UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

Danyel Elias da Cruz Perez

Cirurgião-Dentista

ESTUDO IMUNOHISTOQUÍMICO E ANÁLISE MULTIVARIADA DE FATORES PROGNÓSTICOS EM CARCINOMA ADENÓIDE CÍSTICO DE CABEÇA E PESCOÇO

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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Orientador: Prof. Dr. Luiz Paulo Kowalski

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FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA
Bibliotecário: Marilene Girello – CRB-8ª. / 6159

P415e

Perez, Danyel Elias da Cruz.
Estudo imunohistoquímico e análise multivariada de fatores prognósticos em carcinoma adenóide cístico de cabeça e pescoço. / Danyel Elias da Cruz Perez. – Piracicaba, SP : [s.n.], 2005.

Orientador: Luiz Paulo Kowalski
Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Glândulas salivares. 2. Neoplasias das glândulas salivares. 3. Estudos epidemiológicos. 4. Seio maxilar. I. Kowalski, Luiz Paulo. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

(mg/fop)

Título em inglês: Immunohistochemical study and multivariate survival analysis of prognostic factors in head and neck adenoid cystic carcinoma
Palavras-chave em inglês (*Keywords*): Salivary glands; Salivary glands neoplasms; Epidemiologic studies; Maxillary sinus
Área de concentração: Patologia
Titulação: Doutor em Estomatopatologia
Banca examinadora: Luiz Paulo Kowalski; Albina Messias de Almeida Milani Altemani; Antonio Francisco Durighetto Junior; Márcio Ajudarte Lopes; Oslei Paes de Almeida
Data da defesa: 02/03/2005

DEDICO ESTE TRABALHO

À **Deus,** por tudo de bom que Ele tem me proporcionado todos esses anos de vida, pela presença constante, trilhando-me sempre pelos melhores caminhos.

Aos meus pais, **Wilson e Irismar,** exemplos de vida a serem seguidos, e aos meus irmãos **Luciano, Natalia e Cecília,** por todo apoio, amor e carinho. Amo vocês.

Ao **Prof Oslei Paes de Almeida**, por todo o entusiasmo, competência e seriedade com que sempre conduziu o seu trabalho, pelos ensinamentos transmitidos e pela amizade. Sou eternamente grato.

Ao **Prof Luiz Paulo Kowalski**, exemplo de competência e dedicação ao trabalho, pela orientação, conhecimento transmitido, confiança e oportunidade de ter realizado este e outros trabalhos. Muito obrigado.

À minha querida **Flávia,** fonte constante de inspiração, carinho e amor, por todo o companheirismo e cumplicidade, além do apoio nos momentos mais difíceis. Você é linda!!! Amo-Te.

AGRADECIMENTOS ESPECIAIS

Aos **Profs Márcio Ajudarte Lopes e Pablo Agustín Vargas**, professores e amigos, que sempre me apoiou, dando várias oportunidades para trabalharmos juntos, contribuindo muito para minha formação pessoal e profissional. Muito obrigado.

Ao **Prof Antonio Francisco Durighetto Junior,** grande professor e amigo, obrigado pelo incentivo inicial e confiança depositada em mim.

Aos **Profs Fábio de Abreu Alves e Fábio Ramôa Pires,** grandes amigos e ex-colegas de pós-graduação, obrigado pelo auxílio, incentivo e amizade por todos esses anos, vocês também foram muito importantes na realização deste trabalho.

AGRADECIMENTOS

À Faculdade de Odontologia de Piracicaba, na pessoa do seu diretor, o **Prof. Dr. Thales Rocha de Mattos Filho.**

Ao **Prof. Dr. Pedro Luiz Rosalen**, coordenador dos programas de Pós-Graduação da Faculdade de Odontologia de Piracicaba.

Ao **Prof. Jacks Jorge Júnior**, coordenador do programa de pós-graduação em Estomatopatologia.

À **CAPES**, pela concessão da bolsa de estudos durante o período de realização do trabalho.

Aos médicos e funcionários do **Departamento de Cirurgia de Cabeça e Pescoço e Otorrinolaringologia**, do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo.

Aos funcionários do **Serviço de Arquivo Médico e Estatístico (SAME),** do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo, em especial a **Sra Hirde Contesini** pela prestatividade e simpatia com que sempre nos recebeu.

Às funcionárias de **Centro de Estudos** do Centro de Tratamento e Pesquisa Hospital do Câncer A. C. Camargo, em especial à **Inês Nishimoto** pela análise estatística.

Aos professores das Áreas de Patologia e Semiologia da Faculdade de Odontologia de Piracicaba, **Edgard Graner, Ricardo Coletta, Jacks Jorge e Osvaldo di Hipólito** pelas orientações e aprendizado durante todo esse tempo.

Aos amigos e colegas do programa de pós-graduação em Estomatopatologia, em especial **Chico, Cláudio Maranhão, Jorge Esquiche, Ana Carolina, Lucielma e Andréa,** pelos momentos de descontração, ótima convivência e amizade.

Aos ex-colegas de pós-graduação e grandes amigos **Paulo Bonan, Estela, Eduardo Fregnani, Cleverton Roberto e Hercílio Júnior.** Vocês fazem falta aqui na Semiologia...

Ao amigo **José Divaldo Prado,** pela amizade e companhia nas quintas-feiras em São Paulo.

Aos funcionários e amigos da Área de Patologia e Semiologia, **Adriano, Rosa Fornasier, João Carlos, Dinha, Bete, Cida Campion, Rosa Scalco, Débora, Rogério** e em especial à **Ana Cristina Godoy**, pela realização das reações imunohistoquímicas.

E finalmente, a todos que direta ou indiretamente contribuíram para a realização deste trabalho.

"*Há homens que lutam um dia e são bons. Há outros que lutam um ano e são melhores. Há os que lutam muitos anos e são muito bons. Porém, há os que lutam toda a vida. Esses são os imprescindíveis."*

Bertolt Brecht

SUMÁRIO

RESUMO

Introdução: O objetivo deste estudo foi avaliar as características clínicas, histopatológicas e imunohistoquímicas de 129 casos de carcinoma adenóide cístico (CAC) de cabeça e pescoço, a fim de identificar fatores prognósticos importantes associados a este tumor. Material e métodos: Entre os anos de 1955 e 1997, cento e vinte e nove casos de CAC foram tratados no Departamento de Cirurgia de Cabeça e Pescoço do Hospital do Câncer A. C. Camargo, São Paulo. Os dados clínicos dos pacientes foram obtidos dos prontuários médicos e resumidos em uma ficha clínica padronizada. As lâminas histológicas foram revisadas para confirmação do diagnóstico e os tumores classificados em tipos histológicos cribriforme, tubular ou sólido. Reações imunohistoquímicas contra proteína p53, antígeno nuclear de proliferação celular (PCNA), Ki-67, antígeno carcinoembrionário (CEA), c-erbB-2 e bcl-2 foram realizadas utilizando a técnica estreptavidina-biotina-peroxidase. Resultados: Setenta e um pacientes eram homens (55%) e 58 (45%) mulheres, com uma idade média de 51,5 anos (10-96 anos). As glândulas salivares menores intra-bucais e as parótidas foram as mais acometidas e 96 pacientes (74,4%) foram diagnosticados com tumores em estádios clínicos III ou IV. O tipo histológico mais comum foi o tipo cribriforme (54,2%), seguido pelos tipos tubular (25,2%) e sólido (20,6%). Cirurgia foi a principal forma de tratamento (75,9%) e 42,7% dos pacientes foram submetidos à radioterapia pós-operatória. As taxas de sobrevida global aos 5 e 10 anos foram 56,5% e 32,5%, respectivamente, e a sobrevida livre de doença foi 42.7% e 29.2% para os mesmos períodos. Análise univariada da sobrevida revelou que pacientes com idade superior a 45 anos ($p=0.04$), tempo de queixa inferior a 18 meses ($p=0.007$), presença de parestesia ($p=0.04$), estádio T ($p=0.01$), estádio N ($p=0.04$), estádio M ($p<0.001$), estádio clínico ($p=0.01$), tipo histológico sólido ($p<0.001$), presença de tumor residual após a cirurgia ($p<0.001$) e aumento da expressão de p53 ($p=0.08$) correlacionaram com pobre prognóstico. A análise multivariada de sobrevida mostrou que estádio clínico avançado, tipo histológico sólido e expressão de p53 foram fatores prognósticos significantes independentes em pacientes com CAC. Conclusões: Após análise dos dados, concluímos que estádio clínico avançado, tipo histológico sólido e expressão de p53 foram os fatores prognósticos mais significantes associados ao CAC de cabeça e pescoço.

Palavras chave: carcinoma adenóide cístico; glândulas salivares; imunohistoquímica; fatores prognósticos.

ABSTRACT

Introduction: The aim of this study was to analyze the clinical, histological and immunohistochemical prognostic factors of a large series of adenoid cystic carcinoma treated in a single institution, using univariate and multivariate survival analyses. **Methods:** All cases of head and neck ACC treated between 1955 and 1997, at the Department of the Head and Neck Surgery and Otorhinolaryngology, Hospital do Cancer A. C. Camargo, Sao Paulo, were selected for the study. The clinical data were obtained from the medical records and the histopathological slides reviewed. Immunohistochemical reactions against p53 protein, proliferation cell nuclear antigen (PCNA), Ki-67, carcinoembryonic antigen (CEA), c-erbB-2 and bcl-2 were performed using the streptavidin-biotin-peroxidase method. **Results:** Of 129 cases, seventy-one were male (55%) and 58 female (45%), with mean age of 51.5 years (10-96 years). The palate and parotid gland were the most common sites. TNM stage revealed 96 (74.4%) clinical stages III + IV tumors, and the most common histological type was the cribriform type (54.2%), followed by tubular (25.2%) and solid type (20.6%). Surgery was the main treatment modality (75,9%) and 42,7% of the patients were submitted to postoperative radiotherapy. The overall survival rates at 5 and 10 years were 56.5% and 32.5% respectively, and the free disease survival rates were 42.7% and 29.2% for the same periods. Univariate survival analysis revealed that age older than 45 years ($p=0.04$), period of complaints inferior to 18 months ($p=0.007$), presence of paresthesia ($p=0.04$), T stage ($p=0.01$), N stage ($p=0.04$), M stage ($p<0.001$), clinical stage ($p=0.003$), solid histological type ($p<0.001$), presence of residual tumor ($p<0.001$) and increased expression of p53 ($p=0.08$) correlated with a poor prognosis. In the multivariate survival analyses, clinical stage, solid histological subtype and increased expression of p53 were independent significant prognostic factors. **Conclusions**: According to our findings, clinical stage, solid growth pattern and expression of p53 were the most important prognostic factors in patients with ACC.

Key-words: adenoid cystic carcinoma; head and neck; salivary glands, prognostic factors, immunohistochemistry

1. INTRODUÇÃO

Carcinoma adenóide cístico (CAC) é um tumor incomum originado principalmente em glândulas salivares, representando cerca de 10 a 15% dos tumores da região de cabeça e pescoço (Matsuba *et al.*, 1984; Khan *et al.* 2001). Foi inicialmente descrito por Theodor Billroth em 1859, com o nome de cilindroma. Posteriormente, em 1953, Foote e Frazell propuseram o termo carcinoma adenóide cístico, aceito e utilizado até os dias atuais. Ocorrem principalmente nas parótidas e glândulas salivares menores intra-bucais, particularmente no palato, seguidas das glândulas submandibulares e raramente acometem as glândulas sublinguais (Kim *et al.* 1994; Ellis & Auclair, 1996; Lopes *et al.*, 1999). Tumores que satisfazem os critérios histológicos de CAC podem surgir em uma variedade de outros locais como glândulas ceruminosas do ouvido, glândulas lacrimais, mama, colo uterino, glândula de Bartholin e próstata (Minei *et al.*, 2001; Krasevic *et al.*, 2001; Khan *et al.*, 2001; Millar *et al.*, 2004; Kaku *et al.*, 2004; Shields *et al.*, 2004).

A incidência de CAC varia de acordo com sua topografia. Nas glândulas parótidas, de 1,6 a 2,2% de todos os tumores e 16% dos tumores malignos são CAC. Já na glândula submandibular, essa porcentagem sobe para 12% a 17% e na glândula sublingual representa 40% de todos os tumores (Eveson & Cawson, 1985; Ellis & Auclair, 1996; Ostman *et al.*, 1997; Wahlberg *et al.*, 2002). Considerando todos os tumores malignos de glândula salivar, a incidência de CAC é variável, correspondendo de 7% a 23% deles (Eveson & Cawson, 1985; Ellis & Auclair, 1996).

CAC ocorre mais freqüentemente em pacientes durante a quinta, sexta e sétima década de vida, não sendo observada predileção por gênero (Nascimento *et al.*, 1986; Kokemueller *et al.*, 2004). Em crianças e adolescentes, CAC é extremamente raro (da Cruz Perez *et al.*, 2004). Clinicamente, o tumor apresenta-

se como um nódulo firme, de crescimento lento, infiltrativo e indolente. Em glândulas salivares maiores, dor geralmente ocorre durante o crescimento tumoral (Nascimento *et al*., 1986). Além disso, o paciente pode desenvolver paralisia facial, um indicativo de pobre prognóstico. Em glândulas salivares menores intra-bucais, dor é um achado variável, principalmente na fase inicial do desenvolvimento tumoral (Ellis & Auclair, 1996).

