 de adenocarcinoma sem outra especificação. O principal sintoma apresentado foi aumento de volume indolor, e apenas 5% dos casos tinham história de dor. Os tumores foram classificados de acordo com UICC (União Internacional Contra o Câncer): 14 pacientes tiveram tumores classificados no estádio I, 27 no estádio II, 12 no estádio III, e 16 no estádio IV. Não foi possível estabelecer o estadiamento em 26 pacientes. Comparando a sobrevida entre os subtipos histológicos, o prognóstico foi significativamente melhor nos casos com carcinoma adenóide cístico. No entanto, esta diferença diminuiu após 10 anos, indicando a necessidade de acompanhamento por um longo tempo.

O'BRIEN et al. (1986) analisaram 113 pacientes através da análise multivariada para avaliar os fatores prognósticos. Em 57 casos os tumores eram de parótida, 40 de glândulas salivares menores e 16 de glândula submandibular. Eles observaram que estadiamento clínico, idade do paciente e grau histológico do tumor foram significantes. Os pacientes jovens com carcinoma mucoepidermóide e em estádios iniciais tiveram melhor sobrevida. HOCWALD et al. (2001) avaliaram os fatores prognósticos em tumores de

glândulas salivares maiores e a idade foi um fator importante, 79% dos pacientes com mais de 50 anos de idade tinham tumores mais agressivos enquanto que 56% dos pacientes mais jovens tinham tumores agressivos. LOPES et al. (1998) também demonstraram que a idade estava significativamente associada com as taxas de sobrevida. Pacientes mais jovens tiveram prognóstico melhor em relação aos idosos.

Um total de 128 pacientes com tumores malignos de glândulas salivares menores intra-orais foi avaliado por LOPES et al. (1998). Os tipos histológicos mais comuns encontrados foram carcinomas mucoepidermóide e adenóide cístico, correspondendo a 76 e 34 casos, respectivamente. O palato duro foi o local mais comum com 62 casos, e em seguida a língua com 16 casos. No entanto, os fatores prognósticos mais importantes foram o tipo histológico (Carcinoma adenóide cístico) e a presença de metástase em linfonodos regionais. SPIRO et al. (1991) estudaram 378 pacientes portadores de tumores malignos de glândulas salivares menores e também observaram que estadiamento clínico e grau histológico do tumor foram importantes.

Alguns estudos têm utilizado anticorpos dirigidos a diferentes tipos de citoqueratina, vimentina e actina muscular específica proporcionando recursos para o estudo da diferenciação, histogênese e realização de um perfil imunohistoquímico auxiliando o diagnóstico dos tumores de glândulas salivares (KUMASA et al. 1988, ARAÚJO et al. 1994, ARAÚJO & SOUZA 1996). Entretanto, o uso de reações imunohistoquímicas em tumores de glândulas salivares são mais freqüentemente utilizados na diferenciação entre tumores malignos e benignos ou na tentativa de estabelecer o prognóstico (GALLO et al. 1995, TSUJI et al.1995, ZHAO et al. 1996, TAKAHASHI et al.1998, NAGAO et al. 1998). Todavia, poucos são os estudos clínicos e imunohistoquímicos utilizando exclusivamente

tumores de glândulas submandibulares, a maioria dos dados são da parótida e de glândulas salivares menores. A pesquisa de oncogenes e genes supressores tumorais e marcadores de proliferação celular nestes tumores são importantes e podem explicar melhor as diferenças na evolução clínica dos casos em cada localização.

c-*erb*B-2

O c-*erb*B-2 ou neu-oncogene foi primeiramente identificado em neuroblastoma de rato. Pertence à família dos oncogenes relacionados à tirosina-quinase, codificando uma glicoproteína transmembranosa Mr 185.000, que apresenta estrutura semelhante a do receptor do fator de crescimento epidérmico, no entanto, com composição química distinta (SHIH et al. 1981, STERN et al. 1986).

A amplificação do gene ocorre no sítio do cromossoma humano 17, sendo esta alteração atualmente encontrada em uma variedade de adenocarcinomas originados em diferentes sítios do corpo humano. A amplificação do oncogene c-*erb*B-2 e a expressão de sua proteína correspondente têm sido sugeridas como indicadores de mau prognóstico em carcinomas de mama, ovário, rins e pulmão (TANDON et al. 1989).

Os resultados encontrados na pesquisa de amplificação do c-*erb*B-2 por métodos de genética molecular são semelhantes aos obtidos através da expressão de sua proteína pela técnica imunohistoquímica. O padrão de positividade imunohistoquímica é membranoso, com o delineamento uniforme da membrana citoplasmática (BERGER et al. 1988). CHO et al. (1997) estudaram 25 casos de carcinoma mucoepidermóide de glândulas salivares analisando as características clínicas, fatores prognósticos e expressão da oncoproteína c-*erb*B-2. Os tumores foram graduados histologicamente em grau I (5 casos), grau II (12

casos) e grau III em 8 casos. A expressão de c-*erb*B-2 foi observada em 9 casos (36%), com tendência de estar associada com o mais alto grau histológico, e este tendo grande importância no prognóstico. SUGANO et al. (1992) analisaram a correlação entre a positividade do c-*erb*B-2 e prognóstico de 59 pacientes com tumores malignos de glândulas salivares, sendo 35 de parótida, 20 de glândula submandibular e 4 de glândula sublingual. A positividade foi observada em 13 (22%) dos 59 casos. Interessante ressaltar que os resultados positivos foram observados somente em adenocarcinomas e carcinomas ex-adenoma pleomorfos, e não foi encontrado em nenhum outro tipo histológico como carcinoma adenóide cístico, carcinoma mucoepidermóide e carcinoma de células escamosas. Eles concluíram que a positividade para c-*erb*B-2 é um indicador de agressividade para o adenocarcinoma e carcinoma ex-adenoma pleomorfo de glândulas salivares maiores.

p53

O gene supressor tumoral TP53 está localizado no braço curto do cromossomo 17. Em estudos de biologia molecular foram detectados alterações em nível de RNAm, através da substituição de um nucleotídeo, com conseqüente alteração na cadeia de aminoácidos constituintes da proteína p53. As mutações no gene TP53 são as mais freqüentes anormalidades genéticas encontradas em cânceres humanos (HOLLSTEIN et al. 1991).

A oncoproteína p53 é uma fosfoproteína nuclear de 53kD, incidente na passagem da fase G0 para a fase S (síntese) do ciclo celular. Os seus níveis encontram-se aumentados em células que apresentam crescimento exponencial, comparando-se com as células que se encontram em repouso. A mutação "mis-sense" (rearranjo na seqüência gênica que codifica

os aminoácidos da proteína p53, dando origem a outros aminoácidos, que levam a produção de uma proteína "mutante", não funcional, que fica armazenada no núcleo por tempo superior ao da proteína original) é a alteração genética mais freqüente. Outras alterações são a inativação ou deleção do gene TP53, também associados ao desenvolvimento de câncer e a progressão tumoral (HOLLSTEIN et al. 1991, KAKLAMANIS et al. 1993).

NAKANISHI et al. (1994) verificaram imunopositividade nuclear para a proteína p53 em carcinoma espinocelular e em displasias moderadas e severas de orofaringe. A positividade foi de 44% dos 132 casos de carcinomas invasivos (+58/132) e 20% dos 69 casos analisados de displasia. A imunopositividade em carcinomas espinocelulares dispõe-se em diferenciação zonal, sendo maior em regiões basais do epitélio escamoso displásico e em ilhotas de células escamosas invasivas (periferia do tumor). Tais regiões são consideradas áreas de maior atividade proliferativa.

A expressão da proteína p53 foi analisada em 11 casos de adenocarcinoma de células basais (9 localizados na parótida e 2 na submandibular) e em 9 adenomas de células basais (todos localizados na parótida). Foi considerado imunopositivo quando > 10% das células tumorais estavam marcadas. Seis casos de adenocarcinoma de células basais foram positivos e todos os casos de adenoma de células basais foram negativos (NAGAO et al. 1998). A expressão de p53 em carcinoma mucoepidermóide tem sido bastante variada na literatura, GALLO et al. (1995) e KÄRJÄ et al. (1997) encontraram alta expressão de p53, 77,8% e 69,2%, respectivamente. Entretanto, SOINI et al (1992) e DOI et al. (1999) encontraram baixa expressão. Os autores comentam que esta divergência pode estar relacionada a diferentes anticorpos utilizados.

PCNA

O Antígeno Nuclear de Proliferação Celular (PCNA) foi descrito por MIYACHI et al. (1978), que o definiram como um auto-anticorpo contra o antígeno nuclear em células proliferantes de pacientes com Lupus Eritematoso Sistêmico. O padrão de positividade do PCNA é nuclear, podendo ocorrer diferenças na intensidade de marcação, em decorrência da expressão distinta nas diferentes fases do ciclo celular, portanto, maior positividade nuclear para as células que se encontram na fase S (Síntese) do ciclo celular (GARCIA et al. 1989, ITALL et al. 1990).

A expressão de PCNA e p53 foram avaliadas em 75 tumores benignos e malignos da cavidade oral, compreendendo 50 carcinomas espinocelulares, 14 leucoplasias e 11 adenomas pleomorfos. A expressão da proteína p53 foi significantemente mais alta nos casos de carcinomas espinocelulares (44%) dos que nas leucoplasias (14,3%) e adenomas pleomorfos (9,1%). Contudo, imunopositividade para PCNA foi de 98% em carcinomas, 85% nas leucoplasias e 72,7% nos adenomas pleomorfos (TSUJI et al. 1995).

Em 1996, ZHAO et al. avaliaram a imunopositividade para PCNA em adenocarcinomas de glândulas salivares. A intensidade e a distribuição da coloração variou de acordo com os tipos tumorais, 5 casos de carcinoma de células acinares e 1 adenocarcinoma polimórfico de baixo grau mostraram relativamente baixa proliferação celular. Treze casos de carcinoma adenóide cístico foram positivos, no entanto, não houve diferenças significativas entre os subtipos sólido, cribriforme e tubular. Em seis casos de adenocarcinomas divididos em padrão papilar-cístico bem diferenciados e padrão sólido pobremente diferenciado, os subtipos papilar-cístico foram mais significativamente positivos que os sólidos, mostrando a perda da relação entre a expressão de PCNA e

diferenciação celular.

CEA (Antígeno Carcinoembrionário)

Antígeno carcinoembrionário é uma glicoproteína oncofetal com peso molecular de 200 kD, composta por 40 a 60% de carboidratos (ROGERS 1976). É largamente usado na detecção de recorrência tumoral e para determinar a resposta de tratamento em pacientes com carcinoma do trato digestivo (TABUCHI et al. 1988).