Microscopicamente, três subtipos de CAC são reconhecidos. O tipo clássico ou cribriforme é o tipo mais comum e é formado por células pequenas, com núcleos hipercromáticos e citoplasma escasso, que se arranjam em ninhos de variados tamanhos e formas, formando espaços circulares ou ovóides, denominado padrão de queijo suíço (Eby *et al*., 1972; Perzin *et al*., 1978; Batsakis *et al*., 1990). O estroma é fibroso, podendo apresentar algumas áreas hialinizadas contendo cordões de células neoplásicas. Entretanto, não apresenta células neoplásicas individuais imersas no estroma fibroso ou hialinizado, como é observado no adenoma pleomorfo (Ellis and Auclair, 1996). O segundo tipo mais comum é denominado tubular e apresenta as mesmas células observadas no padrão cribriforme. Entretanto, o arranjo celular é diferente, com as células tumorais formando estruturas ductais simples (Tomich, 1991). E por último, o tipo sólido é o terceiro mais comum e também o que apresenta um comportamento clínico mais agressivo (Perzin *et al*., 1978; Matsuba *et al*., 1986; Nascimento *et al*., 1986; Khan *et al*., 2001). As células apresentam-se maiores e formam ninhos ou blocos de diferentes tamanhos. Comedonecrose é uma característica comum, além de mitoses atípicas e anaplasia celular. Estruturas ductais ou arranjos cribriformes também são encontrados (Batsakis *et al*., 1990; Tomich, 1991; Ellis & Auclair, 1996). Microscopicamente, outros tumores de glândulas salivares podem apresentar características semelhantes ao CAC, como adenocarcinoma polimorfo de baixo grau (APBG), adenoma pleomorfo e tumores com células basais. O APBG é o principal diagnóstico diferencial do CAC, sobretudo nos casos localizados em glândulas salivares menores intra-bucais. APBG apresenta células tumorais uniformes, que se arranjam em lóbulos ou lençóis de células, túbulos e

ilhas com um padrão microcístico ou cribriforme, com freqüente invasão perineural, semelhante ao CAC (Ellis & Auclair, 1996; Speight & Barrett, 2002). Entretanto, as células que compõem o APBG têm os núcleos maiores, apresentam um crescimento intra-ductal e podem formar um padrão de fila indiana na periferia do tumor (Ellis & Auclair, 1996). A detecção do produto do proto-oncogene c-kit (CD 117) pode ser útil na diferenciação entre esses dois tumores, visto que a expressão de CD 117 foi observada em 85,5% dos casos de CAC, enquanto nenhum caso de APBG foi positivo para CD 117 (Jeng *et al*., 2000). Porém, mais estudos utilizando séries maiores devem ser realizados para confirmar esses resultados.

Apesar de várias séries de CAC terem sido publicadas anteriormente, o tratamento ideal para o CAC ainda não está bem estabelecido. Muitos autores têm relatado aumento no controle de doença local com a combinação de cirurgia e radioterapia (Matsuba *et al*., 1984; Garden *et al*., 1995; Avery *et al*., 2000; Umeda *et al*., 2000; Mendenhall *et al*., 2004). Embora aumente a taxa de controle local, essa combinação não necessariamente aumenta a sobrevida (Fordice *et al*., 1999). As indicações para radioterapia freqüentemente dependem de fatores prognósticos mais significativos, como a localização do tumor, estadiamento clínico, tipo histológico e margens cirúrgicas comprometidas (Matsuba et al., 1986; Parsons *et al*., 1996). Pela dificuldade encontrada em se conseguir margens cirúrgicas adequadas, alguns estudos propõem a realização de radioterapia pós-operatória como um procedimento de rotina (Garden *et al*., 1995; Mendenhall *et al*., 2004). Desta forma, a principal forma de tratamento para o CAC descrita na literatura é a ressecção cirúrgica ampla, com margens cirúrgicas macro e microscópicas livres de tumor associada à radioterapia pós-operatória. Esvaziamento cervical eletivo não é indicado, pois o risco de metástases regionais é baixo e metástases à distância são mais comuns (Ellis & Auclair, 1996; Prokopakis *et al*., 1999; Khan *et al*., 2001). Entretanto, alguns autores sugerem ressecção cervical eletiva para tumores situados em regiões ricas em vasos linfáticos como base de língua e rinofaringe (Mendenhall *et al*., 2004). Quando há

disseminação metastática à distância, atualmente não há nenhuma terapia efetiva. Várias alternativas de quimioterapia têm sido empregadas no tratamento de tumores metastáticos, entretanto, apresentam pobres resultados (Spiers *et al*., 1996).

Estudos prévios tentando identificar fatores prognósticos clínicos e histopatológicos importantes associados ao CAC já foram realizados. Entre os fatores clínicos, o estádio do tumor parece ser o fator prognóstico mais importante e confiável em pacientes portadores de CAC (Spiro & Huvos, 1992). Embora outras variáveis como idade e gênero dos pacientes não correlacionam com o prognóstico na maioria das séries, o local do tumor parece ser um fator significante (Nascimento *et al*., 1986). CAC localizados em glândulas salivares menores têm um pior prognóstico que aqueles que surgem nas glândulas salivares maiores (Nascimento *et al*., 1986; Spiro & Huvos, 1992). Isto pode ser explicado pelo fato que tumores situados em glândulas menores têm uma maior chance de ultrapassar os limites da glândula e invadir tecidos moles e osso adjacentes, tornando a ressecção total do tumor mais difícil (Nascimento *et al*., 1986; Kim & Sung., 1994). Alguns autores também observaram que tumores apresentando um curso clínico mais acelerado, com os pacientes queixando de sinais e sintomas por um curto período de tempo, também apresentam um pior prognóstico (Nascimento *et al*., 1986).

Uma característica microscópica freqüente na maioria dos casos de CAC é a presença de invasão perineural. Embora freqüentemente presente em CAC, essa característica também é observada em outros tumores malignos de glândulas salivares (Ellis & Auclair, 1996). Todos os CAC, independentemente da classificação microscópica, apresentam comportamento biológico agressivo e podem apresentar metástases muitos anos após o tratamento. Entretanto, o tipo tubular tem um prognóstico melhor em relação à duração da sobrevida, enquanto o tipo sólido parece ter um pior prognóstico, com múltiplas recidivas, metástases

precoces e alta mortalidade (Perzin *et al*., 1978; Khan *et al*., 2001). Outros autores observaram que o subtipo cribriforme é o mais favorável (Nascimento *et al*., 1986).

Como já mencionado anteriormente, CAC apresenta um crescimento lento e isto se associa a metástases à distância tardias, resultando em uma taxa de sobrevida relativamente favorável em 5 anos, mas baixa em 10 anos (Kim & Sung, 1994; Kokemueller *et al*., 2004). As taxas de sobrevida global variam de 73% a 67% em 5 anos e 51.4% a 44% em 10 anos (Miglianico *et al*., 1986; Garden *et al*. 1995; Khan *et al*., 2001). As porcentagens de recorrência tumoral variam de 37% a 70% entre as séries (Matsuba *et al*., 1984; Nascimento *et al*., 1986; Matsuba *et al*., 1986; Prokopakis *et al*., 1999; Khan *et al*., 2001). Quando há recorrência tumoral, a maior parte dos pacientes apresenta associação de recorrência local e metástases à distância, principalmente nos pulmões (Spiro, 1997; Sung *et al*., 2003). Entretanto, em 31% dos casos apenas metástase à distância é observada, independentemente se há controle local da doença (Spiro, 1997). Comparando os pacientes que nunca haviam apresentado metástase à distância com aqueles que comprovadamente tinham disseminação metastática, não foram observadas diferenças significantes quanto à idade, gênero, local do tumor primário, duração dos sintomas ou estádio tumoral (Spiro, 1997). Entretanto, Bradley (2001) observou que o tamanho do tumor ao diagnóstico e o desenvolvimento de recorrência loco-regional são os dois fatores preditivos mais importantes para ocorrência de metástases à distância. Tratamento para estes tumores metastáticos é um dilema, pois não há nenhuma terapia anti-neoplásica efetiva. Ressecção cirúrgica pode ser considerada para metástases pulmonares isoladas, embora nenhum benefício na sobrevida desses pacientes tenha sido observado (Fordice *et al*., 1999). Altas taxas de recorrência local também estão associadas ao CAC, apresentando índices que variam de 31% a 40% (Khan *et al*., 2001).

Não existem muitos marcadores prognósticos de agressividade biológica definidos para carcinomas de glândulas salivares. Alterações moleculares gênicas e variações na expressão protéica das células tumorais têm sido demonstradas em alguns tumores de glândulas salivares, mas estudos para avaliar seu

significado prognóstico são necessários, visando sua utilização como marcadores prognósticos. Para o estabelecimento destes indicadores, alguns estudos têm pesquisado a possível relevância de marcadores moleculares em CAC e também em outros tumores de glândulas salivares (Norberg-Spaak *et al.*, 2000; Khan *et al.*, 2001; Jia *et al.*, 2004; Alves *et al.*, 2004; Maruya *et al.*, 2004).

p53

O gene TP53 está localizado no cromossomo 17 e o produto da sua expressão, a proteína p53, funciona como inibidor da divisão celular na fase G1 do ciclo celular, podendo induzir apoptose. Alterações nesse gene são freqüentemente observadas em vários carcinomas humanos e são consideradas uma das mudanças mais críticas no início da carcinogênese (Hollstein *et al.*, 1991; Kiyoshima *et al.*, 2001). Nos tumores de glândulas salivares, não há um consenso atual em relação à freqüência de alterações do gene TP53, principalmente em adenocarcinomas de linhagem mioepitelial (Rosa *et al.*, 1997). Em CAC de glândulas salivares, a expressão da proteína p53 varia de 55% a 88% e foi verificado que pacientes portadores de CAC p53-positivos apresentam pior prognóstico, sendo esta proteína, portando, um possível indicador de pobre prognóstico em pacientes com CAC (Zhu *et al.*, 1997; Kiyoshima *et al.*, 2001; Jia *et al.*, 2004).

Marcadores de proliferação celular

O antígeno nuclear de proliferação celular (PCNA) é uma proteína nuclear não-histônica de 36 kD que funciona como uma proteína auxiliar para a DNA polimerase delta, sendo um requisito para a síntese de DNA, cuja expressão tem sido relacionada ao prognóstico de alguns tumores. Em glândulas salivares, apresenta maior expressão em tumores malignos em comparação com os tumores benignos, sendo observada uma expressão significativamente maior nos pacientes que morreram de adenocarcinomas de glândula salivar (Zhu *et al.*,

1999). Em CAC, áreas sólidas expressaram mais PCNA do que áreas cribriformes e tubulares, padrões mais bem diferenciados (Fonseca *et al.*, 1997; Cho *et al.*, 1999). Outra proteína cuja expressão está associada a um aumento na proliferação celular, Ki-67, é uma proteína nuclear com pico de expressão na fase M. A expressão aumentada de Ki-67 em tumores de glândulas salivares correlacionou com um comportamento mais agressivo dos tumores e conseqüentemente pior prognóstico, indicando ser um marcador significante para aumentar a sensibilidade da gradação histológica convencional (Murakami *et al.*, 1992; Skalova *et al.*, 1994). A identificação de marcadores de proliferação celular em CAC de cabeça e pescoço, como Ki-67 e PCNA, está associada a um comportamento clínico agressivo e prognóstico ruim (Norberg-Spaak *et al.*, 2000). Entretanto, outros autores não encontraram resultados similares (Kiyoshima *et al.*, 2001).

CEA

Níveis séricos do antígeno carcinoembrionário (CEA) estão freqüentemente aumentados em tumores do trato gastro-intestinal e em certas condições não-neoplásicas (Alfaro & Carrozza, 1990). Entretanto, há poucos relatos dos valores dos níveis séricos de CEA elevados em associação a carcinomas de glândulas salivares. Níveis séricos de CEA elevados foram descritos em um paciente com metástase abdominal de CAC, dessa forma pode ser utilizado como marcador útil no seguimento de pacientes tratados de CAC de cabeça e pescoço, principalmente aqueles que apresentam metástase a distância, sem recidiva loco-regional (Kuhel *et al.*, 1995). Tem sido observada expressão imunohistoquímica em uma grande variedade de tumores de glândulas salivares, mais comumente em associação a áreas ductais (Kuhel et al., 1995).

c-erbB-2

C-erbB-2 é um proto-oncogene localizado no cromossomo 17p11-q21, sendo primeiramente descrito em estudos de tranfecção de DNA em neuroglioblastomas de ratos e apresenta estreita relação com o receptor do fator de crescimento epidermal (EGF) (Schechter *et al.*, 1984). Codifica um receptor de fator de crescimento transmembrana de 185 kD, que desempenha importante papel na diferenciação, desenvolvimento e sinalização mitogênica em células normais. No câncer de mama feminino, a amplificação de c-erbB-2 parece estar inversamente relacionada a sobrevida livre de doença e a super-expressão deste gene tem significância prognostica em cânceres de mama e ovário (Slalom *et al.*, 1987; Slalom *et al.*, 1989). Em glândulas salivares normais, epitélio ductal apresenta fraca imunopositividade para c-erbB-2, nível de expressão semelhante àqueles tumores de mama que não expressam ou não amplificam esse proto-oncogene (Press *et al.*, 1990). Entretanto, c-erbB-2 tem sido observado em adenocarcinomas de glândulas salivares, levando à hipótese que c-erbB-2 pode induzir e manter neoplasias de glândulas salivares. Embora haja expressão relativamente alta de c-erbB-2 em tumores malignos de glândulas salivares, não foi observada correlação com o comportamento biológico desses tumores (Karja *et al.*, 1994).

Bcl-2

O proto-oncogene bcl-2 codifica uma proteína de 26 kD de mesmo nome, que inibe a apoptose, favorecendo a sobrevida prolongada tanto de células normais quanto de células neoplásicas (Hellquist *et al.*, 1997). Sua expressão aumentada, juntamente com outros oncogenes, tem sido implicada no desenvolvimento de neoplasias malignas. Tem sido demonstrado que a proteína bcl-2 desempenha um papel importante na regulação da apoptose e a super-expressão de bcl-2 como um marcador de supressão de apoptose tem sido relatada em células de tumores de glândulas salivares. Em CAC de glândulas

salivares, a expressão de bcl-2 foi encontrada em 52% dos tumores e não foi observada diferença significante na sobrevida entre pacientes com tumores bcl-2 positivos e pacientes bcl-2 negativos (Norberg-Spaak *et al*., 2000; Jia *et al*., 2004).