Analisando a presença de CEA em tumores de glândulas salivares menores e em glândulas normais, SAITO et al. (1984) relataram que a expressão do CEA foi observada em 5 dos 11 casos de adenomas pleomorfos, 6 dos 7 carcinomas mucoepidermóides e em todos os 3 casos de carcinomas adenóides císticos. As glândulas salivares normais foram negativas para CEA. KUHEL et al. (1995) analisaram os níveis de CEA no soro e a expressões de CEA através de estudo imunohistoquímico em um paciente com carcinoma adenóide cístico de traquéia. Inicialmente, a presença de CEA no soro foi elevada, no entanto, após o tratamento cirúrgico da lesão o nível de CEA diminuiu, e elevou-se novamente quando foi constatada metástase abdominal. Após a ressecção do tumor da região abdominal, o nível de CEA decaiu novamente. O estudo imnuhistoquímico foi realizado no material retirado cirurgicamente e mostrou imunopositividade para CEA. MCDICKEN & SCOTT (1981) analisaram a expressão de CEA em 62 tumores de glândulas salivares menores. Imunoreatividade foi observada em 59% dos adenomas pleomorfos, 57% dos tumores mucoepidermóides, 62% dos carcinomas adenóide císticos e 60% dos adenocarcinomas e sugeriram que a identificação de CEA na saliva e plasma poderia auxiliar na monitoração pós-operatória dos tumores. ALFARO & CARROZZA

(1990) estudaram 23 tumores de glândulas salivares e observaram CEA em tumores benignos e malignos, bem como em glândulas parótidas normais e sugeriram que a presença de CEA não é um marcador adequado para diferenciar tumores benignos e malignos de glândulas salivares. Entretanto, JI (1993) analisou a expressão de CEA, S-100, CK 12 e CK 27 em carcinomas ex-adenomas pleomorfos de glândulas salivares e relatou que a positividade para CEA no citoplasma das células tumorais foi um verdadeiro marcador de malignidade.

bcl-2

A proteína bcl-2 está localizada na membrana mitocondrial e está relacionada a aumento da sobrevida celular, inibindo a apoptose (LU et al. 1996). NAGAO et al. (1998) avaliou a expressão de bcl-2 em adenomas de células basais e em adenocarcinomas de células basais. Todos os adenomas de células basais foram positivos, enquanto que em 27,3% dos adenocarcinomas de células basais a expressão foi negativa.

Ki-67

A expressão de Ki-67 está relacionada a células duplicando ativamente. Tanto tumores malignos quando benignos podem expressar Ki-67, no entanto, sua expressão será maior em lesões biologicamente mais agressivas. HICKS & FLAITZ (2000) estudaram a expressão de Ki-67 em carcinomas mucoepidermóides em crianças e adolescentes, a expressão deste marcador mostrou uma tendência em aumentar dos tumores de baixo grau para os de alto grau. LAZZARO & CLEVELAND (2000) avaliaram a expressão de Ki-67 em biópsias de tumores de glândulas salivares com o objetivo de distinguir os tumores

benignos dos malignos. Mas, em ambos os tumores a porcentagem de células que expressaram Ki-67 foi baixa. Desta forma, este autores concluíram que Ki-67 não deve ser utilizado para diferenciar tumores de glândulas salivares.

OBJETIVOS

- Realizar um levantamento de todos os tumores de glândula submandibular em pacientes atendidos no Departamento de Cirurgia de Cabeça e Pescoço e Otorrinolaringologia do Hospital A.C. Camargo (Hospital do Câncer) no período de 1954 à 1998.

- Analisar as características clínicas, histopatológicas e imunohistoquímicas dos tumores de glândula submandibular e estabelecer os fatores prognósticos destes tumores.

CAPÍTULO 1 (Trabalho submetido para publicação na revista Archives Otolaryngology Head and Neck Surgery)

Pleomorphic adenoma of the submandibular gland: Clinicopathological and immunohistochemical features of 60 cases in Brazil

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ABSTRACT

Submandibular glands are involved in only 5% to 10% of the salivary gland tumors and pleomorphic adenoma (PA) is the most common. This study describes the clinicopathological features and immunohistochemical expression of Ki-67 and p-53 in 60 PA of the submandibular salivary glands. Most of the patients were in the 3rd and 5th decades of life and 61.7% were females. Tumor size varied from 1 to 10cm and the mean time of the initial symptoms was 52 months. Local recurrence occurred in only one patient after 3 years of treatment. Microscopically, the majority of cases presented vast stromal component, mainly chondromyxoid. The stroma-rich PAs were larger than stroma-poor PAs ($p < 0.017$). All cases were negative for Ki-67 and p-53. These results show that PA of submandibular glands are microscopically similar to PA of other salivary glands and have a low proliferative rate and good prognosis.

Key words: pleomorphic adenoma; submandibular; salivary gland tumor.

INTRODUCTION

Salivary gland tumors comprise 1% to 4% of all human neoplasias. Parotid accounts for more than 70% of the cases, submandibular salivary gland is involved in 5% to10%, sublingual in 1% and the minor glands in 5% to 15%[1]. Pleomorphic adenoma (PA) is the most common tumor involving both major and minor salivary glands. It accounts for about 60 to 70% of all parotid neoplasms, 40 to 60% of all submandibular tumors, and 40 to 70% of minor salivary gland tumors[1,2]. PA is characterized by great histological diversity with myoepithelial cells considered to be responsible for the production of the extracellular matrices[3,4].

Expression of genes related with cell proliferation and oncogenesis seems to be associated with the prognosis of some oral tumors[5]. The expression of Ki-67 evaluated by immunohistochemical methods has been correlated with mitotic activity, histological grade and clinical behavior of tumors, including salivary gland tumors[5,6]. In addition, the mutation of the tumor suppressor gene p-53 is the most common genetic alteration in human cancer[5].

Many studies of PA are of parotid and minor salivary glands and few series of submandibular glands have been reported. Therefore, the objective of this study is to describe the clinical, histopathological and immunohistochemical features of PA of the submandibular salivary gland.

MATERIAL AND METHODS

A total of 61 cases of benign tumors of the submandibular salivary glands were reviewed. The specimens and clinical data were obtained from the files of the Cancer Hospital AC Camargo, São Paulo, Brazil. The study was carried out with approval of the

Human Research Ethics Committee of the hospital. All cases except one Basal Cell Adenoma (BCA) were diagnosed as PA. The paraffin-embedded specimens were stained with haematoxylin and eosin and classified according to Seifert et al. (1980)[7] in 4 subtypes as follows: I- approximately half of their bulk (30-50%) composed of stroma, II- most of their bulk (greater than 80%) composed of stroma, III- little of their bulk (less than 30%) composed of stroma and IV- less than 30% of stromal component but with a predominant monomorphic epithelial cellular component (plasmacytoid or hyaline cellular appearance).

Immunohistochemistry using Ki-67 and p53 was performed in 3?m paraffin sections, mounted on coated glass slides. Antigen was retrieved in citrate buffer (pH 6) with the help of a microwave oven (2 cycles of 12 min each). Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum to reduce nonspecific binding, the slides were incubated overnight at room temperature with primary antibodies against Ki-67 (Dako, MIB-1; 1:200), and p-53 (Dako, DO-7; 1:200). Peroxidase activity was visualized with 0.02% diaminobenzidine hydrochloride containing 0.03% hydrogen peroxidase. All slides were counterstained with haematoxylin. After each step, the sections were washed with phosphate buffered saline. Negative control was obtained using nonimmune serum instead of the primary antibody. Samples of squamous cell carcinoma of floor of the mouth were used as positive control. The statistical analysis was performed using chi-squared test. A p value below 0.05 was considered significant.

RESULTS

Most of the patients (64%) were between the 3rd and 5th decades of life, with a mean age of 36.32?16.3 years and 62.3% were females. Tumor size varied from 1 to 10cm, with a mean of 4.08?1.9 cm. The main symptom was a swelling at the submandibular region and the time of complaint varied from 2 to 240 months, with a mean of approximately 54.6 months (Table 1).

Fifty eight cases of PA out of 60 were treated by surgery, 54 cases for primary tumor and 4 due to local recurrence. These 4 patients were initially treated in another service. The other two patients did not receive any treatment; one patient died after one month of diagnosis of pulmonary infection and the other patient refused treatment and did not return. The treatment of all 54 patients with primary tumor consisted of surgical resection of the tumor and the submandibular gland. The 4 patients with recurrent PA, 2 were also treated by excision of the submandibular gland and the tumor. The other 2 cases, only the tumor was extirpated because the gland had been removed previously. Analyzing the 54 cases with primary PA, local recurrence was observed in only one case after 3 years of treatment. This patient underwent another surgery and is asymptomatic for 8 years. Considering the patients with recurrent PA, three out of four are asymptomatic. However, the other one developed high-grade mucoepidermoid carcinoma (MEC) in the parapharyngeal space, 20 years after the treatment of pleomorphic adenoma and died. The MEC was considered not associated with the previous PA.

The majority of the PA presented vast stromal component, mainly chondromyxoid areas and classified as subtypes I and II (Fig.1). To verify the association between tumor

size and histological subtype, we correlated the value below and above the median size (3.5cm) with the subtypes. The majority of the PA (70.4%) classified as subtype 2 was ? 3.5cm, 68.2% of the subtype 1 were ? 3.5cm and 63.6% of the subtype 3 were ? 3.5cm (Table 2). The stroma-rich tumors were significantly larger than cellularized tumors ($?^2$ test, $p < 0.017$). Regarding time of complaining and tumor size, it was not observed correlation between them. Other microscopic features were also observed as necrosis in a hyalinized area in one case, squamous differentiation in other 9 cases (Fig.2) and capsular infiltration in 7 cases. The 4 recurrent cases showed multilocular areas, but without features of malignancy. Considering the immunohistochemical analysis, all cases were considered negative for Ki-67 and p-53, although 7 tumors showed few positive cells (< 1% of the tumor cells) for Ki-67 and 11 cases for p-53.

One case of our series of 61 cases of benign tumors of the submandibular gland was classified as basal cell adenoma (BCA) solid type. The patient was 58 years old, female, and the main complain was a swelling present at the submandibular region with 36 months of evolution. Surgical treatment consisted of resection of the tumor and the submandibular gland. The patient is asymptomatic for 10 years.

DISCUSSION

PA is the most common neoplasm of the salivary glands, involving more frequently the parotid gland (63.4%), followed by minor salivary glands (18,4%), submandibular (9,5%), and sublingual glands (0,1%)[8]. Chidzonga et al.[9] also observed higher prevalence of PA in the parotid, followed by minor and submandibular glands, and none case was found in the sublingual gland. The present study describes 60 cases of PA of the

submandibular gland. In fact, we reviewed all benign submandibular salivary gland tumors and only one case was not diagnosed as PA, but BCA. The mean age of the patients at the time of the diagnosis was 36.3 years. Eveson and Cawson[2] evaluated 2.410 tumors affecting the salivary glands, 72.9% involved the parotid, 14% the minor salivary glands, 10.7% the submandibular, 0.3% the sublingual and 2.2% the site was unknown. The majority of the submandibular gland tumors (59.5%) were PA and the mean age of the patients was 44.5 years. Laskawi et al.[10] analysed 38 cases of benign tumors of submandibular gland, being 35 PA, 2 lipomas and 1 hemangioma. The mean age of the patients with PA was 47 years, similar to Eveson and Cawson[2] series, however one decade older than our cases. Chau & Radden[11] and Lopes et al.[12] reported that the mean age of patients with benign minor salivary gland tumors was 42 and 43.5 years, respectively. According to the AFIP series[8], considering all salivary glands, the mean age of patients with PA is 41.2 years. As regards the gender, it is well accepted that women are more affected by PA[2,9,11,12]. In our series, females were more affected as well, corresponding to 62.3% of the cases. In general, benign salivary gland tumors present as painless swelling. However, in the oral cavity, ulceration and pain can be present difficulting speech and swallowing[11,12]. Our study also showed that PAs of the submandibular glands have few clinical signs or symptoms besides the painless swelling.

Resection of the tumor plus the involved gland is the treatment of choice for benign submandibular gland tumors[2,10]. In all 54 primary tumors of our series, the treatment was the resection of the tumor and the involved gland, and only one case presented recurrence. Of the 4 cases treated for recurrence, 3 patients are asymptomatic and the other patient developed a high grade MEC in the parapharyngeal space 20 years after treatment of PA.

The tumors probably were not related, because the second primary tumor was located at a distinct place, and the histopathological features showed a high grade MEC, without areas remembering PA.