Como comentado anteriormente, embora haja alguns estudos abordando o uso de marcadores imunohistoquímicos no prognóstico do CAC de cabeça e pescoço, os resultados obtidos ainda são conflitantes, sendo necessários mais estudos, onde os dados clínicos, histopatológicos e imunohistoquímicos possam ser relacionados.

2. PROPOSIÇÃO

O objetivo deste estudo foi analisar as características clínicas, histopatológicas e imunohistoquímicas de uma grande série de CAC da região de cabeça e pescoço tratados em uma única instituição, para identificar fatores prognósticos. Além disso, nós objetivamos relatar os achados clínico-patológicos de CAC e outros tumores glandulares localizados em regiões anatômicas raramente acometidas, como glândulas lacrimais, seio maxilar e glândulas sublinguais.

3. CAPÍTULO 1

PROGNOSTIC FACTORS IN HEAD AND NECK ADENOID CYSTIC CARCINOMA

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* Artigo submetido à Archives of Otolaryngology – Head and Neck Surgery, em fevereiro de 2005.

ABSTRACT

Objective: The aim of this study was to analyse the clinical, histological and immunohistochemical prognostic factors of a large series of adenoid cystic carcinoma treated in a single institution, using univariate and multivariate survival analyses. **Design**: Inception Cohort. **Setting**: Referral center **Patients:** All cases of head and neck ACC treated in the A. C. Camargo Cancer Hospital, Sao Paulo, Brazil, between 1955 and 1997 were selected for the study. **Main outcome measures:** Rates of local recurrence, regional and distant metastasis, and overall survival. **Results:** Of 129 cases, 71 were male and 58 female, with mean age of 51.5 years (range of 10-96 years). The palate and parotid were the most common sites. TNM stage revealed 96 (74.4%) clinical stages III and IV tumors, and cribriform was the most common histological type. Overall survival rate at 5 years was 56.5%. Univariate survival analysis revealed that age older than 45 years ($p=0.04$), period of complaints inferior to 18 months ($p=0.007$), presence of paresthesia ($p=0.04$), T stage ($p=0.01$), N stage ($p=0.04$), M stage ($p<0.001$), clinical stage ($p=0.003$), solid histological type ($p<0.001$), presence of residual tumor ($p<0.001$) and expression of p53 ($p=0.08$) correlated with a poor prognosis. In the multivariate survival analyses, clinical stage, solid histological subtype and increased expression of p53 were independent significant prognostic factors. **Conclusions**: According to our findings, clinical stage, solid growth pattern and expression of p53 were the most important prognostic factors in patients with ACC.

Key-words: adenoid cystic carcinoma; head and neck; salivary glands, prognostic factors

INTRODUCTION

Adenoid cystic carcinomas (ACC) are uncommon head and neck tumors, corresponding to 4 % to 10% and 7.5% to 23% of all epithelial tumors and malignancies of the salivary glands respectively.[1,2] ACC typically presents an indolent and slow growth associated to frequent late distant metastases, which are together with local recurrences, the reasons for the low long-term survival rate.[2-4]

Standard treatment has not yet been established, although many series of ACC have been described. Some authors have reported an increase local control of the disease with combined surgical and radiotherapy treatments.[5] However, other studies were not able to demonstrate a significant effect for postoperative radiotherapy.[4,6] Several studies showed that advanced clinical stage and solid histological subtype are relevant prognostic factors associated with survival of the patients.[4,6,7] Some immunohistochemical markers, such as p53, bcl-2 and cell proliferation markers have been associated to survival of patients with ACC.[8-11] Nevertheless few studies have associated survival with the clinical, histological and immunohistochemical features in a large series of head and neck ACC. Therefore, the aim of this study was to analyse the clinical, histological and immunohistochemical prognostic factors of 129 patients with ACC treated in a single institution.

MATERIAL AND METHODS

Between the years of 1955 and 1997, all patients diagnosed as having ACC, from the Department of Head and Neck Surgery and Otorhinolaryngology, Hospital

do Câncer A. C. Camargo, Sao Paulo, Brazil, were included in this study. This study was performed with the approval of the Human Research Ethics Committee of this Hospital. Clinical, epidemiological, treatment and follow-up data were obtained from the medical records. All cases were histologically reviewed to confirm the diagnosis and the tumors were classified as tubular, cribriform or solid. Cases sited in the lacrimal glands were excluded of this study.

Immunohistochemical reactions against p53 protein (clone DO-7, dilution 1:200), proliferation cell nuclear antigen (PCNA) (clone PC10, dilution 1:16000), Ki-67 antigen (clone MIB-1, dilution 1:200), c-erbB-2 protein (dilution 1:200), carcinoembryonic antigen (CEA) (clone II-7, dilution 1:500) and bcl-2 protein (clone 124, dilution 1:50) (all DakoCytomation Norden A/S, Glostrup, Denmark) were performed in 3-μm histological sections. Microwave antigen retrieval was carried out using citrate buffer, overnight incubation with the primary antibodies, and secondary antibodies conjugated to a streptavidin-biotin-peroxidase system (strept ABComplex/HRP duet, mouse/rabbit; DakoCytomation Norden A/S), followed by application of diaminobenzidine as the chromogen. Slides were counterstained with Carazzi hematoxylin, mounted, and analyzed by two of us (DECP, FAA). Positive and negative controls were used in all reactions. Percentage of positive cells in ten high power fields was used to classify each tumor, using the following values: PCNA (negative, < 5% positive cells; weak 6%-25% positive cells; or strong > 26% positive cells); p53, Ki-67, CEA, bcl-2 and c-erbB-2 (negative, < 5% positive cells; or positive, > 6% positive cells). Final score was established by a mean of all values obtained.

The Kaplan-Meier method and log-rank test were used for univariate survival analysis. Overall survival was calculated from the date of the first treatment (surgery, radiotherapy or chemotherapy) or first consultation (for the untreated cases) until the date of the last follow-up information. The data were considered uncensored when death was the outcome, independent of its cause. For multivariate survival analysis, all variables were analyzed using the Cox proportional hazards regression model.

RESULTS

Clinical

Of the 129 patients with ACC included in this study, 71 were male (55%) and 58 female (45%), giving a male-female ratio of 1.22:1. The mean age of the patients was 51.5 years (range 10-96 years), being the women older (mean 53.4 years) than men (mean 50.2 years). The mean time of complaints was 39.4 months (range 1-360 months), and the most common signs and symptoms were the presence of a nodule (92.1%), pain (59.8%), paresthesia (12.6%) and nasal congestion (11.8%). Most tumors involved the intraoral minor salivary glands (41.9%), followed by major salivary glands (37.2%) and extraoral minor glands (20.9%) (Table 1). The mean size of the tumors was 4.3 cm (range 1.0-8.5 cm), the larger ones involving the extraoral minor glands (mean 5.3 cm) and submandibular glands (mean 5.2 cm). Intraoral tumors presented a mean size of 4.1 cm, sublingual 3.8 cm and parotid 3.6 cm. In addition, most tumors were fixed to deep structures (77.3%), and invaded adjacent structures (55.8%). At diagnosis, 88

patients (68.2%) presented T3 or T4 tumors, 29 (22.7%) had metastatic neck nodes (N+) and 11 (8.5%) distant metastasis (M+) involving the lungs (7 cases), bone (3 cases) and liver (1 case). Final clinical stage demonstrated that 51.9% of the patients had stage IV and 22.5% stage III disease (Table 2).

Histology and Immunohistochemistry

Twenty-two cases were not submitted to histological analysis because of the limited amount of available tissue. Microscopically 58 tumors (54.2%) were classified as cribriform, 27 (25.2%) as tubular, 22 (20.6%) as solid. Fifty-eight cases (54.2%) revealed perineural invasion, 33 (30.8%) necrosis and 30 (28.0%) vascular invasion.

Most of the cases expressed PCNA (weak in 33.4% and strong in 50%), while expression of bcl-2, p53, Ki-67, CEA and c-erbB-2 was found in 63.1%, 21.3%, 30%, 13.6% and 50%, respectively.

Treatment

The patients were mainly treated by local resection associated to postoperative radiotherapy (RT) (42.7%) and local resection alone (30.2%). Other therapeutic modalities included RT alone (10.1%), RT and chemotherapy (3.9%), local resection and chemotherapy (2.3%) and chemotherapy alone (1.5%). Twelve patients received only supportive care. Thirty-two patients were submitted to neck dissection, with 13 of them showing pathologically positive lymph node metastasis (pN+). After surgical treatment performed in 97 patients, 19 (19.6%) presented surgical margins involved by tumor, and 6 (6.2%) had residual tumor macroscopically.

Local recurrence and regional and distant metastases

A total of 71 cases (55%) presented local recurrence and/or regional and distant metastases. Time of the diagnosis of local, regional or distant failures ranged from 1 to 109 months, with a mean time of 27.2 months. Of the 71 cases with tumoral recurrence, 33.8% presented local recurrence and distant metastases, 25.4% only distant metastases, 19.7% only local recurrence, 9.9% local recurrence, regional and distant metastases, 5.6% distant and regional metastases, 2.8% local and regional recurrences and 2.8% of the patients only lymph node metastases. Distant metastases involved mainly the lungs and bones, followed in decreasing order by brain, liver and epiploon.

Follow-up and survival rates

The mean follow-up time was 70.8 months (range 1-450 months), where 18.7% of the patients are alive without evidence of disease, 4.6% alive with recurrent disease, 48% died by causes related to disease, 16.3% died during treatment or other causes not related to the disease, and 12.4% had the follow-up lost. Overall survival rates were 56.5% and 32.5% for 5 and 10 years, respectively, while disease free survival rates for the same periods were 42.7% and 29.2%.

Univariate and multivariate survival analyses

Univariate survival analysis of clinical and epidemiological data revealed that patients older than 45 years ($p=0.04$), period of complaints inferior to 18 months ($p=0.007$), presence of paresthesia ($p=0.04$), T stage ($p=0.01$), N stage ($p=0.04$), M stage ($p<0.001$) and clinical stage ($p=0.003$) statistically correlated with a poor prognosis (Table 3). Patients presenting fixed tumors ($p=0.27$) and/or invasion of adjacent structure ($p=0.12$), and tumors sited in extraoral glands ($p=0.10$)

presented lower survival, but for these parameters the differences were not statistically significant. Microscopically, solid histological type ($p<0.001$) and presence of residual tumor ($p<0.001$) also statistically correlated with poor prognosis (Table 4). Adjuvant RT improved survival of cases showing microscopical residual tumor, however this finding was not significant ($p=0.07$). Expressions of p53 ($p=0.08$), Ki-67 ($p=0.18$), PCNA ($p=0.19$) and CEA ($p=0.76$) also correlated with a lower survival, however these differences were not statistically significant (Table 4). On the other hand, expressions of bcl-2 and c-erbB-2 did not show differences in the survival rate. Multivariate survival analysis revealed that clinical stage, solid histological subtype and increased expression of p53 were independent significant prognostic factors (Table 5 and Fig 1).

COMMENTS

Adenoid cystic carcinoma most commonly occurs during the fifth and sixth decades of life,[3,4,7] as in our series. In children and adolescents, ACC is extremely rare.[12] We found a slight male predilection, although other studies showed a female or no gender predilection.[2,4,6,7] ACC occurs mainly in the intraoral salivary glands, particularly in the palate, and parotid, followed by submandibular, extraoral, and sublingual salivary glands,[7,13] similar to our findings. However, other sites in the head and neck region can be affected, including lacrimal glands, esophagus and tracheobronchial tree.[14,15]

Several authors have retrospectively studied the clinicopathological features of ACC, attempting to identify significant prognostic factors, but the findings still remain controversial. Age of the patient, location of the tumor, type and duration of

the symptoms, clinical stage, modality of treatment, histological subtype, perineural and/or vascular invasion, and presence of positive surgical margins have been considered. [5-7,13,14,16-21]

In ACC, age seems be an important prognostic factor, although some authors did not confirm this observation.[4,6,22] In our series, age, considering patients older than 45 years, was a significant prognostic factor in the univariate survival analysis. Similarly, patients presenting signs and symptoms in period inferior to 18 months had lower survival rate, as also shown by Nascimento et al[7]. Paresthesia, usually associated with parotid, maxillary sinus and nasal cavity tumors, was a statistically significant prognostic factor, as also observed by Spiro et al[16]. Pain has also been associated with poor prognosis, but we did not confirm such observation.

Advanced clinical stage has been reported as a significant predictor of outcome, and this was confirmed in our series, since patients who had stages III and IV presented a worse prognosis in the univariate and multivariate survival analyses.[7,22] Spiro et al[14,16] postulated that clinical stage is the most important indicator of ACC behavior.

Solid histological subtype was an important indicator of outcome in the present and in other series.[6,7,18] Nevertheless this was not so clear in other reports.[17,21,22] Positive surgical margin was also a significant prognostic factor in our series, confirming other studies.[4,21] Perineural invasion is common in ACC. It is interesting that similarly to most other series, we did not find a correlation between perineural invasion and poor prognosis.[7,16,18] Most ACC present perineural invasion, although not observed in some cases, harming the evaluation of this

parameter. On the other hand, a decreased rate of local control was found when a major nerve was involved by tumoral cells.[20]

Regarding to modalities of treatment in the present series, combined surgery and radiotherapy was the most common form, followed by surgery alone, as also described in most series.[5,17,20] As in other reports, we were not able to demonstrate significant differences between groups that received postoperative radiotherapy or surgery alone.[4,6] As mentioned by Khan et al[6], this can be due the nonrandomization of patients for various treatment modalities and the inclusion of palliative cases. However, some studies showed better local control of the disease with the combined use of surgery and radiotherapy, mainly in cases with surgical positive margins.[5,18,19,23] There are reports of local control rate of more than 80% in 10 years in patients with surgical margins compromised by tumor treated with adjuvant radiotherapy.[20] Elective neck dissection is not indicate, because the risk of lymph node metastases is relatively low and distant metastases are most common.[6.21,23] Our cases that underwent to a neck dissection, there was clinical or surgical suspicion of lymph node involvement. However, some authors indicate the elective treatment of the first-echelon lymph nodes for patients with tumors sited in base of the tongue and nasopharynx, which are regions with high amount of capillary lymphatics.[23] In the present series, of the cases with lymph node metastasis, it was not observed predilection for site. Prospective multicentric studies must be performed to establish the standard treatment for ACC.