PA characteristically shows a variable amount of myxochondroid stroma produced by myoepithelial cells[3,4]. Chau & Radden[11] studied 53 cases of intra-oral PA and 29 were stroma-poor (subtypes III and IV). These authors compared the histological subtypes of PA with non-encapsulated lesions, but there were no significant differences. In our series, most of the cases also were stroma-rich (subtypes I and II). Cesinaro et al.[13] also found high incidence of the subtypes I and II (78.5%) PA, but the authors did not described the localization of the tumors. The stroma-rich PAs were significantly larger than the other PAs, these findings are similar to those of Naeim et al[14], who suggested that PAs are highly cellular in early stages of development. However, Chau and Radden[11] described that stroma-poor PAs are larger than the stroma-rich tumors and the authors suggested that the cellular tumors may grow in a faster rate.

Microscopical findings as necrosis, nuclear atypia, hyalinization, and invasion of adjacent tissue, increased abnormal mitotic activity have been related with aggressive behavior or malignant transformation of PA[15]. One case in our series presented necrosis in a hialynized area but the tumor was encapsulated and there were no other features indicating malignancy. Allen et al.[16] described areas of necrosis in 5 cases of benign tumors of the salivary glands and considered as caused by ischaemia. Other characteristic that may lead to histopathological problems in PA is extensive squamous differentiation, mainly in small biopsy or in fine needle aspiration biopsy. In this situation, diagnosis of MEC or squamous cell carcinoma may be done wrongly[17].

In our cases, expression of Ki-67 and p-53 was negative in all tumors. Lazaro & Cleveland[6] analysed the expression of Ki-67 and p-53 in benign and malignant intraoral salivary gland tumors. All benign tumors were negative for Ki-67 and in some cases there was a low expression of p-53. Tsuji et al.[5] evaluated the immunoreactivity for p-53 in 11 PA, and only one case was positive. These findings suggest that PA present a low proliferative index and mutant p-53 is only eventually expressed.

In conclusion, PA is the most common tumor in submandibular glands. Clinically, as in other glands, PA of the submandibular gland grows slowly. Only one case presented recurrence after 3 years of the treatment. Microscopically, most cases of PA were rich in chondromyxoid areas (subtype II) and all cases were negative for p-53 and Ki-67. The larger tumors presented rich stromal components, while smaller tumors were cellularized ($p<0.017$).

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Table 1. Clinical characteristics and histological subtypes* of 60 cases of pleomorphic adenoma of the submandibular salivary gland in Brazil.

Variables	Nº of cases (%)
Age (years)	
10-20	11 (18.3)
21-30	15 (25.0)
31-40	10 (16.7)
41-50	14 (23.3)
> 50	10 (16.7)
Time of symptoms (months)	
< 5	39 (65.0)
5-10	13 (21.7)
11-15	4 (6.7)
> 15	4 (6.7)
Size (cm)	
< 3	10 (16.7)
3-6	43 (71.7)
> 6	7 (11.7)
Gender	
Male	23 (38.3)
Female	37 (61.7)
Histological Subtype	
I (30-50% stroma)	22 (36.7)
II (> 80% stroma)	27 (45.0)
III (< 30% stroma)	7 (11.7)
IV (< 30 % stroma with plasmocytoid or hyaline cells)	4 (6.7)

* Histological subtypes were considered according to Seifert et al.[7]

Table 2. Correlation between tumor size and histological subtypes of 60 cases of pleomorphic adenoma of the submandibular salivary gland in Brazil.

Size (cm)	Subtypes			p
	I	II	III and IV*	
? 3.5	15 (68.2%)	8 (29.6%)	7 (63.6%)	0.017
? 3.5	7 (31.8%)	19 (70.4%)	4 (36.4%)	

* For statistical analysis the subtype 4 was considered together with subtype 3.

Legends

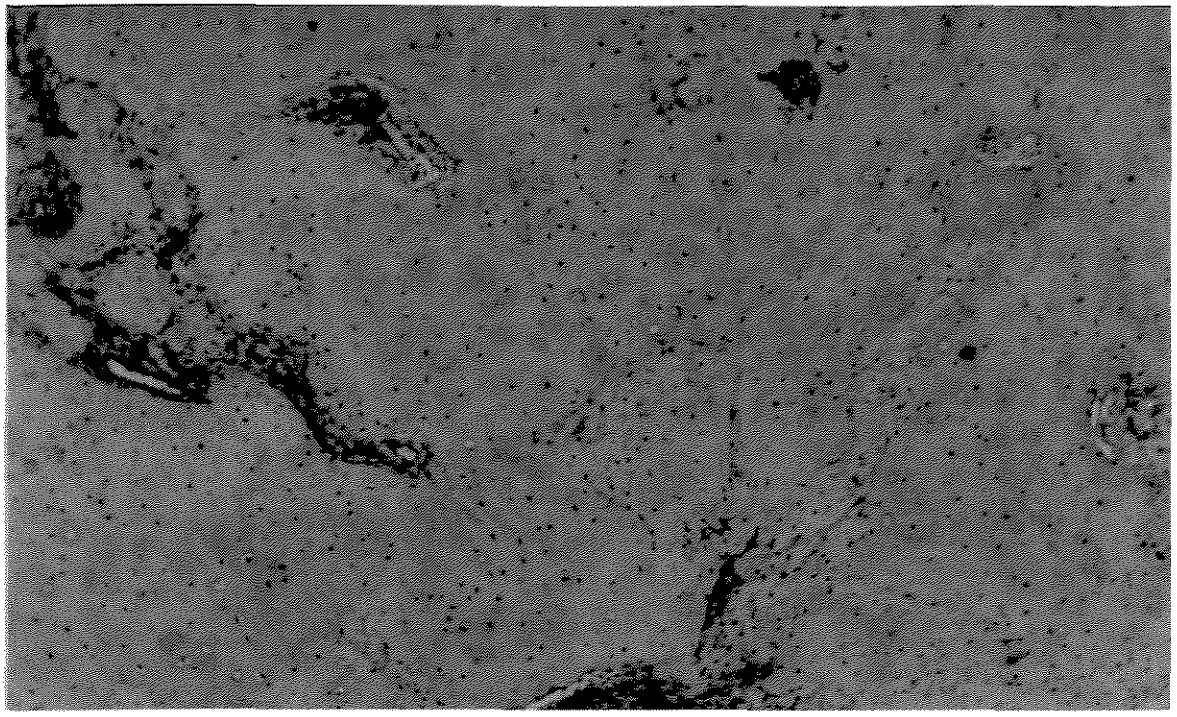

Fig. 1. Pleomorphic adenoma subtype 2 with extensive chondromyxoid component (HE x60).

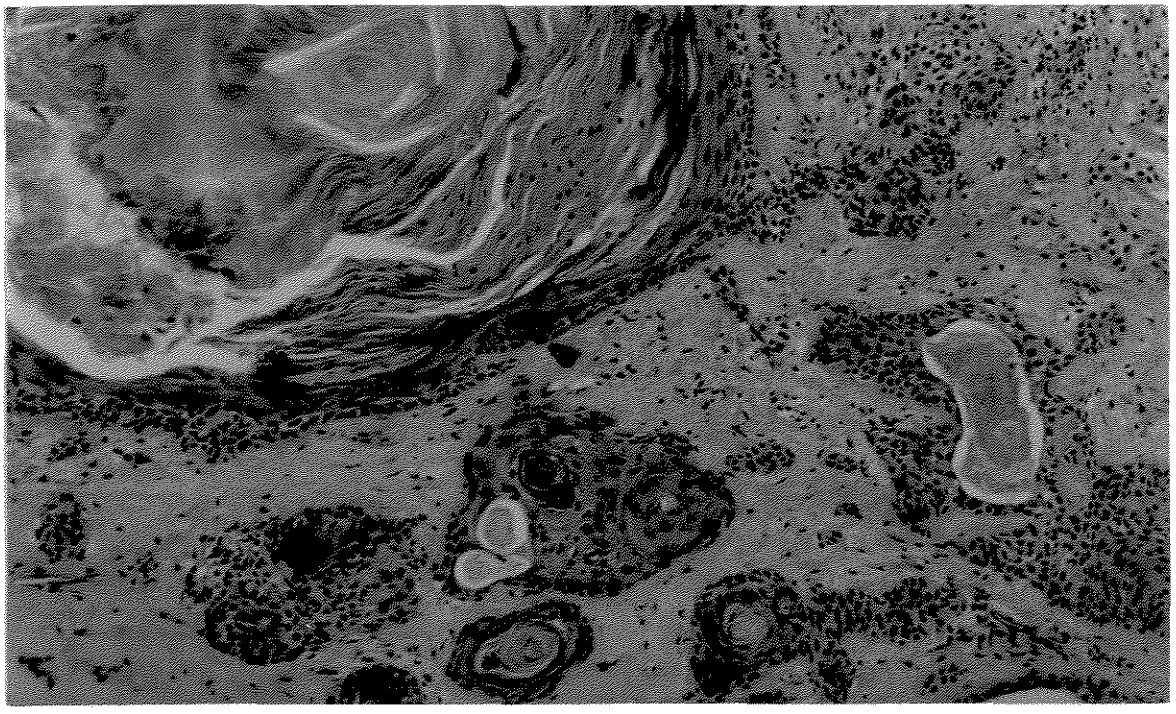

Fig. 2 Plemorphic adenoma showing squamous differentiation. (H&E x100).

CAPÍTULO 2 (Trabalho submetido para publicação na revista Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics)

PCNA, Ki-67 and p53 expressions in pleomorphic adenoma, adenoid cystic carcinoma and mucoepidermoid carcinoma of the submandibular salivary gland

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ABSTRACT

Salivary gland tumors are uncommon with a broad heterogeneity. The most common benign tumor is pleomorphic adenoma, whereas mucoepidermoid carcinoma and adenoid cystic carcinoma predominate among the malignants. Most of the salivary gland tumors occur in the parotid, and consequently clinical and biological data are normally derived from this site. This work describes the expressions of PCNA, Ki-67 and p53 in 15 pleomorphic adenomas, 15 mucoepidermoid carcinomas and 15 adenoid cystic carcinomas of the submandibular gland. Our results showed that all pleomorphic adenomas were negatives for p53 and Ki-67, and 66.6% were positives for PCNA. On the other hand, p53 and Ki-67 were positives in 53% and 50% of the mucoepidermoid carcinomas and in 40% and 20% of the adenoid cystic carcinomas, respectively. All malignant tumors were positives for PCNA. These results indicate that proliferative rate analyzed with PCNA and Ki-67 and the expression of p53 in pleomorphic adenoma and adenoid cystic carcinoma of the submandibular gland were similar to those described in the parotid and minor salivary glands. However, mucoepidermoid carcinomas showed higher expression of these markers than those of other salivary glands. This work is the first describing the expression of these immunohistochemical markers exclusively in submandibular salivary gland tumors.

INTRODUCTION

Expression of proteins related to cell proliferation and oncogenesis seems to be associated with the clinical prognosis of several tumors, including salivary gland tumors[1-4]. Proliferating cell nuclear antigen (PCNA) and Ki-67 are involved in cellular cycle[5,6], and can be identified in replicating cells of both benign and malignant lesions. Higher expression of these markers has been shown in more aggressive tumors[7-9]. Mutation, inactivation and deletion of p53 gene are involved in the pathogenesis of several types of cancers as well[10,11]. In many tumors, the accumulation of mutant p53 protein is correlated with aneuploidy, tumor stage and prognosis. Many works have correlated the expression of normal and mutant proteins with the aggressiveness, differentiation and prognosis of salivary gland tumors, but the results are still controversial[4,7,12-14]. In addition, most of the publications are from the parotid and minor salivary gland tumors. The aim of this study is to describe and compare the expression of p53, Ki-67 and PCNA in 45 tumors of the submandibular salivary gland.