Local and distant recurrences are common in ACC, leading to low long-term survival rates.[1,2] Total failure rate varies from 43% to 70%, with local failures ranging from 31% to 52% and distant metastases from 19% to 52%.[4,6,14,18,21] We

found overall, local and distant failure rates of 55%, 36.3% and 41.0%, respectively. In agreement to various reports, metastases to the lungs were the most common.[4,6,7,14,24] Among our patients, the mean time to total failure was 27.2 months, but there were cases that recurred after 9 years of treatment. Late recurrence is frequent in ACC and recurrence after 20 years of treatment were reported.[1] In the present study, 48% of the patients died of causes related to the disease. This high rate of mortality is explained by the advanced stage of the disease when diagnosed, as occurred in other series.[7]

Expressions of proliferation cell nuclear antigen (PCNA) and Ki-67 (MIB-1) are correlated with aggressive clinical behavior, and poor prognosis in patients with ACC.[11] However, this was not confirmed by Kiyoshima et al[9]. In the present report, PCNA and Ki-67 expressions were associated with lower survival, but the difference was not statistically significant. Elevated serum CEA level was described in a patient with abdominal metastasis of ACC, and it was suggested it could be a useful marker for distant metastases.[15] We found expression of CEA in 13.6% of our cases, and no significant correlation with survival of the patients was noted. We also did not confirm that expression of c-erbB-2 is related to survival.[25] Similar to other reports bcl-2 expression was not a prognostic factor.[10,11] Expression of p53 protein in ACC varies from 55.5% to 88% of the cases, however we found only 21.3% of positive tumors. Patients with p53 positive tumors presented worse prognosis and therefore it can be useful as indicator of poor prognosis.[8-10] In fact, in our series p53 was an independent significant prognostic factor.

In summary, advanced clinical stage, solid growth pattern and expression of p53 protein were the independent significant prognostic factors, found in our series

of 129 cases of ACC. Although ACC is one of the most studied salivary gland malignancies, further studies are necessary to better establish relevant prognostic factors and treatment modalities.

ACKNOWLEDGEMENTS

This work was supported by The State of Sao Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq), Brazil.

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Table 1- Sites of incidence of 129 Head and Neck adenoid cystic carcinomas

Site	**No. (%)**	**Total %**
Intraoral minor salivary glands	54 (100)	41.9
Palate	29 (53.7)	22.5
Tongue	10 (18.5)	7.7
Buccal mucosa	5 (9.3)	3.9
Floor of the mouth	5 (9.3)	3.9
Upper lip	4 (7.4)	3.1
Lower lip	1 (1.8)	0.8
Major salivary glands	48 (100)	37.2
Parotid	25 (52.1)	19.4
Submandibular	20 (41.6)	15.5
Sublingual	3 (6.3)	2.3
Extraoral minor salivary glands	27 (100)	20.9
Maxillary sinus	18 (66.6)	14.0
Nasal cavity	3 (12.5)	2.3
Oropharynx	3 (12.5)	2.3
Nasopharynx	2 (7.4)	1.5
Ethmoid sinus	1 (3.7)	0.8
Total	**129**	**100**

Table 2- TNM Stage of 129 Head and Neck adenoid cystic carcinomas

TNM Stage Criterion	**No. (%)**
Size of tumor (T)	
T1	13 (10.1)
T2	23 (17.8)
T3	30 (23.2)
T4	58 (45)
TX	5 (3.9)
Regional metastasis (N)	
N+	29 (22.5)
N0	100 (77.5)
Distant metastasis (M)	
M1	11 (8.5)
M0	118 (91.5)
Clinical stage (TNM)	
I	10 (7.8)
II	18 (13.9)
III	29 (22.5)
IV	67 (51.9)
Not staged	5 (3.9)

Table 3- Univariate survival analysis of clinical and epidemiological data of 129 adenoid cystic carcinomas

Variable	No.	Survival, %		P Value*
		5y	10y	
Age, y (n=129)				
≤ 45	45	65.3	33.5	0.04
> 45	84	48.8	26.8	
Complaints, months (n=106)				
≤ 18	60	43.8	16.6	0.007
> 18	56	59.4	39.9	
Paresthesia (n=124)				
Yes	16	27.3	9.1	0.04
No	106	57.6	30.2	
Size of tumor (T) (n=124)				
T1 and T2	36	70.8	38.7	0.01
T3 and T4	88	45.6	23.9	
Regional metastasis (N) (N=129)				
N+	29	32.0	22.8	0.04
N0	100	60.5	31.4	
Distant metastasis (M) (n=129)				
M1	11	13.1	0.0	<0.001
M0	118	57.7	30.8	
Clinical stage (TNM) (N=124)				
I and II	28	70.8	38.7	0.003
III and IV	96	45.6	23.9	

*All statistically significant

Table 4- Univariate survival analysis of histological and immunohistochemical variables of head and neck adenoid cystic carcinomas

Variables	No.	Survival, %		P Value
		5 y	10 y	
Histological subtype (n=107)				
Cribriform	58	60.1	40.4	<0.001*
Tubular	27	55.9	34.1	
Solid	22	25.4	0.0	
Residual tumor (n=91)				
No	66	58.3	30.7	<0.001*
Microscopical	19	42.3	14.1	
Macroscopical	6	0.0	0.0	
Proliferation cell nuclear antigen (n=84)				
No	14	71.6	51.1	0.19
Weak	28	35.7	23.8	
Strong	42	28.0	18.6	
Ki-67 (n=80)				
Yes	24	38.9	12.0	0.18
No	56	55.1	33.8	
p53 (n=80)				
Yes	17	37.9	7.6	0.08
No	63	53.4	33.8	
CEA (n=82)				
Yes	11	50.2	12.6	0.7
No	70	53.4	28.6	

*Statistically significant

Table 5- Independent significant prognostic factors after cox multivariate survival analysis of 129 head and neck adenoid cystic carcinomas

Variable	**Categories**	**Relative Risk (95% Confidence Interval)**	**P Value**
Clinical stage	III and IV	2.3 (1.3-4.1)	0.004
Histologic subtype	Solid	3.6 (2.1-6.1)	<0.001
Expression of p53	Yes	1.7 (1.0-3.7)	0.05

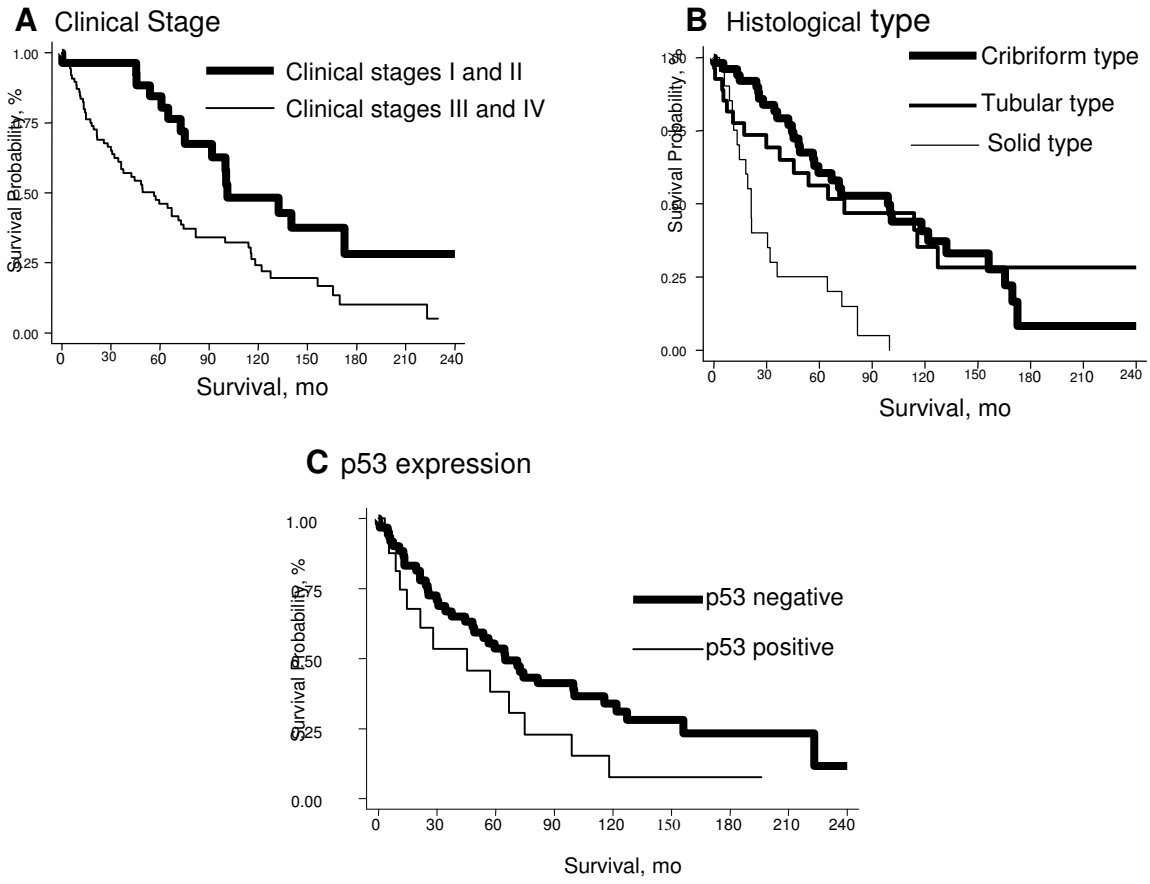

Figure 1- Multivariate survival analysis of patients with head and neck adenoid cystic carcinoma. Overall survival curves for: A, clinical stage; B, histological subtype of the tumor and C, expression of p53.

4. CAPÍTULO 2

ADENOID CYSTIC CARCINOMA AND MUCOEPIDERMOID CARCINOMA OF THE MAXILLARY SINUS: REPORT OF A 44-YEAR EXPERIENCE OF 25 CASES FROM A SINGLE INSTITUTION

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*Artigo submetido a Journal of Oral and Maxillofacial Surgery em fevereiro de 2005.

ABSTRACT

PURPOSE: The aim of this study was to analyze the clinicopathological characteristics of all cases of adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC), arising in the maxillary sinuses, treated in a single institution. **PATIENTS AND METHODS**: From 1953 to 1997, eighteen ACC and seven MEC from the maxillary sinus were studied. Clinical data were obtained from the medical records and microscopical slides were reviewed. **RESULTS**: Mean age was 45.9 years (range 13 - 77) and TNM staging revealed 88.9% of ACCs at advanced clinical stages, while 57.1% of the MECs were initial clinical stages. Surgery combined to radiotherapy was the most common treatment. Follow-up showed 88.8% of ACC patients died by the tumor, while 42.8% of the patients with MEC are alive without disease. **CONCLUSIONS**: Maxillary sinus ACC and MEC were uncommon tumors and the prognosis depends particularly of the clinical stage and of the histological type, where MEC has better outcome.

Key-words: adenoid cystic carcinoma; glandular carcinomas; mucoepidermoid carcinoma; maxillary sinus

INTRODUCTION

Maxillary sinus malignant tumors are relatively uncommon malignancies and can include several histologic types such as squamous cell carcinomas (SCC), adenocarcinomas, undifferentiated carcinomas, melanomas, sarcomas, lymphomas and metastatic tumors.[1] Carcinomas are the most common tumors, with SCC, followed by the glandular tumors, such as adenoid cystic carcinomas (ACCs) and mucoepidermoid carcinomas (MECs), representing about 60% and 13% of the carcinomas in this site, respectively.[2] Maxillary sinus carcinomas usually present as advanced T stage tumors and are reported to be associated with a moderate to poor prognosis.[3] However, apart from TNM staging, histological type seems to be very important in the prognosis of the maxillary sinus carcinomas, with better survival rates for glandular carcinomas.[2]

Most reported studies have analyzed paranasal sinus as a group, including maxillary, ethmoid, sphenoid and frontal sinuses, and also including nasal tumors, difficulting the interpretation of its individual characteristics.[2,4] In addition, most papers have analyzed squamous and non-squamous cell carcinomas together, reporting different survival rates. The aim of our study was to evaluate clinical and histopathological findings as well as the management and prognosis of maxillary sinus ACCs and MECs treated in a single institution during a 44-year period.

PATIENTS AND METHODS

The files of the Head and Neck Surgery and Otorhinolaryngology Department, AC Camargo Cancer Hospital, São Paulo, Brazil were reviewed and

all cases diagnosed from 1953 to 1997 as ACC and MEC arising from the lining of maxillary sinus were selected for this study. Cases whose antral origin was not completely established with clinical, radiological and tomographical findings, as well as cases supposed to be originated from palatal or other glands were excluded from the study.

Clinical information about age, gender, duration of signs and symptoms, type of complaints, clinical stage, management and follow-up with current status of the patients were obtained from the medical charts. TNM re-staging was established according to Sobin and Wittekind.[5] All cases were histologically reviewed in HE stained slides and MECs were also stained with PAS and mucicarmine and graded according to Ellis & Auclair.[6] ACCs were histologically classified in cribriform, tubular and solid type.

Overall survival was calculated from the date of the first treatment (surgery or radiotherapy) or first consultation, until the date of the last follow-up information. The cases were considered uncensored when death was the outcome independent of its cause.

RESULTS

In the 44-year interval from 1953 to 1997, 25 cases, including 18 ACCs and 7 MECs were selected from a total of 129 ACCs and 173 MECs of the head and neck. The mean age of the patients was 45.9 years (range 13 to 77), and it was similar to MECs (44.7 years) and ACCs (46.4 years). ACCs were more frequent in men, while MECs predominated in women (Table 1).

Mean time of complaint was 31.6 months, ranging from 1 to 180 months in the total, and it was higher in MECs than ACCs. The most common complaint was the presence of a mass or tumor in the area (100%), pain (72%), nasal obstruction (36%), epistaxis (24%), visual disturbances (20%) and paralysis (16%). All complaints were more frequent in ACCs. Tumors similarly affected right and left maxillary sinus, but none of the tumors were bilateral (Table 1).

Twenty-three cases (92%) showed invasion of one or more adjacent structures, as the maxillary bone (23 cases), nasal cavity (10 cases), floor of the orbit (6 cases), ethmoid (4 cases) and pterigo-palatine fossae (4 cases). TNM staging showed that most ACCs were diagnosed at T3 or T4 stages and at clinical stages III and IV, while MECs were more diagnosed in initial clinical stages I and II. Regional metastasis at the diagnosis occurred in one case of ACC and one MEC, while two cases of distant metastasis (lung and bone) were in ACCs (Table 1).

Histopathology of the surgical specimens revealed 72.2%, 16.7% and 11.1% cribriform, solid and tubular ACCs, respectively; and 28.6%, 28.6% and 42.8% low, intermediate and high grade MECs, respectively. Perineural, perivascular, and muscle invasion were more common in ACCs, while anaplasia and atypical mitosis were more common in MECs (Table 2).

Regarding treatment, seven patients (4 ACCs and 3 MECs) reported previous oncologic treatment, including surgery and radiotherapy (RT). All but three patients, that received only supportive care, received treatment in our Hospital, that included surgery and RT (11 cases), surgery alone (4 cases), RT alone (4 cases), RT and chemotherapy (CT) (2 cases) and CT alone (1 case). Surgeries included total maxillectomy (13 cases, being 9 ACCs and 4 MECs) and

craniofacial resection (2 ACCs). A neck dissection was performed in only one ACC patient, and revealed 5 metastatic lymph nodes out of 34 evaluated. The patient with MEC that presented lymph node involvement received only supportive care. Residual microscopical and macroscopical tumor was present in 7 cases (6 ACCs and 1 MEC) and 2 cases (2 ACCs), respectively. RT was employed in 13 ACCs and 4 MECs (Table 3).

Follow-up showed local recurrence in 11 ACCs, after a mean of 35 months (range 2 to 83 months). Regional metastases were found in 2 ACCs as homolateral masses after 32 and 128 months. Distant metastases were diagnosed in 7 ACCs after a mean of 45.8 months (range 6 to 156 months). In 4 cases distant metastasis were found after local recurrence/regional metastasis, and in 2 cases distant metastases were found before local recurrence/regional metastasis. One patient presented only distant metastasis. The sites of distant metastasis included lung (5), bone (1), liver (1), brain (1), cerebellum (1), abdomen (1) and epiploon (1), with one patient showing multiple distant metastases (lung, liver, brain, cerebellum and epiploon). Local recurrences, regional and distant metastasis were managed by surgery (9 cases), CT (6 cases) and RT (5 cases) (Table 4).

From the 18 cases of ACC, 16 (88.8%) died by the disease after a mean follow-up of 54.7 months (range 1 to 170 months). From 7 MECs, 3 patients (42.9%) were alive without clinical evidence of disease after a mean follow-up of 57.3 months (range 1 to 224 months), while 2 patients died by persistence of the tumor. Overall 5-year and 10-year survival rates for all cases were 46% and 25%, respectively. Overall 5-year and 10-year survival rates for MECs were 70%. For

ACCs, overall 5-year and 10-year survival rates were 40% and 0%, respectively. Follow-up was lost in two cases of MEC out of the 25 cases (Table 4).

DISCUSSION

Malignancies of the nasal cavity and paranasal sinuses represent only 3 to 5% of all head and neck carcinomas, with an annual incidence of 0.5 to 1.0 cases per 100,000 population.[1,2,7] Maxillary sinus is the most common paranasal sinuses affected, representing about 70% of the cases[1,2,7] and SCC is the most common histological type.[1,7-9] Maxillary sinus non-SCC include glandular carcinomas (e.g. ACC and MEC), and undifferentiated carcinomas.[3] These groups represent, respectively, 15% and 13% of maxillary sinuses carcinomas, and ACC is considered the most common non-SCC carcinoma of the maxillary sinus. [1-3,7,10,11]

Maxillary sinus non-SCCs affect mainly adults with male predilection.[1-3,11] Although both our ACCs and MECS affected adults, ACCs were more common in males and MECs in females. Maxillary sinus and nasal cavity ACCs represent only about 5 to 10% of all ACC,[12,13] although our report shows a slighter higher value (14%). MECs are also uncommon in the maxillary sinus, and represented 4% of all MECs from our Institution for the same period.

Maxillary sinus MECs presented as long-lasting asymptomatic tumors, as shown by the longer complaint interval and fewer symptoms reported by the affected patients. On the other hand, ACCs showed a shorter mean complaint interval and all complaints, such as pain, nasal obstruction and nasal bleeding, were more common in this tumor. We can suppose that ACCs presented faster growth rate than MECs and, consequently, presented symptoms earlier. Although

the literature reports symptoms for a mean period of 4 months,[1] our cases showed a much longer interval between the onset of complaints to diagnosis. Initial symptoms of maxillary sinus carcinomas are highly unspecific and could be misdiagnosed as allergic rhinitis and sinusitis, delaying correct diagnosis and consequently, proper treatment .[4,9,11]

Tumor growth in the sinus cavity delays some complaints until it reachs one of the cortical plates and invades bone. After bone invasion, the complex anatomy of the region, associated with invasion of adjacent structures (especially the pterygomaxillary fossae) and its proximity to vital structures, such as eyes, brain and cranial nerves, have a significant direct negative impact on prognosis and survival.[2,14] These features were very common in our ACCs and could be responsible, at least in part, for their poor outcome.

Most maxillary sinus non-SCCs present at T3 or T4 stages and local advanced disease, so early diagnosis is essential for successful management.[1-3,9,11] However, this seems not to be true for all glandular carcinomas. Our ACCs were diagnosed in advanced clinical stages (III + IV) in 89% of the cases, but MECs were almost equally diagnosed in early and advanced stages. This finding can explain, at least partially, the differences in their prognosis. In contrast to advanced local staging in non-SCC of the maxillary sinus, regional metastasis (2% to 16% of the cases) and distant metastasis (up to 2% of the cases) are uncommon at diagnosis,[1-3] as it was confirmed by our series.

Surgery is the treatment of choice for maxillary sinus carcinomas, and prognosis is better for patients managed by surgery (associated to RT) than for patients submitted to RT and/or CT alone.[2] Several reports demonstrated

increased local control rates with the use of postoperative RT, achieving local control rates of 60 to 76%.[4,15,16] RT alone seems to be indicated only in T4 unresectable tumors.[9] Nishino et al.[8] reported the treatment of maxillary sinus carcinomas with conservative surgery combined with RT and regional chemotherapy, demonstrating an overall 5-year and 10-year survival rates of 76% and 66%, respectively.

Failure in controlling local disease is the most frequent cause of death in patients with maxillary sinus carcinoma, and positive surgical margins are directly related to a poor prognosis.[2,8] Destruction of maxillary sinus bone walls with local spreading of the tumor is common, making it difficult to reach adequate complete resection and tumor-free margins, leading to high local recurrence rates.[4] We found positive margins in 9 out of 15 cases (60%) submitted to surgical treatment and eight of these tumors were ACCs. This finding reinforces that tumors presenting with complaints are probably more aggressive and locally destructive, consequently leading to invasion of adjacent structures and more difficult surgical approach, resulting in incomplete resection. Local recurrence, regional metastasis and distant metastasis rates can reach 76%, 10% and 32%, respectively, in maxillary sinus carcinomas,[1,2,16] and this was confirmed by our results. It is relevant to consider that local recurrence, regional and distant metastases were exclusive of ACCs in our cases.

Maxillary sinus non-SCC carcinomas have an overall 5-year survival rate of 45.6%.[3] Among the glandular tumors, ACCs is considered to have the worst prognosis, but some authors have claimed that overall 5-year survival for maxillary sinus ACC and MEC are 57 and 36%, respectively.[1,3] In our series, ACCs patients

had a poorer prognosis than those with MECs, and this difference in the survival may well reflect the different clinical stage at presentation between the two tumors. In addition, as expected by the late regional and distant metastasis associated with ACCs, overall 10-year survival rate for this tumor is 35%.[1,16] When comparing these data to overall survival rates of ACCs and MECs from all sites including major and minor salivary glands, it seems clear that maxillary sinus ACCs and MECs have a worst prognosis.[13,17] As staging and histological features from both maxillary sinus ACCs and MECs, are similar to correspondent salivary gland tumors, failure in controlling local disease and distant metastasis are probably the important factors in the survival rate, specially for ACCs. However, there is paucity of prognostic data regarding exclusively maxillary sinus ACC and MEC in the literature, so it is difficult to compare our results.

Loco-regional control and cancer survival rates are better for maxillary sinus glandular tumors (78%), including ACC and MEC, than for SCC (60%).[2,3] This seems even more clear when comparing to undifferentiated carcinoma, a histological subtype prone to develop distant metastasis,[2,14] although Myers et al[1] showed that survival rates were similar for SCC, ACC and adenocarcinoma. As the literature suggests that there are differences in the behavior and prognosis in SCC and non-SCC of the maxillary sinus, it is important to accurately distinguish histologically both groups. This usually is easy when dealing with ACC, but it can be somewhat difficult in high-grade MEC and undifferentiated carcinomas.

Several authors have claimed that advanced T stage, as well as neck and distant metastasis have adverse effects on prognosis of maxillary sinus carcinomas.[1,2,18,19] In contrast, Bhattacharyya[3] supported that T stage has less

effect than tumor histology on the prognosis of maxillary sinus non-SCC. We were not able to evaluate the relevance of tumor histological subtypes on prognosis, due to the limited amount of each subgroup, but it seems that local invasion and staging features are the most important features in prognosis of maxillary ACCs and MECs. This is even more suggestive when we observe that invasion parameters were much more frequent in ACCs than in MECs, and in contrast, cellular anaplasia was more common in MECs than in ACCs.

In summary, ACC and MEC are uncommon maxillary sinuses malignant tumors, typically present with advanced T stage, especially ACCs. ACCs seem to have a more aggressive behavior, presenting a greater potential of invading adjacent structures, consequently leading to positive surgical margins, favoring local recurrences and distant metastasis. As primary symptoms of maxillary sinus carcinomas are many times unspecific, they can be misdiagnosed as inflammatory diseases from paranasal sinuses, delaying correct diagnosis. Therefore, early diagnosis and proper surgical approach, eventually associated to postoperative RT, are important in order to improve overall survival in maxillary sinuses ACC and MEC.

ACKNOWLEDGEMENTS

This work was supported by The State of Sao Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq), Brazil.

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Table 1. Clinical parameters of adenoid cystic carcinomas (ACC) and mucoepidermoid carcinomas (MEC) from the maxillary sinus.

Parameter	ACC (n=18)	MEC (n=7)	Total (n=25)
Mean age (range)	46.4 (27-72)	44.7 (13-77)	45.9 (13-77)
Gender			
Male	12 (66.7%)	3 (42.9%)	15 (60%)
Female	6 (33.3%)	4 (57.1%)	10 (40%)
Time of complaints (months)	21.7 (1-60)	59.5 (2-180)	31.6 (1-180)
Complaints			
Presence of a mass	18 (100%)	7 (100%)	25 (100%)
Pain	14 (77.8%)	4 (57.1%)	18 (72%)
Nasal obstruction	8 (44.4%)	1 (14.3%)	9 (36%)
Nasal bleeding	5 (27.8%)	1 (14.3%)	6 (24%)
Visual disturbances	4 (22.2%)	1 (14.3%)	5 (20%)
Paralysis	4 (22.2%)	0	4 (16%)
TNM staging			
T1 + T2	2 (11.1%)	4 (57.1%)	6 (24%)
T3 + T4	16 (88.9%)	3 (42.9%)	19 (76%)
N+	1 (5.6%)	1 (14.3%)	2 (8%)
M+	2 (11.1%)	0	2 (8%)
Clinical stages I + II	2 (11.1%)	4 (57.1%)	6 (24%)
Clinical stages III + IV	16 (88.9%)	3 (42.9%)	19 (76%)

Table 2. Histopathological subtypes and features of 18 adenoid cystic carcinomas (ACCs) and 7 mucoepidermoid carcinomas (MECs) from the maxillary sinus.

Parameter	ACC (n=18)	MEC (n=7)
ACC		
Cribriform	13 (72.2%)	-
Solid	3 (16.7%)	-
Tubular	2 (11.1%)	-
MEC		
Low grade	-	2 (28.6%)
Intermediate grade	-	2 (28.6%)
High grade	-	3 (42.8%)
Histopathological parameters		
Perineural invasion	7 (38.9%)	0
Necrosis	5 (27.8%)	1 (14.3%)
Anaplasia	3 (16.7%)	3 (42.9%)
Perivascular invasion	4 (22.2%)	0
Atypical mytosis	2 (11.1%)	2 (28.6%)
Muscle invasion	4 (22.2%)	0

Table 3. Treatment modalities of adenoid cystic carcinomas (ACCs) and mucoepidermoid carcinomas (MECs) from the maxillary sinus.