MATERIAL AND METHODS

A total of 45 cases of submandibular salivary gland tumors, comprising 15 pleomorphic adenomas (PA), 15 mucoepidermoid carcinomas (MEC) and 15 adenoid cystic carcinomas (ACC) were studied. All specimens and clinical data were obtained from the files of the Hospital do Câncer A.C. Camargo, São Paulo, Brazil. The specimens were fixed in 10% formalin solution, and 5?m paraffin sections were stained with hematoxylin and eosin for routine histopathological examination. The MECs were graded according to the criteria described by Ellis & Auclair[15].

Immunohistochemistry using antibodies against p53, Ki-67 and PCNA was performed in 3?m paraffin sections, mounted on coated glass slides. Antigen was retrieved in citrate buffer (pH 6.0) microwave digestion (2 cycles of 12 min each). Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum to reduce nonspecific binding, the slides were incubated overnight at 4°C with primary antibodies against p53 (Dako, Clone DO-7; 1:200), Ki-67 (Dako, MIB-1; 1:200) and PCNA (Dako-patts, PC-10; 1:16.000). Secondary antibodies associated to a streptavidin-biotin-peroxidase method were used (Dako A/S, Strept ABComplex Duet, mouse/rabbit), complemented with diaminobenzidine as the cromogen. All slides were counterstained with hematoxylin. After each step, the sections were washed with phosphate buffered saline. Negative controls were obtained using nonimmune serum instead of the primary antibody. Samples of squamous cell carcinoma of the floor of mouth were used as positive control. Immunoreactivity was classified as: (-) negative ? 5%, (+) low 6-25%, (++) moderate 26-50% and (+++) high > 50% of positive tumor cells, counting at least 1000 cells at high magnification (X40 objective and X10 eyepiece). Intensity of staining was not considered for evaluation.

RESULTS

All clinical and immunohistochemical results are summarized in the table 1. Most of the patients with PA were in between the 3rd and 5th decades (mean age 32.3?15.6 years), and the majority were females (66.7%). Tumor size varied from 2 to 9cm, with a mean of 4.3?2.0 cm. All cases of PA were negatives for p53 and Ki-67, and 66.6% were positives for PCNA. Although, no case the expression was high.

Regarding patients with MEC, the mean age was 58.8?8.3 years and 80% were males. The tumor size ranged from 3 to 16cm (mean 6.2?3.6cm). The majority of the patients with MEC presented advanced clinical stage (T3 and T4), being 6 cases with regional metastases and one case with bone metastasis. Microscopically, 73.3% of the MECs were classified as high-grade, 20% as intermediate-grade and 6.7% as low-grade. All cases of MECs showed immunoreactivity for PCNA, being high expression in 66.7%. p53 was expressed in 8 cases (53.3%) of MECs and in all of these cases the expression was high (Fig. 1). Almost 50% of the MECs were positives for Ki-67, but 26.7% of the cases showed low expression.

Patients with ACC presented a mean age of 56.0?14.4 years, 53.3% were males and tumor size ranged from 2 to 9cm (5.2?1.9cm). As regards TNM stage, approximately 50% of patients with ACC presented tumors with stages T1 e T2, none case had lymph node metastasis and 3 cases presented distant metastases. Concerning immunohistochemistry analysis, all ACCs were positives for PCNA, being 46.7% of cases classified as high (Fig. 2). Only 3 cases (20.0%) of ACCs were positives for p53 and 6 cases (40%) for Ki-67.

DISCUSSION

Submandibular salivary gland tumors are relatively rare comprising only 5 to 10% of all salivary gland tumors and consequently there are few studies describing the expressions of proliferative markers and tumor suppressor genes. PA is the most common salivary gland tumor, with well-known clinical and microscopical characteristics. Nevertheless, its pathogenesis is still unclear, as the expression of oncogenes and the eventual transformation to malignancy. It is interesting that although PA is considered

negative for p53, mutation of this suppressor gene seems to be involved in the transformation of PA to carcinoma ex-PA[16]. Furthermore, p53 is negative for basal cell adenoma and positive in 55% of the basal cell adenocarcinomas[12]. Tsuji et al.[7] showed PCNA expression in about 70% of the cases of PA and only one out of 11 cases was positive for p53. Gallo et al.[14] also found similar results, verifying p53 expression in 2 out of 13 PA of parotid gland. Lazzaro & Cleveland[8] analyzed the expression of Ki-67 and p53 in benign and malignant intraoral salivary gland tumors. All benign tumors were negatives for Ki-67 and in some cases there was a low expression of p53. Most of the works indicate that p53 has low expression in PA of parotid and minor salivary glands. We analyzed in the present work the expression of PCNA, p53 and Ki-67 in benign and malignant submandibular salivary gland tumors and observed that in PA, p53 and Ki-67 were not immunohistochemically detected, whereas PCNA was expressed in 66.6% of the cases. Our 15 cases were considered negatives, but this difference could be probably related to different criteria in considering borderline cases as low-positive. p53 mutation in PA could be better determined by other techniques as PCR.

MECs showed intense reactivity for p53 in 8 cases (53.3%) and the other 7 were negatives. Considering only high-grade MECs, 7 out of 11 cases presented high p53 expression. In the literature, different expressions have been demonstrated for p53 in MEC. Gallo et al.[14] and Kärjä et al.[17] found a relative higher expression of p53 in MEC, 77.8% and 69.2% of the cases, respectively. However, Soini et al.[18] and Doi et al.[19] found low expression. Considering MECs of parotid and intraoral minor salivary glands, p53 was negative in 92.0% and 90.3%, respectively (data not shown). Our results indicate that p53 is involved in about half of the MECs, particularly in high-grade tumors. PCNA was positive

in all cases and in 53.3% were classified as high. Cardoso et al.[9] evaluated the relationship between the grade of MEC with the PCNA expression. There was significant difference in PCNA expression in high-grade MEC and intermediate-grade and low-grade MECs. However, there were no differences between intermediate and low-grade. In our series, 72.7% of the high-grade MEC and 66.6% of the intermediate-grade MEC presented high PCNA expression. In the only one low-grade MEC the PCNA expression was low. Ki-67 expression is increased with cell cycle disregulation. In MECs affecting infants, the expression of Ki-67 seems to increase from low to higher grades tumors[20]. Our results showed that Ki-67 was negative in 53.3% of MECs, being low in 26.7%, moderate in 13.3% and high in 6.7% (one case). Although most of our cases were high-grade MECs, these data confirm that these tumors are slow growing, as most of the salivary glands tumors.

p53 expression was negative in 80% of ACC, in 13.3% was low and in 6.7% was considered high. Lazzaro & Cleveland[8] compared p53 and Ki-67 expressions in benign and malignant intraoral and perioral salivary tumors. ACCs showed low or no expressions for p53 in 76.5% of cases and for Ki-67 in 88.2%. In contrast, Gallo et al.[14] described higher positivity for p53 (8 out of 10 cases) for ACC of the parotid. These authors reported that the difference is probably due to the antibody used. Fonseca et al.[21] also found low expression of Ki-67 in ACC. In our cases, the expressions of p53 and PCNA were higher in ACCs than in PA but lower than in MEC. Daniele et al.[22] also found PCNA indexis lower in PA than in ACC. Kim et al.[23] found PCNA expression in all 28 cases studied, but the index ranged from 1.0% to 44%.

Our PCNA and Ki-67 results confirm that PA has slower growth, in relation to MEC and ACC. Takahashi et al.[4] observed that PCNA marked 0.7%, 2.0% and 23.1% of cells of benign, low and high-grade tumors of the parotid, respectively. Cell proliferation is related to aggressiveness of the tumor and to prognosis, although this has not been confirmed for all types of cancer using PCNA or Ki-67. Although, polymorphous low grade adenocarcinoma (PLGA) affects almost exclusively the minor salivary glands, it is interesting to consider it on this context as it bears histological similarities with PA and ACC. Kelsch et al.[24] found positivity for p53 in 4 of 15 PLGA, which lies between the values for PA and ACC, and this is according to the low clinical aggressiveness of these tumors.

In summary, p53 and Ki-67 were not detected in PA of the submandibular salivary glands, and were expressed in low levels in ACC. MECs showed higher expression these markers than ACCs. However, almost 50% of cases of MEC were negatives for p53 and Ki-67. PCNA was expressed in all tumors studied, being very low in PA, intermediate for ACC and high in most of the MEC. Although many data are available in salivary gland tumors, larger series are needed to better determine the biological and clinical significance of PCNA, p53, Ki-67 and other markers in the various types of salivary gland tumors affecting the major and minor glands.

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Table 1. Clinical and immunohistochemical features of PA, MEC and ACC of the submandibular salivary glands. (n= 45).

Parameters	PA	MEC	ACC
Mean age (years)	32.3?15.6	58.8?8.3	56.0?14.4
Mean size (cm)	4.3?2.0	6.2?3.6	5.2?1.9
Gender			
Male	5 (33.3%)	12 (80.0%)	8 (53.3%)
Female	10 (66.7%)	3 (20.0%)	7 (46.7%)
T Stage			
T1/T2	*	3 (20.0%)	7 (46.7%)
T3/T4		12 (80.0%)	8 (53.3%)
N Stage			
N0	*	9 (60.0%)	15 (100.0%)
N1/N2/N3		6 (40.0%)	0.0 (0.0%)
M Stage			
M0	*	14 (93.3%)	12 (80.0%)
M1		1 (6.7%)	3 (20.0%)
PCNA			
-	5 (33.3%)	0 (0.0%)	0 (0.0%)
+	5 (33.3%)	3 (20.0%)	4 (26.7%)
++	5 (33.3%)	2 (13.3%)	4 (26.7%)
+++	0 (0.0%)	10 (66.7%)	7 (46.7%)
p53			
-	15 (100.0%)	7 (46.7%)	12 (80.0%)
+	0 (0.0%)	0 (0.0%)	2 (13.3%)
++	0 (0.0%)	0 (0.0%)	0 (0.0%)
+++	0 (0.0%)	8 (53.3%)	1 (6.7%)
Ki-67			
-	15 (100.0%)	8 (53.3%)	9 (60.0%)
+	0 (0.0%)	4 (26.7%)	5 (33.3%)
++	0 (0.0%)	2 (13.3%)	0 (0.0%)
+++	0 (0.0%)	1 (6.7%)	1 (6.7%)