Parameter	ACC (n=18)	MEC (n=7)	Total (n=25)
Treatment modalities *			
Surgery + RT	8 (44.5%)	3 (42.8%)	11 (44%)
Surgery	3 (16.7%)	1 (14.3%)	4 (16%)
RT	3 (16.7%)	1 (14.3%)	4 (16%)
RT + CT	2 (11.1%)	0	2 (8%)
CT	1 (5.5%)	0	1 (4%)
Supportive care	1 (5.5%)	2 (28.6%)	3 (12%)
Radiotherapy (RT)			
Local	12	4	16
Local + regional	1	0	1
Median Local dose (cGy)	5360 (3000-6500)	4475 (2000-6000)	5151 (2000–6500)
Regional dose (cGy)	4500		-

* – RT – radiotherapy; CT – chemotherapy.

Table 4. Follow-up information of adenoid cystic carcinomas (ACCs) and mucoepidermoid carcinomas (MECs) from maxillary sinus.

Parameter	ACC (n=18)	MEC (n=7)	Total (n=25)
Local recurrences/Persistence	11 (61.1%)	2 (28.6%)	11 (44%)
Regional neck metastasis	2 (11.1%)	0	2 (8%)
Distant metastasis	7 (38.9%)	0	7 (28%)
Follow-up (months)	54.7 (1 – 170)	57.3 (1 – 224)	55.4 (1 - 224)
Last information			
Alive without disease	1 (5.6%)	3 (42.8%)	4 (16%)
Alive with disease	1 (5.6%)	0	1 (4%)
Dead by the disease	16 (88.8%)	2 (28.6%)	18 (72%)
Lost follow-up	0	2 (28.6%)	2 (8%)

5. CAPÍTULO 3

Epithelial lacrimal gland tumors: a clinicopathological study of eighteen cases

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ACKNOWLEDGEMENTS: This work was supported by The State of Sao Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq), Brazil.

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Running title: Lacrimal gland tumors

Artigo submetido a Head & Neck em novembro de 2004.

ABSTRACT

Background: The aim of this study is to report the clinicopathological features of 18 epithelial tumors of the lacrimal gland treated in a single institution. **Methods**: Clinical data and treatment were recorded and histological features reviewed. **Results**: Twelve tumors (66.7%) were adenoid cystic carcinoma (ACC), five (27.8%) pleomorphic adenoma (PA), and one (5.5%) carcinoma ex-pleomorphic adenoma (CXPA). All patients with ACC presented with advanced clinical stage, and most were treated by wide surgical resection followed by adjuvant radiotherapy. No recurrence was observed in four cases of ACC, however, seven patients died by persistence of disease or by local and/or distant recurrence. All PA patients were successfully treated by surgical resection. **Conclusions**: Epithelial lacrimal gland tumors are mainly PA and ACC, and malignancies are more common than benign tumors. Although ACC presented typically with advanced clinical stage, thirty-three percent of the patients survived without tumor recurrence or metastasis.

Key words: adenoid cystic carcinoma, carcinoma ex pleomorphic adenoma, lacrimal glands, lacrimal gland tumors, pleomorphic adenoma.

INTRODUCTION

Lacrimal gland tumors are rare and constitute approximately 9% of all orbitary lesions.[1] Lacrimal gland lesions are divided in four groups, the inflammatory and lymphoid are the most common, followed by metastatic, and primary epithelial tumors.[2,3] Primary epithelial lacrimal gland tumors are histologically similar to those arising in the salivary glands, and pleomorphic adenoma and adenoid cystic carcinoma are the most common benign and malignant tumors respectively.[4,5] Differently from the salivary glands, mucoepidermoid carcinoma and acinic cell carcinoma are extremely rare in the lacrimal glands.[2-4]

Because of the rarity of lacrimal gland tumors, most papers describe single cases or small series, and only a few large series have been reported in the English language literature.[1,4,5] The aim of this study is to report the clinical and histopathological features of eighteen epithelial lacrimal gland tumors treated in a single institution in Brazil during a period of 36 years.

MATERIALS AND METHODS

From 1966 to 2002, eighteen cases of lacrimal gland tumors were treated at the Department of Head and Neck Surgery and Otorhinolaryngology, Hospital do Cancer A. C. Camargo, São Paulo, Brazil. All were intrinsic tumors of the lacrimal glands, being excluded cases of the major and minor lacrimal ducts and extrinsic tumors that only invaded the lacrimal gland or were metastasis. Data about age,

gender, duration of signs and symptoms, size of the tumor, treatment and outcome were collected from the medical records. Malignant tumors were clinically re-staged according to UICC TNM Classification.[6] All cases were histologically reviewed for confirmation of the diagnosis. This study was carried out with the approval of the Human Research Ethics Committee of this Hospital.

RESULTS

Five (27.8%) out of eighteen cases of lacrimal gland tumors were pleomorphic adenoma (PA), twelve (66.7%) adenoid cystic carcinoma (ACC) and one (5.5%) carcinoma ex-pleomorphic adenoma (CXPA). Ten patients were females (three PA; six ACC; one CXPA) and eight males (two PA; six ACC). The mean age of the patients was 41.3 years (ranging from 16 to 63 years old), being 40.2 years (ranging from 19 to 63 years old) for malignant tumors and 44.2 years (ranging from 16 to 61 years old) for PA. The clinical data of all tumors are summarized in the Tables 1 and 2.

In PA there was no predilection for gender, with two males and three females affected. All five patients complained of eyelid ptosis or proptosis (Figure 1); one also complained of pain and two presented loss of mobility of the eye. The mean time of complaints was 28.8 months (range of 12 to 60 months), and the tumors presented a mean size of 3.4 cm. All patients with PA were treated by surgical excision of the tumor, except one case treated in 1974 that was submitted to orbital exenteration. There was no tumoral recurrence after treatment. Microscopically, the tumors were composed mainly by epithelial cell sheets and ductal structures, with few myxoid and chondroid areas. Only one case (case 2)

was predominantly formed by myxoid areas. All cases were well circumscribed, and three encapsulated. In addition, we observed squamous differentiation (Figure 2) in three cases. Mitotic figures and necrosis were absent.

No predilection for gender was observed in ACC, and the patients presented a mean age of 40.4 years. Most patients with ACC complained of swelling (ten cases), and eight patients also presented exophthalmia, six had pain, six loss of ocular mobility and vision and one showed facial paralysis. The mean time of complaints was 26.6 months (ranging from 3 to 144 months). All patients were diagnosed with advanced clinical stage, six cases T4N0M0, four T3bN0M0, one T3aN0M0, and one T4N0M1 (with lung and bone metastasis at presentation). Eight cases showed destruction of adjacent bone and three presented intracranial invasion. One patient (case 16) reported previous surgical and RT treatments in another institution. Patients with ACCs were mainly treated by wide surgical resection and radiotherapy (RT) (four cases), and surgery, RT and chemotherapy (CT) (three cases). Other treatment modalities included wide surgical resection alone (two cases), RT alone (one case) and RT and CT (one case). One case received only supportive care. No tumoral recurrence was observed in four cases after 24, 27, 67 and 171 months of treatment. However, five cases showed local recurrence and/or metastases (lung, bone and brain) and all five patients died of the disease after a mean time of 77.8 months of the treatment. One patient was lost to follow-up and two died because of persistence of the disease (cases 6 and 12).

Histologically, seven cases of ACC were classified as cribriform, three as solid (basaloid type) and two as tubular. Five cases presented necrosis and six

perineural invasion (Figure 3). ACC solid type was associated with the lowest survival, all had intracranial invasion, and two out of three showed distant metastases.

The other malignant tumor was a CXPA, which affected a 38 year-old woman. The patient complained of a painless right orbital swelling, with 10 months of evolution. Clinically, it was observed a hard subpalpebral lesion, fixed to orbitary wall, measuring approximately 3 cm of extension. There was no loss of ocular mobility and vision. Magnetic resonance imaging revealed a tumor sited on right lacrimal gland, which caused erosion of the upper orbitary wall. The patient was submitted to wide surgical resection, presenting tumor-free margins, and adjuvant RT, remaining without recurrences after 29 months of treatment. Microscopically, benign and malignant areas composed the tumor. The benign component was PA, while the malignant part was an adenocarcinoma NOS, presenting invasive features, atypical mitoses, capsular and vascular invasion.

DISCUSSION

The lacrimal glands are formed by the palpebral and orbital lobes, superficially and deeply sited respectively. Lacrimal gland tumors are more common in the deep orbital lobe, than in the palpebral. Most palpebral lobe tumors are PA, although malignant tumors also can arise on this site.[7] In our series, it was not possible to identify the location of the tumor in the gland because it was not recorded, and most cases were treated before the use of computed tomography or resonance magnetic imaging in our hospital.

Lacrimal gland masses are inflammatory in about 63% of the cases, followed by epithelial tumors with approximately 30% of the lesions.[2,8] Epithelial tumors present a large histopathological spectrum, as in the major and minor salivary glands.[2,4,9] Benign epithelial tumors, mainly PAs, are more common than malignancies, but the data of the literature are variable.[2-4] In our series, malignant tumors predominated, possibly because our institution is a tertiary cancer referral center.

PA presents clinically as a well circumscribed, slow growing, painless swelling, which can cause ptosis, proptosis and mobility restriction of the eye.[3] The majority of the patients of our series presented these complaints. The mean age of the patients with PA is 41 years,[10] however we found a slightly higher value, 44.2 years. Lacrimal gland tumors, whether benign or malignant, commonly affects middle aged patients and are rare in children and adolescents.[11-13] One of our patients was a 16 years old girl with a history of PA for five years, causing palpebral ptosis when diagnosed.

Orbital masses that present clinical and radiographic features of benign epithelial tumors, as well as circumscribed tumors with smooth limits, should be submitted to dacryoadenectomy and excision of the tumor.[3,14] This is very important because a diagnostic biopsy before the excision of the tumor may result in a recurrence rate of 30% over five years.[3,11] In this series, all cases were treated by excision of the tumor without previous biopsy, except in one case (case 1) that was submitted to orbital exenteration. This was the first case treated in our hospital and such aggressive approach can be credited to the inexperience with the management of this tumor at the time. For preoperative diagnosis, fine needle

aspiration biopsy can be an important tool, as the accurate diagnosis of such lesions, can result in less invasive and safer surgical treatment.[15]

Malignant transformation in a benign PA has been reported. This transformation can occur in a PA with long time of evolution or in a recurrent PA that was submitted to multiple surgical resections. Therefore, an appropriate surgical approach is fundamental in the treatment of PA. Our case of CXPA probably have arisen de novo, because there was no previous history of PA and the patient reported short time of evolution. The malignant areas of the CXPA can represent an adenoid cystic carcinoma, undifferentiated carcinoma, or epithelial-myoepithelial carcinoma.[16,17] In our case, the malignant component was composed by adenocarcinoma NOS.

In the head and neck region, the major and minor salivary glands are the most commonly affected sites by ACC. Lacrimal glands are responsible for approximately 7.2% of head and neck ACC.[18] Although rare in the orbital region, ACC is the most common malignant lacrimal gland tumor, affecting usually men in the fourth and fifth decade of life.[2,4] In our series, all malignant tumors but one (CXPA), were diagnosed as ACC. The majority of our patients with malignancies were in the fourth and fifth decades of life (mean age of 40.4 years), and no predilection for gender was observed. Nevertheless, other authors found a female predilection.[19] In large series of lacrimal gland tumors, patients with CXPA and other adenocarcinomas were older than those with ACC.[2,4] Generally, patients with PA or ACC present similar mean age,[4] however in our series, ACC patients were slightly younger than PA patients.

Clinically, malignant tumors present aggressive features, with bone and intracranial involvement, and patients complain more frequently of pain, exophthalmia and loss of mobility of the eye.[5] Our data confirm this finding, since most patients with ACC presented with advanced tumors (80% with bone and 30% with intracranial invasion). In addition, pain, exophthalmia and facial paralysis were also common.

The optimal treatment of the malignant lacrimal gland tumors, particularly ACC, remains controversial. Aggressive approach, as orbital exenteration followed by local RT, is recommended by some authors.[2,9] However, Polito & Leccisotti[20] did not find improved survival in patients submitted to extensive surgery, when compared with those with more limited local excision. In our opinion, the major problem for the treatment of these tumors is the advanced clinical stage at presentation, making it difficult to reach adequate complete resection with tumor-free margins, leading to high local recurrence rates. Combined craniofacial approaches and microvascular free-flaps have been used more recently in the treatment of such cases. It seems to offer better exposure of the tumor, improving our ability to remove the lesion en bloc, with wide surgical margins. Nevertheless its efficacy remains to be demonstrated in rare tumors such as lacrimal gland adenocarcinomas. Due to limited number of cases in our casuistic, it was not possible to determine whether postoperative radiation therapy improved local control rates or not. There are reports of other therapeutic options, as neoadjuvant chemotherapy using cisplatin and doxorubicin hydrochloride, associated with postoperative RT supplement by intravenous cisplatin and doxorubicin.[21]

In spite of the aggressiveness of the treatment for malignant lacrimal gland tumors, there are high rates of local recurrences and distant metastasis.[9,19] In one series of malignant tumors of the lacrimal gland, all 12 patients with ACC developed recurrences.[2] We observed similar findings, with 41.6% of the cases of ACC presenting local recurrences, 33.3% distant metastasis, and 58.3% of our patients died of cancer related causes. A factor that seems to be important in the prognosis is the histological type of the ACC. Solid growth pattern or basaloid differentiation is associated with more aggressive tumors and a reduction in disease-free survival.[9,22] In our series, there were 3 cases with solid growth pattern and all presented a rapid evolution, with intracranial involvement, distant metastasis and a fatal outcome.