* Not applicable; - negative; + low; ++ moderate; +++ high expression

Legends

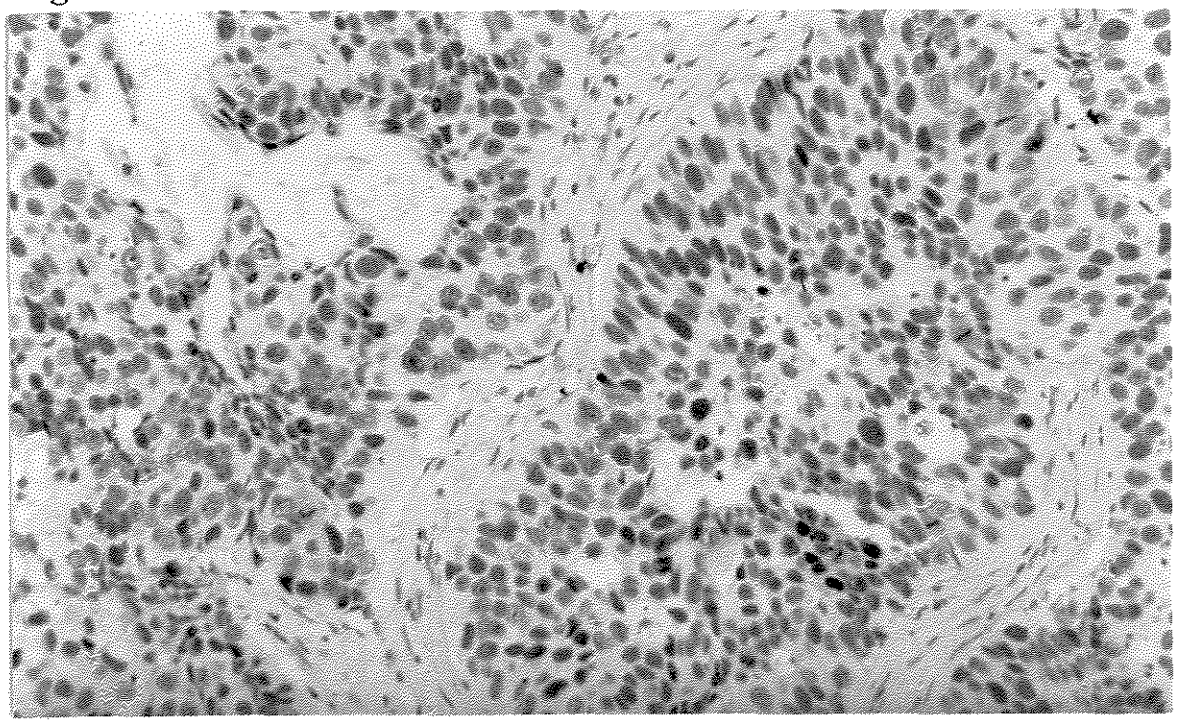

Fig. 1. High-grade mucoepidermoid carcinoma with high p53 expression. (Immunostaining for p53 X100).

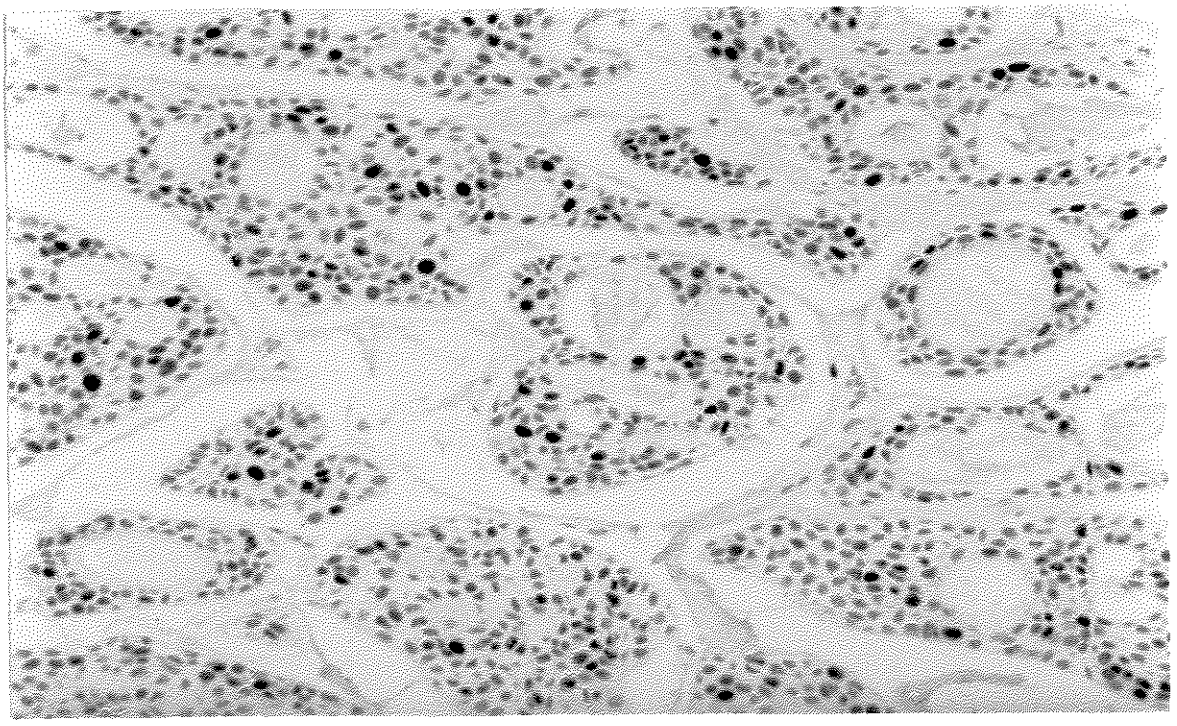

Fig. 2 Cribriform area of adenoid cystic carcinoma showing high immunoreactivity for PCNA. (Immunostaining for PCNA X200).

CAPÍTULO 3

Carcinomas of the submandibular salivary gland: Clinicopathological and immunohistochemical features

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Abstract

Submandibular salivary gland tumours are very uncommon, and most of the available data are from parotid and minor salivary glands. This work presents the clinicopathological and immunohistochemical features of 51 carcinomas of the submandibular salivary glands. The patients' age ranged from 25 to 79 years (mean 55.4?13.2 years) and 33 patients (64.7%) were males. The time of symptoms varied from 2 to 120 months (mean 20.1?26.7 months). Most of the tumours were diagnosed in advanced clinical stages (T3 and T4) and ACC was the most common tumour (45.1%). Nineteen patients did not present recurrences, 19 patients had recurrences (local, lymph nodes or distant), 9 patients showed residual disease after treatment and 4 patients were considered untreatable. Distant metastases were found in 14 out of 51 cases, being 8 adenoid cystic carcinomas, 3 mucoepidermoid carcinomas, 2 adenocarcinomas NOS and 1 salivary duct carcinoma. The 5 and 10-years overall survival rates were 39.8% and 26.8% and 5 and 10-years disease free survival rates were 57.5% and 44.9%. p53 expression showed important association with prognosis, correlating with T and N stages, death and overall survival. Our results demonstrated that submandibular gland carcinomas had poor prognosis and the diagnosis in initial stages and the evaluation of the p53 expression is could be important to estimate the survival of patients with submandibular salivary gland carcinomas.

Introduction

Salivary gland tumours comprise 1% to 4% of all human neoplasias. Parotid accounts for more than 70% of the cases, submandibular is involved in 5% to10%, sublingual in 1% and minor salivary glands in 5% to 15% (Gates 1982, Spiro 1986). The percentage of malignant tumours varies according to the involved gland, representing approximately 25% in the parotid, 35% to 55% in the submandibular, 40% to 85% in the minor glands and more than 80% in the sublingual gland (Eneroth 1971, Lopes et al. 1999). Because of the diversity of histologic subtypes, biological and clinical characteristics of the malignant salivary tumours are not yet well established. The main prognostic factor is clinical stage, but histological type, tumour grade and location are also relevant (Borthne et al. 1986, O'Brien et al.1986, Lopes et al. 1998).

Recently, immnohistochemical markers related to cell proliferation and expression of oncoproteins have been associated with aggressiveness and prognosis of human cancer (Hollstein et al. 1991, Kaklamanis et al. 1993), including salivary gland tumours (Gallo et al. 1995, Nagao et al. 1998). However, most of the available data are from parotid and minor salivary gland tumours. We evaluated the clinicopathological and immunohistochemical features of 51 carcinomas of the submandibular salivary gland tumours.

Patients and methods

A total of 51 patients with submandibular salivary gland carcinomas were evaluated. All patients were attended in the Department of Head and Neck Surgery and Otorhinolaryngology, Hospital do Câncer A.C. Camargo, São Paulo, Brazil, between 1954 to 1998. The tumours were staged according to the UICC (TNM stage).

The specimens were fixed in 10% formalin solution, and 5?m paraffin sections were stained with hematoxylin and eosin for histopathological examination. Mucoepidermoid carcinomas (MECs) were also stained with periodic acid of Schift (PAS) and mucicarmin to confirm the diagnosis, and graded according to the criteria described by Ellis & Auclair (1996).

Immunohistochemistry using antibodies against PCNA, p53, Ki-67, c-erbB-2, CEA bcl-2 was performed in 3?m paraffin sections, mounted on coated glass slides. Antigen was retrieved in citrate buffer (pH 6.0) with microwave digestion (2 cycles of 12 min each). Endogenous peroxidase was blocked with 0.05% hydrogen peroxide for 30 min. After incubation with a 1:20 dilution of normal horse serum to reduce nonspecific binding, the slides were incubated overnight at 4°C with primary antibodies against PCNA (Dako-patts, PC-10, 1:16000), p53 (Dako, DO-7, 1:200), Ki-67 (Dako, MIB-1, 1:200), c-erbB-2 (Dako, 1:200), CEA (Dako, II-7, 1:500) and bcl-2 (Dako, 124, 1:50). Secondary antibodies associated to streptavidin-biotin-peroxidase method were used (Dako A/S, Strept ABComplex Duet, mouse/rabbit), complemented with diaminobenzidine as the cromogen. All slides were counterstained with hematoxylin. After each step, the sections were washed with phosphate buffered saline. Negative and positive controls were used for all used primary antibodies. Positivity for PCNA was classified in low (? 50% of the tumour cells) and high (>50% of the tumour cells). For the other markers, the immunoreactivity was classified as (-) negative when < 5% of the tumour cells were marked and (+) positive when > 5% of the tumour cells were stained. Intensity of immunostaining was not considered.

The statistical analysis to verify the associations of immunohistochemical markers with clinicopathological parameters were performed using Fisher's exact test and non-parametric Mann-Whitney U-test with significance of 95% ($p<0.05$). Overall survival and disease free survival were estimated by the Kaplan-Meier method and the log-rank test was applied to compare survival curves with the confidence interval of 95% ($p<0.05$).

Results

Thirty-three patients were males and 18 females. The patients' age ranged from 25 to 79 years (mean 55.4?13.2 years) and the main complain was a swelling in the submandibular region (Fig.1). The time of symptoms varied from 2 to 120 months (mean 20.1?26.7 months). The tumour size ranged from 2 to 16cm (mean 5.6?2.6cm) (Table 1).

The histopathological diagnoses were reviewed by 2 pathologists, and classified as follows: Twenty-three (45.1%) adenoid cystic carcinomas (ACCs) (Fig. 2), 16 (31.4%) MECs, 4 adenocarcinomas not otherwise specified (adenocarcinoma NOS), 4 squamous cell carcinomas (SCC), 1 undifferentiated carcinoma (UC), 1 salivary duct carcinoma (SDC), 1 basal cell adenocarcinoma (BCA) and 1 malignant myoepithelioma (MM) (Table 1). Twelve cases of MECs were classified as high-grade, 2 cases as intermediate-grade (Fig.3) and 2 cases as low-grade. Neural invasion was presented in 10 tumours; 7 ACC, 1 MEC, 1 SDC and 1 adenocarcinoma NOS.

Most of the patients (88.2%) were treated with surgery, of these 43.1% underwent only surgery, 37.3% surgery associated with radiotherapy and 7.8% (4 patients) surgery and chemotherapy. Exclusive radiotherapy was used in 1 patient with ACC and 1 patient with high-grade MEC, because refused the surgical treatment. Both patients died after 8 and 15

months, respectively. Three patients with MEC and 1 patient with SDC were considered untreatable and underwent only supportive therapy. These 4 patients (3 with unresectable MECs and 1 with lung metastasis of SDC) died between 1 and 4 months after the initial diagnosis. During the follow-up period, 5 patients had local recurrences, 2 regional lymph node metastases, 7 distant metastases and in 5 patients more than one site was affected for recurrence. Fourteen patients (27.5%) were alive without evidence of disease, and 37 (72.6%) died, being 32 due the cancer and 5 of other causes. The 5 and 10-years overall survival rates were 39.8% and 26.8%, and 5 and 10-years disease free survival rates were 57.5% and 44.9% (Table 1).