Our findings confirm that epithelial lacrimal gland tumors are mainly PA and ACC, and malignancies are more common than benign tumors. PA presented good prognosis and ACC showed advanced clinical stage, with a poor prognosis.

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Table 1- Clinical data of five cases of pleomorphic adenoma of the lacrimal gland

Case	Age (years)	Gender	Complaints (months)	Size (cm)	Treatment
1	51	Male	12	3.0	Orbital exenteration
2	34	Male	24	5.0	Excision
3	61	Female	12	3.0	Excision
4	59	Female	36	3.0	Excision
5	16	Female	60	2.5	Excision

Table 2- Summary of clinical and outcome data of 13 malignant lacrimal gland tumors

Case	Age (years)	Gender	Complaints (months)	TNM	Treatment †	Outcome‡ (time in months)
6	53	Male	18	T4N0M0	Supportive care	DOD (7)
7	35	Male	144	T3bN0M0	Orbital wide resection	Local recurrence and lung metastasis/DOD (34)
8	52	Male	12	T4N0M0	RT	Local recurrence and bone metastasis/DOD (80)
9	19	Female	36	T3bN0M0	Wide resection and RT	Local recurrence/DOD (221)
10	26	Female	4	T3bN0M0	Orbital exenteration and RT	NED (67)
11	42	Male	5	T4N0M0	Surgery, RT and CT	NED (171)
12	63	Male	24	T4N0M0	RT and CT	DOD (48)
13	39	Female	31	T4N0M0	Orbital exenteration, RT and CT	Lung metastasis/DOD (10)
14	52	Male	18	T4N0M1	Wide resection, RT and CT	Lost follow-up
15	34	Female	18	T3bN0M0	Orbital exenteration and RT	NED (24)
16	40	Female	7	T4N0M0	Wide resection	Local recurrence and lung and brain metastases/DOD (44)
17	30	Female	3	T3aN0M0	Wide resection and RT	NED (27)
18*	38	Female	10	T2N0M0	Wide resection and RT	NED (29)

*- All cases are ACC, except case 18 (carcinoma ex pleomorphic adenoma)

†- RT- radiotherapy; CT- chemotherapy

‡- DOD- Died of disease; NED- No evidence of disease

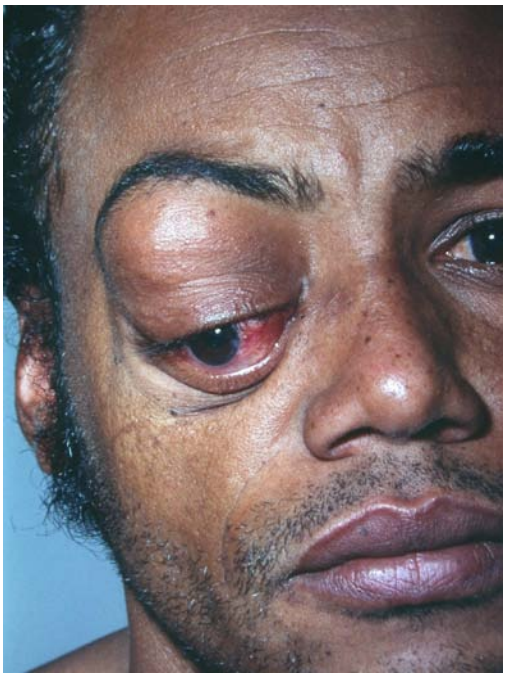

Figure 1- Patient with pleomorphic adenoma of the lacrimal gland showing a painless nodule with 5 cm of extension, causing palpebra ptosis.

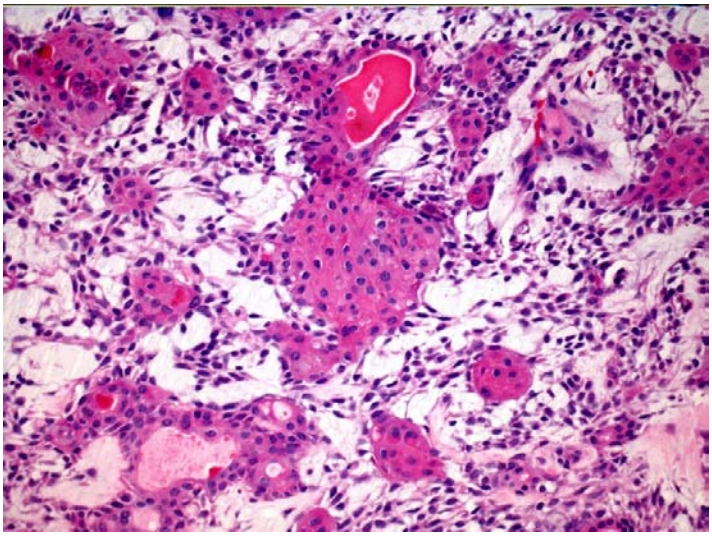

Figure 2- Pleomorphic adenoma of the lacrimal gland with areas of squamous differentiation (hematoxylin-eosin, original magnification, x200).

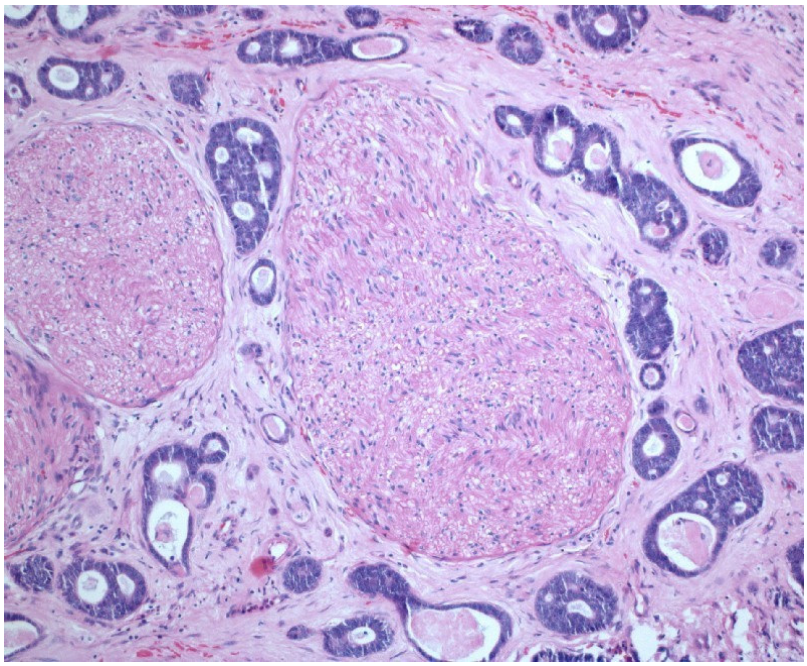

Figure 3- Adenoid cystic carcinoma of the lacrimal gland presenting extensive perineural invasion (hematoxylin-eosin, original magnification, x100).

6. CAPÍTULO 4

SUBLINGUAL SALIVARY GLAND TUMORS: CLINICOPATHOLOGICAL STUDY OF SIX CASES

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Artigo submetido a Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology em fevereiro de 2005.

ABSTRACT

Objective: The aim of this study was to assess the clinical and histological features of 6 cases of sublingual salivary gland tumors treated in a single institution. **Study design**: The clinical data were obtained from the medical records and the histopathological slides were reviewed. **Results**: Four cases were adenoid cystic carcinoma (ACC) and 2 mucoepidermoid carcinoma (MEC). Most cases were clinically staged as T3N0M0. Histologically, 3 cases of ACC were of the cribriform subtype, one MEC was classified as intermediate and one as high grade of malignancy. After surgical treatment, three patients (2 MEC and 1 ACC) did not show tumoral recurrence after a mean time of 162 months of follow-up (ranging from 120 to 216 months). **Conclusions**: Sublingual salivary gland tumors are very rare, most are malignant and in our series, 3 cases of ACC had poor prognosis, but the patients with MEC were considered cured of the disease.

Key-words: adenoid cystic carcinoma; mucoepidermoid carcinoma; sublingual salivary gland; salivary gland tumors

INTRODUCTION

Salivary gland tumors are uncommon, representing about 3% to 10% of all neoplasms of the head and neck region.[1] Sublingual salivary gland neoplasms are extremely rare, accounting for only 0.3% to 1% of all epithelial salivary gland tumors. Most of the sublingual tumors are malignant, being adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC) the most common histological types.[1-3] Other malignant tumors have been rarely described in the sublingual gland, such as acinic cell carcinoma, clear cell carcinoma, carcinoma ex pleomorphic adenoma, basal cell adenocarcinoma, salivary duct carcinoma, carcinosarcoma, mucinous adenocarcinoma, adenocarcinoma NOS, and polymorphous low-grade adenocarcinoma.[4-12] Benign tumors, although extremely uncommon, have also been reported.[13,14]

Apart from single case reports, few series of sublingual salivary gland tumors have been published in the English-language literature.[2,3,15] The aim of this study is to describe the clinicopathological features of six cases of sublingual salivary gland tumors, considering the differential diagnosis, treatment and outcome.

PATIENTS AND METHODS

Between 1953 and 2001, of the 1330 salivary gland tumors treated at the Department of Head and Neck Surgery and Otorhinolaryngology, Hospital do Cancer A. C. Camargo, Sao Paulo, Brazil, only 6 (0.45%) were sited at the sublingual salivary glands. Clinical data, including age, gender, time of complaints, size of the tumor, clinical stage, treatment and outcome were obtained from the

medical records. Only tumors unequivocally arising in the sublingual salivary glands were included in this study. Tumors were re-staged according to UICC TNM Classification of Malignant Tumors[16] and the histopathological slides stained with hematoxylin-eosin, Periodic Acid Schiff (PAS) and mucicarmim, were reviewed to confirm the diagnosis. MECs were histologically graded in low-, intermediate- or high-grade and ACCs classified as cribriform, tubular or solid.[8]

RESULTS

Four cases out of 6 sublingual salivary gland tumors were ACC and two were MEC. Four patients were female and two male, with a mean age of 55.2 years. Mean time period of complaints was 21 and 61 months for ACC and MEC, respectively. All patients, but one (case 4) who had only pain, complained of an asymptomatic submucous nodule sited in the floor of the mouth. The nodules were firm, presenting a mean size of 3.8 cm (ranging from 2 to 5 cm). Three cases were clinically staged as T3N0M0, two as T2N0M0 and one T1N0M0. Clinical data of all cases are summarized in Table 1.

Histologically, 3 cases of ACC were cribriform and one tubular (Fig 1), all showing perineural invasion. In one case (case 4), squamous differentiation was also observed. One MEC was classified as intermediate (Fig 2) and one as high-grade tumor. All patients were submitted to wide surgical excision with resection of the floor of the mouth, and involved sublingual and ipsilateral submandibular salivary gland. Case 3 was also submitted to segmental mandibulectomy because of mandibular involvement, and case 1 to marginal mandibulectomy due to periosteoum involvement by the tumor. In addition, in case 2 it was necessary a

resection of a segment of the lingual nerve, since it was macroscopically involved by the tumor. Neck dissection was carried out in five patients, being levels I-III (supraomohyoid neck dissection) in two and level I in three. Two patients received adjuvant radiotherapy (cases 3 and 6). Previous diagnosis to surgical procedures was performed by incisional biopsy in 2 cases. The other 4 cases were submitted to surgical treatment and transoperative frozen section analysis. Treatment data are shown in Table 2.

Of the 4 patients with ACC, one is alive without disease after 12.4 years of follow up, one presented local and distant (lung) recurrences and died of the disease 13 years after initial treatment, one had a lung metastasis 2.5 years after initial treatment and currently is alive with disease and the other died of postoperative complications. Regarding MEC, one patient died of colon adenocarcinoma 18 years after treatment of the sublingual tumor and the other is alive without disease after 10 years of follow up.

DISCUSSION

Sublingual salivary gland tumors are uncommon, representing up to 1% of all salivary gland tumors.[2] Most of them are malignant, and as shown on the present series, ACC and MEC are the most common.[3,15] However, a higher percentage (5.2%) of tumors sited at the sublingual glands have been described.[17] Differently, most parotid tumors are benign (64%), predominantly pleomorphic adenoma.[1] Although ACC and MEC are the most common sublingual tumors, only about 2% of all ACC and 1.6% of MEC are sited in sublingual gland.[18-20] Additionally, there is a large series of ACC without a single case in the sublingual

gland.[21] Patients with sublingual tumors present a mean age of about 57 years, without gender predilection.[3,15] We found similar data, with a mean age at diagnosis of 55.2 years, and slight female predilection, although the number of cases is limited. Salivary sublingual tumors in adolescents are extremely rare, with few previously reported cases.[22] One of our patients was a 16 years old boy who presented an intermediate-grade MEC. He was cured of the sublingual tumor but eventually died of a colon cancer at the age of 34 years.

Clinically, a malignant sublingual salivary gland tumor presents as an asymptomatic submucous nodule in the floor of the mouth, more exactly in the sublingual space, which can cause some inconvenience.[15] Pain, difficulties with dental prosthesis adaptation and tongue numbness can also be reported.[23] In our series, all patients but one complained of a painless swelling in the floor of the mouth. Other glandular lesions, neoplastic or non-neoplastic, can also present at the floor of the mouth, such as ranula, inflammatory conditions, and minor salivary gland tumors. Ranula refers to salivary extravasation phenomena involving the sublingual space, causing swelling in the floor of the mouth and occurring most commonly during childhood. This lesion frequently shows smooth, brilliant and bluish surface, thin mucosa, sessile base and flaccid consistency.[24] On the other hand, inflammatory processes cause significant swelling, most patients complain of pain and exudate and purulence can be observed.[25] To establish the site of origin of a submucous nodule in the floor of the mouth, it is important to distinguish between lesions arising in the sublingual or in the minor salivary glands.[26] Frequently, it is not easy to determine the tumor's origin based only on clinical features,[11] being necessary to correlate with computed tomography and magnetic

resonance imaging, as well as surgical and histopathological findings. In our cases, the sublingual origin was confirmed by clinical, surgical and histological features. However, as the present study is retrospective, it was not possible to report the radiographic characteristics because they were not recorded. Depending of the localization, salivary gland tumors can be diagnosed before surgery by incisional or fine needle aspiration biopsies, with high index of accuracy,[27] helping the surgeon in the planning of the surgical procedure. Nevertheless in the present series, only 2 cases were diagnosed prior to surgery.