Immunohistochemistry was performed in 46 tumours, because 3 ACCs and 2 MECs had not sufficient material available. PCNA was expressed in all tumours, with high expression in 65.3% of the tumours. p53, Ki-67, c-erbB-2, CEA, and bcl-2 were expressed in 39.1%, 41.3%, 45.7%, 19.6%, and 47.8% of the tumours, respectively. Regarding the correlation of immunohistochemical markers with clinicopathological variables, the expressions of PCNA, Ki-67, c-erbB-2, CEA e bcl-2 were not associated with any of the analysed features. However, p53 correlated with T and N stages, death and overall survival ($p<0.05$), these values are demonstrated in table 2.

To verify the associations between histological subtypes with clinical variables and immunohistochemical expressions, we divided the tumours in 3 groups: 23 ACCs, 16 MECs and 12 other tumours, comprising 4 adenocarcinomas NOS, 4 SCCs, 1 UC, 1 SDC, 1 BCA and 1 MM. Most of the ACCs (14 cases) at the moment of the diagnosis were in the stage clinical T1 and T2, however 13 MECs (Fig.3) and 11 other tumours were in advanced clinical stage (T3 and T4). Regarding the N stage, all cases of ACCs were N0, however 6

MECs and 2 other tumours showed lymph node metastases. These correlations showed statistical significant difference (p=0.01). However, there was no correlation between histological subtypes with age, gender, recurrence, M stage and perineural invasion. CEA, c-erbB-2 and bcl-2 expressions presented association with histological subtypes (p<0.05) (Fig. 4, 5 and 6). bcl-2 expression was associated with ACCs, while CEA was more frequent in MECs and c-erbB-2 in MECs and other tumours. p53, PCNA and Ki-67 expressions were not associated with histological subtypes (p<0.05) (Table 3).

In the group of other tumours, PCNA was expressed in all cases, being ? 50% of the cells labelled in 2 adenocarcinomas NOS and 1 BCA. p53 was expressed in 3 cases of adenocarcinoma NOS, 2 SCC and 1 undifferentiated carcinoma. Ki-67 was positive in only 1 adenocarcinoma NOS and 1 SCC; CEA in 1 adenocarcinoma NOS and SDC and c-erbB-2 was not expressed in only 2 adenocarcinomas NOS and 2 SCCs. No case expressed bcl-2.

Discussion

Salivary gland tumours are relatively rare and present a large diversity of histologic subtypes. Considering the histologic subtype, the most common malignant tumour affecting the parotids, minor glands and sublingual is MECs. However, in the submandibular gland ACCs are more frequently found (Eveson & Cawson 1985, Lopes et al. 1999, Sykes et al. 1999). The incidence of ACCs in submandibular glands range from 31% to 63% of all malignancies (Bissett & Fitzpatrick 1988, Andersen et al. 1991, Sykes et al. 1999). In our 51 malignant tumours affecting the submandibular glands, ACC was also the most common, present in 45.1% of the cases, followed by MEC (31.4%).

The main clinical feature of salivary gland tumours is a painless mass with progressive slow growing (Lopes et al. 1998). In our series of malignant tumours of submandibular gland, painless swelling was also the main complain. However, 37.3% of the patients presented pain at the moment of diagnosis. This fact may be explained because majority of the tumours (64.7%) was in advanced clinical stage (T3 e T4). Sykes et al. (1999) evaluated 30 patients with carcinoma of the submandibular gland, being 16 males and 14 females, and their ages ranged from 23 to 80 years, with a mean age of 55 years. Our patients presented the same mean age of 55 years (range from 25 to 79). However, 64.7% of the patients were males.

In our series, 8 patients present lymph node metastases at the moment of diagnosis, and 6 patients during the follow-up period. The presence of regional lymph node involvement was associated with the development of subsequent distant metastases in parotid tumours (Gallo et al 1997). Considering, the 3 patients with MECs and the 2 patients with adenocarcinomas NOS that presented distant metastases in our study, the lymph node was also involved firstly. However, all the 8 ACCs and 1 SDC had only distant metastases.

Distant metastasis is an important factor associated with survival. In our study, 4 patients presented distant metastasis at the time of diagnosis (2 ACC, 1 MEC and 1 SDC) and 10 patients developed during the follow-up period. Six out of these 10 cases were ACCs, and the others were 2 MECs and 2 adenocarcinoma NOS. Three ACCs metastasised to the lungs, 1 to lung and bone, 1 case to brain and another to the liver. Both MECs metastasised to the lungs, 1 adenocarcinoma to bone and another case to kidney. Bradley (2001) reviewed the literature about distant metastasis in salivary gland neoplasms and

reported that the incidence of metastases ranged from 20 to 40%. The lungs were the more affected site, followed by bones, liver and brain. SDC is considered a high-grade salivary malignancy, some works have shown high rate of distant metastases of this tumour, ranging from 33.3 to 61.5% (Lewis et al. 1996, Martinez-Barba et al. 1997, Guzzo et al. 1997). Recently, We reported 1 case of SDC in the palate of 63-year-old man and the patient developed metastases to lymph nodes and liver (Lopes et al. 2001). The unique case of SDC in our series, it was observed lung metastasis at the initial diagnosis. Considering the tumour sites, minor salivary gland tumours located in the tongue and submandibular glands had higher records of distant metastases (Yu & Ma 1987). However, few series in literature reported only submandibular tumours, difficulting the determination of the incidence of distant metastases in tumours of this site. Sikes et al (1999) reported that 20% of the submandibular tumours presented distant metastases, being 80% to the lungs and all cases were ACC. In our series, 27.5% of the tumours developed distant metastases.

Some clinical and histological features as advanced clinical stage, location, high grade tumours and perineural invasion are associated with poor prognosis (Spiro et al. 1989, Camilleri et al. 1998, Lopes et al. 1998, Hocwald et al. 2001). We also confirmed that tumours with advanced clinical stage presented ominous prognosis, but we did not find correlation between histological subtype, perineural invasion and prognosis. ACCs showed lower 5 and 10-years overall and disease free survival than MECs and other tumours, but this difference was not significant. Huang et al. 1997 demonstrated that ACCs in the stages III and IV, located in the submandibular gland, with perineural invasion and the solid histological type have been associated with poorer prognosis. In our series, 23 cases (45.1%) were ACC and most of MECs (12 out of 16 cases) were high-grade tumours. In the

group of other tumours, all other tumours are considered high-grade neoplasms, except 1 malignant myoepithelioma and 1 BCA, these data are in according to Seifert & Sobin (1992). Other authors have also considered that ACC of submandibular and high-grade tumours of salivary glands are associated with poorer prognosis (Huang et al. 1997, Camilleri et al. 1998, Bradley 2001). The fact that submandibular salivary gland tumours presented poor prognosis could be explained due to the higher incidence of ACC and high-grade malignancies. However, larger series using exclusively submandibular tumours should be reviewed to confirm this information.

Many works have associated the expressions of immunohistochemical markers with aggressive clinical manifestations of the disease and poor prognosis (Tsuji et al. 1995, Lazzaro & Cleveland 2000). Cho et al. (1997) studied c-erbB-2 expression in MECs, and described that this marker was expressed in 36% of the cases and was associated with high-grade MECs. Sugano et al. (1992) evaluated the immunoreativity of c-erbB-2 in 59 tumours of the major salivary glands; only adenocarcinoma NOS and carcinoma ex-pleomorphic adenoma were positives and all ACCs and MECs were negatives. In our series, 3 ACCs expressed c-erbB-2, but we did not find association between c-erbB-2 expression and prognosis. bcl-2 protein has been shown to increase cell survival by inhibiting apoptosis (Lu et al. 1996). Nagao et al. (1998) evaluated bcl-2 expression in benign and malignant basaloid tumours; all basal cell adenomas were positives, whereas 3 of 11 BCAs were negative. Unique case of BCA in our work was negative for bcl-2. In contrast, 18 out of 20 ACCs were positive for bcl-2. For estimation of proliferative activity, we evaluated the PCNA and Ki-67 expression. It was observed that 60.0% of the ACCs and 64.3% of the MECs showed > 50% of the positive cells for PCNA and 50% of the ACCs and MECs

were positive for Ki-67. These results indicated that ACCs and MECs of the submandibular glands presented similar proliferative rates, although we did not find association of these markers with prognosis.

Inactivation, deletion and mutation of *p53* gene are involved in the pathogenesis of several types of human cancers (Hollstein et al. 1991). The assessment of immunohistochemical expression of the p53 in parotid gland tumours has been an indicator of clinical aggressiveness (Gallo et al. 1995). Doi et al. (1999) demonstrated that p53 expression might be an important factor in determining the prognosis of salivary gland carcinomas. We also found strong relation of p53 expression with T and N stages, death and overall survival ($p<0.05$).

In summary, we evaluated 51 submandibular salivary gland carcinomas. The tumours showed slow growth rate, although most of the patients presented advanced clinical disease at time of diagnosis. ACC was the most frequent histological subtype followed by MEC. Nineteen patients had recurrence (local, lymph nodes or distant) and 9 patients showed residual disease after treatment and 4 patients were considered untreatable. The submandibular tumours had poor prognosis with low overall survival and disease free survival rates in 5 and 10 years. p53 expression showed high association with advanced clinical stage, death and overall survival ($p<0.05$). Diagnosis in initial stages and the evaluation of the p53 expression could be important to survival of submandibular salivary gland carcinomas.

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Table 1. Clinicopathological data of 51 malignant submandibular salivary gland tumours.

Parameters		n (%)
Age (years)		
Range: 25-79 (55.4 ? 13.2)		
Time of symptoms (months)		
Range: 2-120 (20.1 ? 26.7)		
Size (cm)		
Range: 2-16 (5.6 ? 2.6)		
Gender		
Male		33 (64.7%)
Female		18 (35.3%)
Histological type		
Adenoid cystic carcinoma		23 (45.1%)
Mucoepidermoid carcinoma		16 (31.4%)
Adenocarcinoma NOS		4 (7.8%)
Squamous cell carcinoma		4 (7.8%)
Undifferentiated carcinoma		1 (2.0%)
Salivary duct carcinoma		1 (2.0%)
Basal cell adenocarcinoma		1 (2.0%)
Malignant myoepithelioma		1 (2.0%)
T Stage	T1	2 (3.9%)
	T2	16 (31.4%)
	T3	19 (37.3%)
	T4	14 (27.5%)
N Stage	N0	43 (84.3%)
	N1	1 (2.0%)
	N2	6 (11.8%)
	N3	1 (2.0%)
M Stage	M0	47 (92.2%)
	M1	4 (7.8%)
Recurrence		
No recurrence		19 (37.3%)
Residual disease		13 (25.5%)
Local		5 (9.8%)
Neck (lymph nodes)		2 (3.9%)
Distant		7 (13.7%)
Local and neck		2 (3.9%)
Local and distant		1 (2.0%)
Neck and distant		1 (2.0%)
Local, neck and distant		1 (2.0%)
Status		
Alive		14 (27.5%)
Death-cancer		32 (62.7%)
Death-other causes		5 (9.8%)
Overall survival		
5 years		39.8%
10 years		26.8%
Disease free survival		
5 years		57.5%
10 years		44.9%

Table 2. Clinicopathological variables associated with p53 expression in 46 submandibular salivary gland tumours.

Parameters	p-53		
	Negative	Positive	*P* value
Age			
? 55 years	9 (52.9%)	8 (47.1%)	0.39
> 55 years	19 (65.5%)	10 (34.5%)	
Gender			
Male	17 (58.6%)	12 (41.4%)	0.683
Female	11 (64.7%)	6 (35.3%)	
T Stage			
T1/T2	13 (86.7%)	2 (13.3%)	**0.038**
T3	9 (52.9%)	8 (47.1%)	
T4	6 (42.9%)	8 (57.1%)	
N Stage			
N0	27 (69.2%)	12 (30.8%)	**0.006**
N1/N2/N3	1 (14.3%)	6 (85.7%)	
M Stage			
M0	24 (57.1%)	18 (42.9%)	0.093
M1	4 (100.0%)	0 (0.0%)	
Recurrence			
No	18 (64.29%)	10 (35.7%)	0.554
Yes	10 (55.6%)	8 (44.4%)	
Death			
No	9 (90.0%)	1 (10.0%)	**0.033**
Yes	19 (52.8%)	17 (47.2%)	
Perineural invasion			
No	22 (61.1%)	14 (38.9%)	0.949
Yes	6 (60.0%)	4 (40.0%)	
Overall Survival			
5 years	44.1%	22.2%	**0.035**
10 years	27.5%	11.1%	
Disease free Survival			
5 years	65.0%	37.6%	0.211
10 years	44.0%	37.6%	

Table 3. Histological subtypes tumours of the submandibular salivary gland correlated with clinical (n=51cases), pathological (n=46) and immunohistochemical expressions of PCNA, p53, Ki-67, CEA, c-erbB-2 and bcl-2 (n=46).

Parameters	ACC nº (%)	MEC nº (%)	Others nº (%)	Total	*P*
Age (years)					
? 55	12 (57.1%)	4 (19.1%)	5 (23.8%)	21	0.24
> 55	11 (36.7%)	12 (40.0%)	7 (23.3%)	30	
Gender					
Male	12 (36.4%)	12 (36.4%)	9 (27.7%)	33	0.23
Female	11 (61.1%)	4 (22.2%)	3 (16.7%)	18	
Stage T					
T1 and T2	14 (77.8%)	3 (16.7%)	1 (5.6%)	18	**0.01**
T3	5 (26.3%)	9 (47.4%)	5 (26.3%)	19	
T4	4 (28.6%)	4 (28.6%)	6 (42.9%)	14	
Stage N					
N0	23 (53.5%)	10 (23.3%)	10 (23.3%)	43	**0.01**
N1/N2/N3	0 (0.0%)	6 (75.0%)	2 (25.0%)	8	
Stage M					
M0	21 (44.7%)	15 (31.9%)	11 (23.4%)	47	0.96
M1	2 (50.0%)	1 (25.0%)	1 (25.0%)	4	
Recurrence					
No	12 (35.5%)	11 (34.4%)	9 (28.1%)	32	0.35
Yes	11 (57.9%)	5 (26.3%)	3 (15.8%)	19	
Overall survival					
5 years	46%	31%	42%	*	0.80
10years	26%	31%	25%		
Disease free survival					
5 years	52%	61%	71%	*	0.84
10 years	32%	61%	71%		
Perineural invasion					
No	13 (36.1%)	13 (36.1%)	10 (27.8%)	36	0.13
Yes	7 (70.0%)	1 (10.0%)	2 (20.0%)	10	
PCNA					
? 50	8 (50.0%)	5 (31.3%)	3 (18.8%)	16	0.69
> 50	12 (40.0%)	9 (30.0%)	9 (30.0%)	30	
P53					
Negative	16 (57.1%)	6 (21.4%)	6 (21.4%)	28	0.06
Positive	4 (22.2%)	8 (44.4%)	6 (33.3%)	18	
Ki-67					
Negative	10 (37.0%)	7 (25.9%)	10 (37.0%)	27	0.13
Positive	10 (52.6%)	7 (36.8%)	2 (10.5%)	19	
CEA					
Negative	19 (51.4%)	8 (21.6%)	10 (27.0%)	37	**0.02**
Positive	1 (11.1%)	6 (66.7%)	2 (22.2%)	9	
c-erbB-2					
Negative	17 (68.0%)	4 (16.0%)	4 (16.0%)	25	**< 0.001**
Positive	3 (14.3%)	10 (47.6%)	8 (38.1%)	21	
bcl-2					
Negative	2 (8.3%)	10 (41.7%)	12 (50.0%)	24	**< 0.001**
Positive	18 (81.8%)	4 (18.2%)	0 (0.0%)	22	

significance $p<0.05$ * data not disposable

Legends

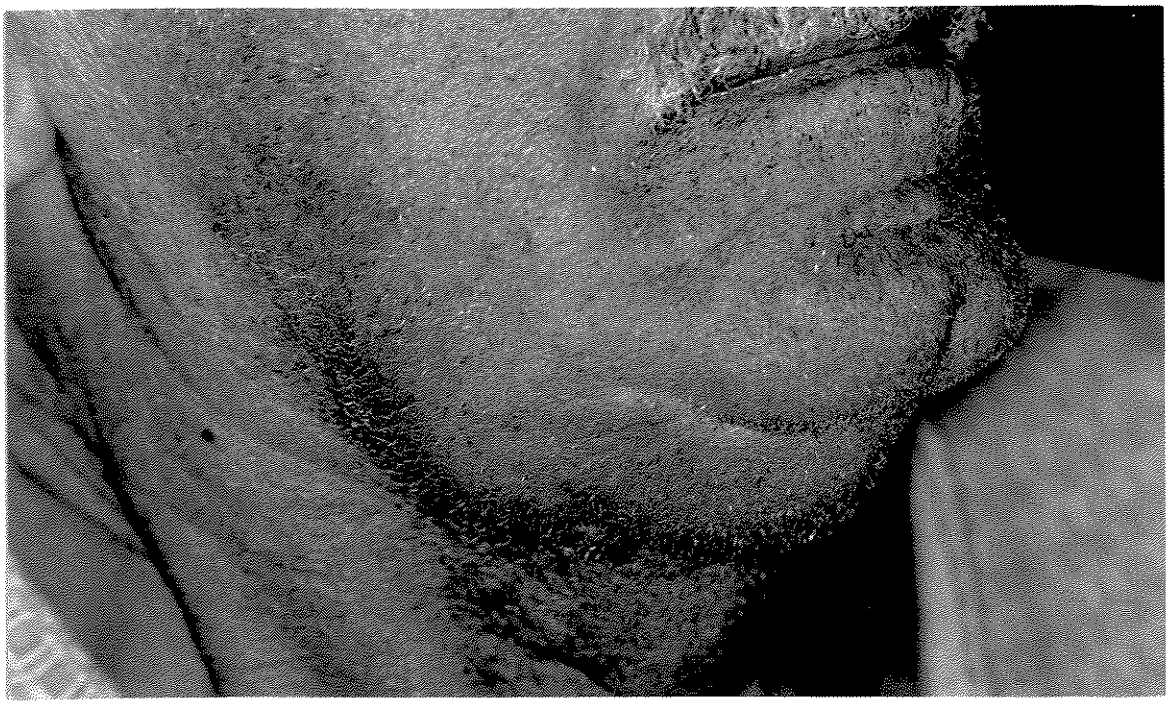

Fig.1- Clinical view of a patient with ACCs of submandibular gland.

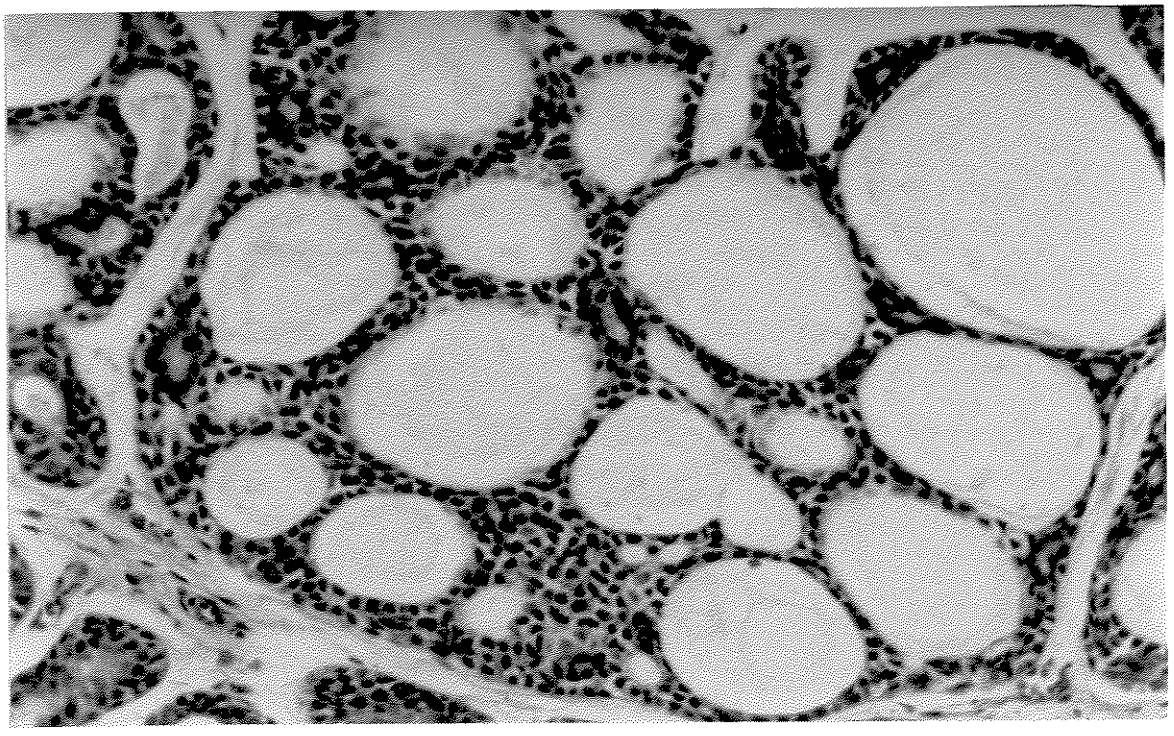

Fig. 2- High power view of cribriform area of adenoid cystic carcinoma (H&E x200).

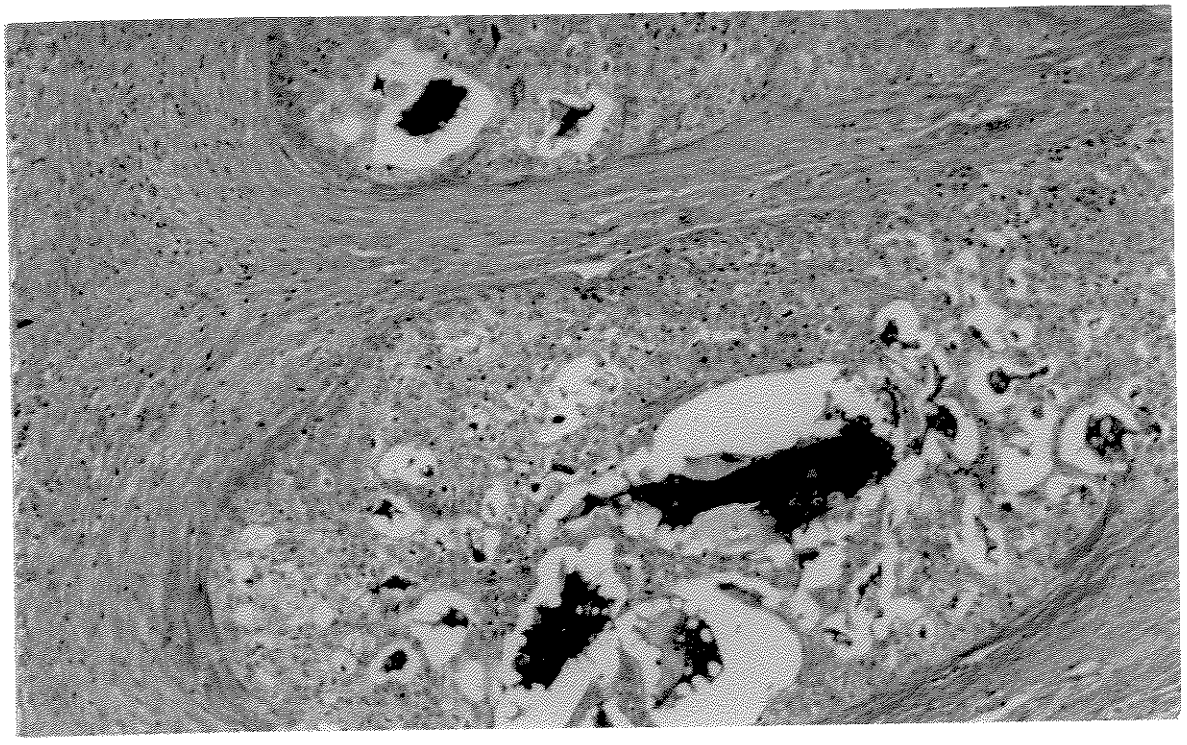

Fig. 3- Intermediate-grade MEC showing small cystic spaces and tumor cells secreting PAS+ material (PAS x100).

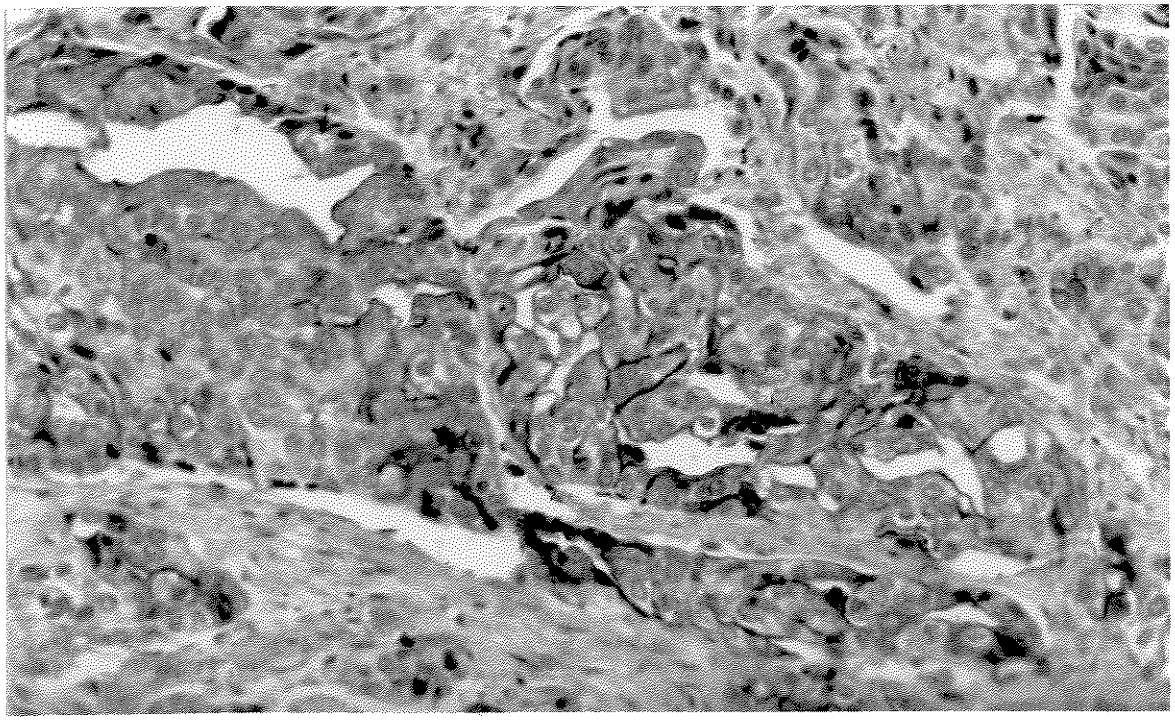

Fig. 4- Expression of CEA in high-grade MEC (Immunostaining for CEA x200).

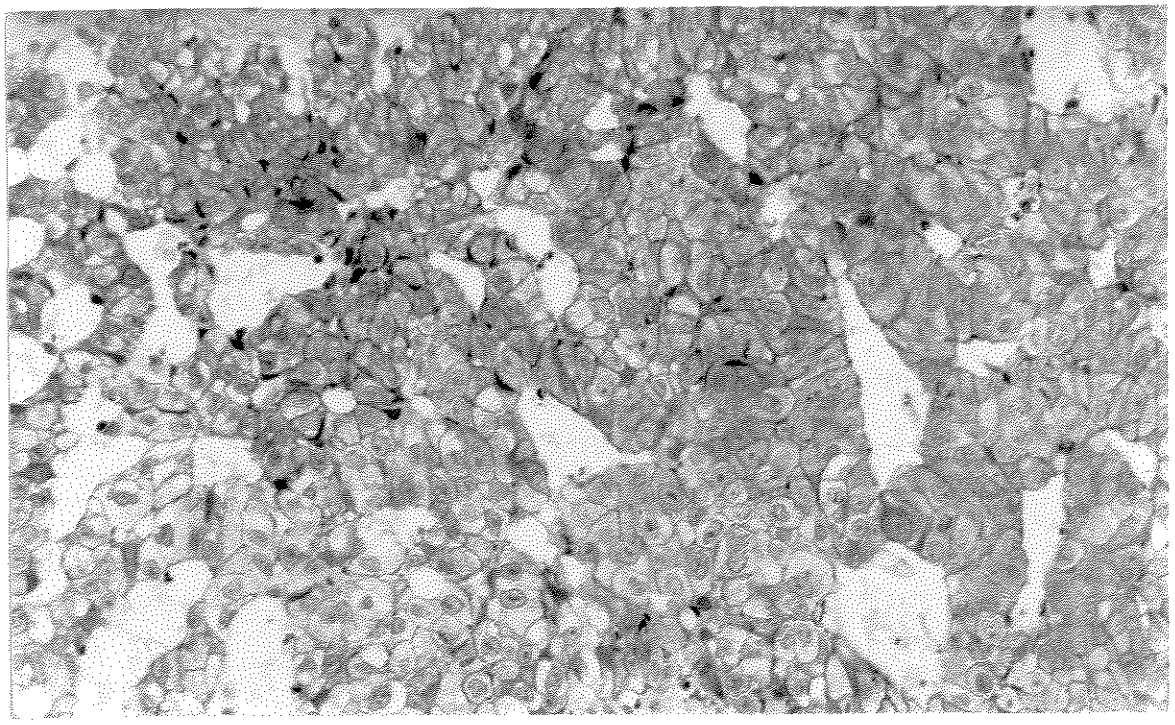

Fig. 5- Membrane positivity of c-erbB-2 in high-grade MEC of submandibular gland (Immunostaining for c-erbB-2 x200).

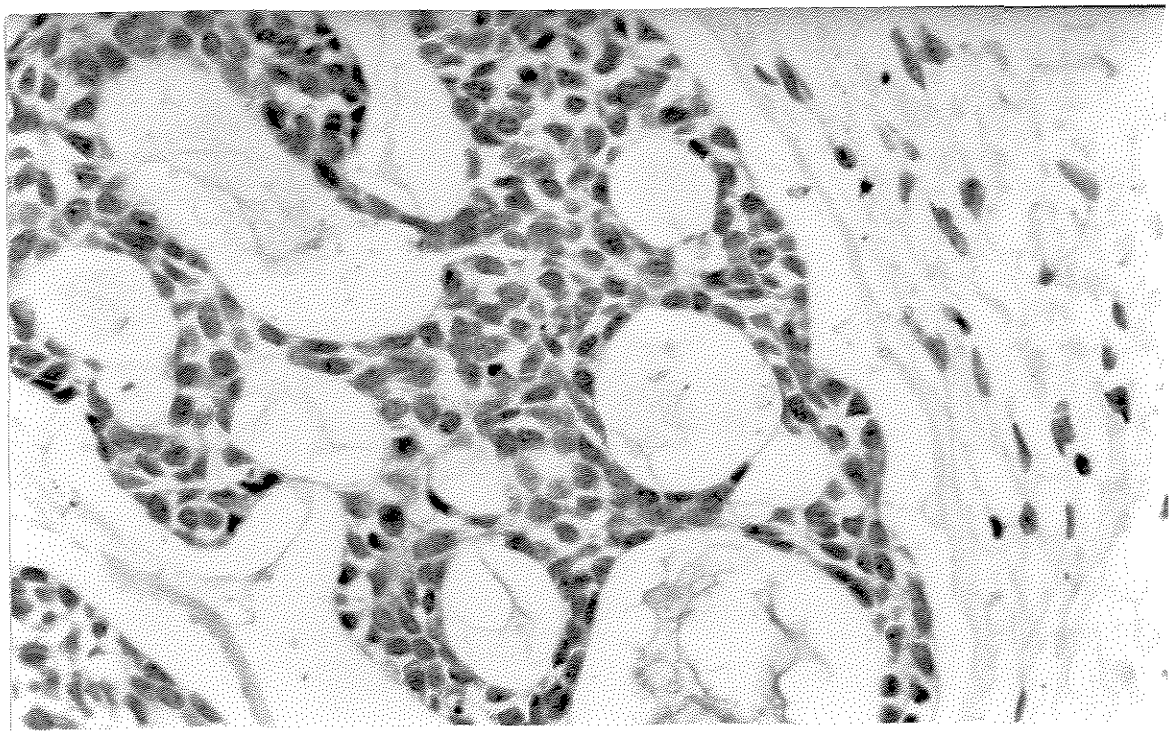

Fig. 6- bcl-2 expression in cribriform area of ACC of submandibular gland (Immunostaining for bcl-2 x200).

CONCLUSÕES

- Adenoma pleomorfo é o tumor benigno mais comum da glândula submandibular.

- Adenomas pleormorfos de glândula submandibular apresentando grande tamanho são ricos em componente estromal, enquanto tumores menores são mais celularizados.

- A expressão de PCNA, p53 e Ki-67 em adenoma pleomorfo e carcinoma adenóide cístico afetando a glândula submandibular é semelhante a expressão descrita para estes tumores localizados na parótida e glândulas salivares menores. Entretanto, o carcinoma mucoepidermóide de glândula submandibular apresenta maiores índices para estes marcadores.

- Tumores malignos de glândula submandibular apesar de apresentarem crescimento lento, são diagnosticados em avançado estádio clínico, sendo o carcinoma adenóide cístico o tumor maligno mais freqüente.

- Metástases a distância são mais freqüentes em carcinomas adenóides císticos.

- Tumores malignos de glândula submandibular apresentam prognóstico desfavorável, com baixos índices de sobrevida global e sobrevida livre de doença.

- Tumores malignos que expressam p53 apresentam maior tamanho, metástases regionais e menor sobrevida global. Avaliação deste marcador pode ser importante na avaliação do prognóstico de tumores de glândula submandibular.

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