Due to absence of symptoms of these tumors, most them are diagnosed when relatively large, as in our series with half of the cases classified as T3N0M0. Surgery remains the main modality of treatment for malignant sublingual gland tumors, however there are different types of surgical interventions according to extent of the primary tumor.[23] For small tumors restricted to the floor of the mouth, it has been suggested a wide surgical resection, including the involved sublingual and also the ipsilateral submandibular salivary gland, because the ductal system is often affected even with limited resection.[3] Similar treatment was employed in our cases. In tumors larger than 2.0 cm, it has been recommended a more aggressive approach, using en-bloc resection like pull-through procedure. Additionally, resection of the lingual nerve and marginal mandibulectomy are necessary when there is involvement of this nerve and of the periosteum, respectively.[3] In the present study, in one case a marginal mandibulectomy was performed and in other the lingual nerve was removed, because of the reasons explained above. In cases with unequivocal mandibular involvement, a segmental mandibulectomy must be done, as it was performed in our case 3. Although the risk of lymph node

metastasis is low, a selective cervical lymphadenectomy including levels I-III seems to be useful and it can be included in the surgical procedure without difficulties.[3] Two of our cases were submitted to neck dissection of levels I-III, and three cases to level I, although there were no evidences of lymph node involvement. Adjuvant postoperative radiotherapy (RT) has been indicated for patients with advanced clinical stage, high-grade tumors or when surgical margins are closed or involved by tumor.[3,23] Of our cases, two received postoperative RT, one ACC that presented invasion of the adjacent bone tissue and a high-grade MEC; both patients are alive without evidence of disease after long-term follow-up.

Proper reconstruction of the floor of the mouth after surgery, especially after a marginal or a segmental mandibulectomy, is one of the major challenges for the surgeon. The current use of microvascular free-flaps has increased the function and outcome of the patients. For marginal mandibulectomy or wide surgical excision of the floor of the mouth, radial forearm or liberal arm flap are the most used. In patients where it is necessary to perform a segmental mandibulectomy, reconstruction by microvascular fibular free-flap is the most used option.[23]

The malignant sublingual salivary gland tumors show various degrees of malignancy and present difficulties in relation to diagnosis and treatment, as previously commented. Although sublingual gland tumors usually show poor prognosis due to advanced clinical stage or high grade of malignancy, our cases of MEC did not present tumoral recurrence after long-term follow up, even being classified as high and intermediate-grade.[23] Local recurrence is relatively low, and this was confirmed in this series, where only one case (16.6%) had local

recurrence.[3,15] Distant metastasis seems to be related to the histological type of the tumor, being more commonly seen in cases of ACC.[3]

In summary, sublingual salivary gland tumors are very rare, most are malignant and ACC and MEC are the most common. Wide surgical excision, neck dissection and postoperative radiotherapy seem to be efficient to achieve local and regional control of the disease. Nevertheless on long term, distant metastasis is common in cases of ACC.

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Table 1- Summary of clinical data and diagnosis of 6 sublingual salivary gland tumors

Case	Age (years)	Gender	Complaints (months)	Size (cm)	Diagnosis*
1	67	Female	12	3.5	Cribriform ACC
2	58	Male	24	5.0	Cribriform ACC
3	70	Female	36	3.0	Cribriform ACC
4	65	Female	12	2.0	Tubular ACC
5	16	Male	3	4.0	Intermediate grade MEC
6	55	Female	180	5.0	High grade MEC

*ACC- Adenoid cystic carcinoma; MEC- mucoepidermoid carcinoma

Table 2- Details of surgical treatment of sublingual salivary gland tumors

Case	Clinical stage	Surgical Treatment	RT*	Outcome** (time in months)
1	T2N0M0	Pull-through procedure with marginal mandibulectomy, neck dissection (levels I-III), and submandibulectomy	No	DOC (10 days)
2	T3N0M0	Ressection of sublingual gland including segment of lingual nerve, submandibulectomy and neck dissection (levels I-III)	No	Local recurrence and lung metastasis/DOD (156)
3	T3N0M0	Ressection of sublingual gland and segmental mandibulectomy	Yes	NED (149)
4	T1N0M0	Ressection of sublingual gland, submandibulectomy and neck dissection (level I)	No	Lung metastasis/ AWD (40)
5	T2N0M0	Resection of sublingual gland, submandibulectomy and neck dissection (level I)	No	DOC (216)
6	T3N0M0	Ressection of sublingual gland, submandibulectomy and neck dissection (level I)	Yes	NED (120)

*RT- Radiotherapy

**DOC- Died of other causes; DOD- Died of disease; AWD- Alive with disease; NED- No evidence of disease

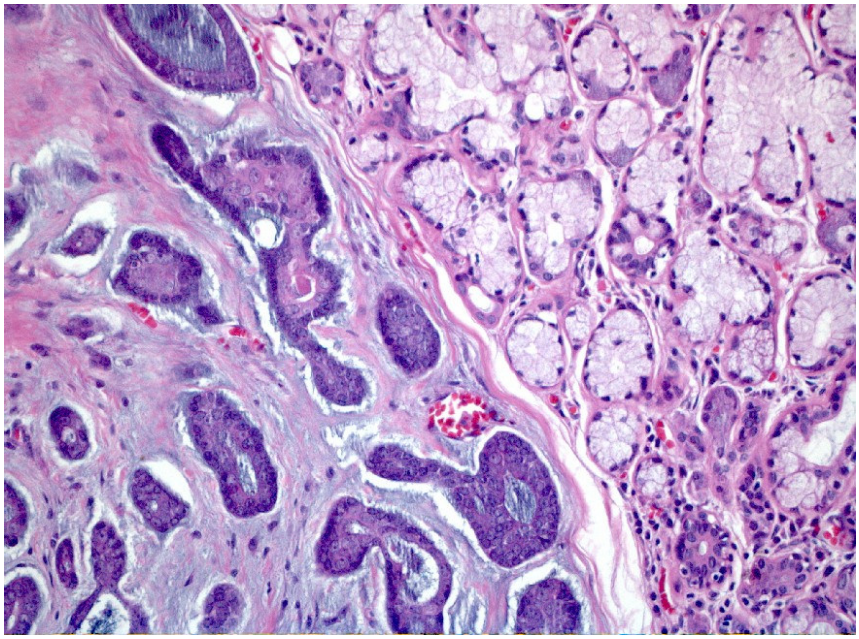

Figure 1- Tubular adenoid cystic carcinoma compressing the adjacent sublingual salivary gland (hematoxylin-eosin, original magnification, x200).

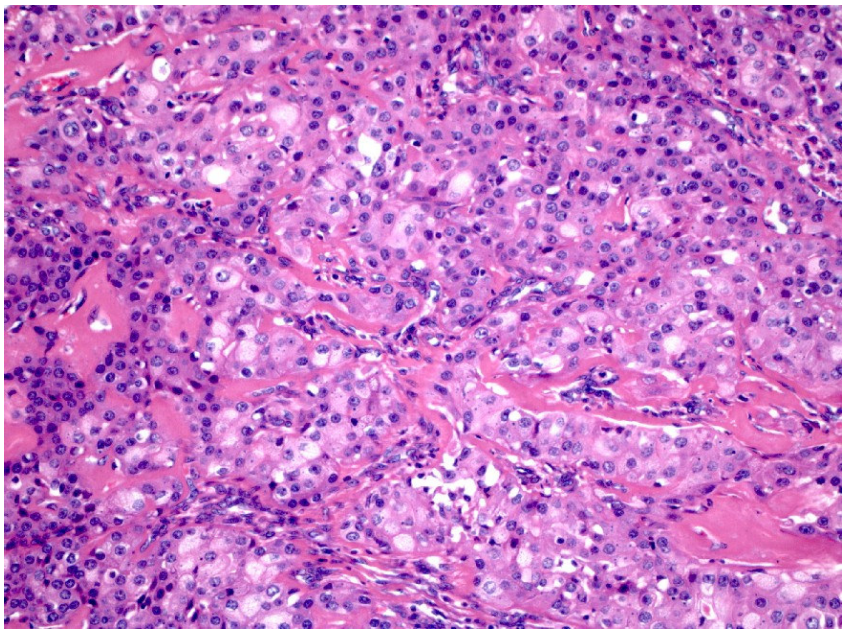

Figure 2- Intermediate grade mucoepidermoid carcinoma. The clear cells were positives for Periodic Acid Schiff (PAS) and mucicarmim staining (hematoxylin-eosin, original magnification, x200).

7. CAPÍTULO 5

ESTROGEN RECEPTOR EXPRESSION IN SALIVARY GLAND MUCOEPIDERMOID CARCINOMA AND ADENOID CYSTIC CARCINOMA

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ACKNOWLEDGEMENTS: This work was supported by FAPESP, Brazil.

Artigo publicado em **Pathology Oncology Research**, 10 (3): 166-168, 2004.

ABSTRACT

Estrogen receptor (ER) expression in salivary gland carcinomas is controversial, and each one of most published studies considered no more than 10 cases. We analyzed ER expression by immunohistochemistry in 136 mucoepidermoid carcinomas and 72 adenoid cystic carcinomas. All cases were negative. These results do not support a role for estrogens in salivary gland mucoepidermoid carcinoma and adenoid cystic carcinoma.

KEY-WORDS

Mucoepidermoid carcinoma; adenoid cystic carcinoma; salivary gland tumors; immunohistochemistry; estrogen; estrogen receptor.

INTRODUCTION

Estrogen receptor (ER) expression has been demonstrated in hormone-dependent organs such as breast, endometrium, colon, prostate and their cancers.[3,9] In salivary gland tumors ER expression has been reported in a limited number of cases.[4,7,10,13] Salivary gland tumors refractory to conventional therapy (surgery, radiotherapy and chemotherapy), would be possibly benefited by hormonal therapy, but it is important to identify therapeutic targets to these agents. The aim of this study was to evaluate the expression of ER in a large series of salivary gland mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC).

MATERIAL AND METHODS

A hundred and thrity six MECs and seventy two ACCs, from the files of the AC Camargo Cancer Hospital, São Paulo, Brazil, were used on this study. Clinical data were obtained from the patients records. MECs were graded according to Ellis and Auclair[5] in low, intermediate and high grade, and ACCs classified in cribriform, tubular and solid types. Immunohistochemical reactions against ER (Mouse monoclonal antibody against estrogen receptor, clone NCL-ER-6F11, Novocastra Laboratories, UK, dilution 1:50) were performed in 3μm histological sections. Microwave antigen retrieval using EDTA pH 8.0, overnight incubation with the primary antibody and secondary antibodies conjugated to a streptavidin-biotin-peroxidase system (Strept ABComplex/HRP Duet, Mouse/Rabbit, Dako A/S, Denmark) were used, followed by diaminobenzidine as the chromogen. Slides

were counterstained with Carazzi hematoxyllin. Positive (benign fibrocystic disease of breast) and negative (absence of the primary antibody) controls were included in all reactions.

RESULTS

For MECs, the mean age of the patients was 45 years (ranging from 6 to 96 years) and 77 (57%) were males. Site of the tumors included 69 minor (50.7%) salivary glands and 67 major (49.3%), with parotid affected in 50 cases (37%). TNM staging revealed 59% clinical stages I + II and 41% clinical stages III + IV patients. MEC histological grade showed 58 (45%) low-grade tumors, 24 (18%) intermediate-grade tumors and 48 (37%) high-grade tumors. For ACCs, the mean age was 50 years (ranging from 10 to 82 years), and 37 were males (51.4%). Minor and major salivary glands were affected in 45 (62.5%) and 27 (37.5%) cases, respectively. Clinical stages were 53 (73.6%) stages III + IV and 19 (26.4%) stages I + II tumors. Microscopically, 34 cases (47.2%) were cribriform, 21 (29.2%) tubular and 17 (23.6%) solid. ER expression was not detected in none of the MEC nor ACC cases.

DISCUSSION

Hormone therapy has been succesfully used as adjunctive treatment in some cancers, but its efficacy in salivary gland tumors has not yet been demonstrated.[8] Ozono et al.[12] demonstrated the expression of ER in DMBA-induced epidermoid carcinoma of the submandibular glands in rats, experimentally supporting its participation in tumorigenesis and the use of hormone therapy in salivary gland tumors. However, studies in humans have shown that the

expression of hormone proteins and their receptors in salivary gland tumors is not frequent or even absent.[4,6,7,9,10,13,14] These findings do not support hormones role in salivary gland function and tumorigenesis.[4,9,10] Nevertheless, hormone therapy trials have been suggested as adjunctive protocols in salivary duct carcinoma and adenoid cystic carcinoma unresponsive to conventional therapeutic strategies, in view of their aggressive behavior.[2]

ER expression in MEC is controversial. Jeannon et al.[8] have shown ER in 3 out of 10 MECs, supporting the use of hormone therapy in some tumors, and Dimery et al.[3] found ER expression in one of their 2 MECs studied. More recently Nasser et al.[11] have shown ER expression in one of their 10 MECs studied. In contrast, Gaffney et al.[6] studying 6 parotid MEC, Lamey et al.[9] and Wilson et al.[14] in one case of MEC each, found no estrogen expression. Our 136 cases of MEC were negative for ER expression (Table 1).

ER expression in ACCs was negative in most reports.[4,6,8,10,11,13] We confirmed these results in 72 cases of ACC. However, Dimery et al.[3] found ER expression in 3 out of 4 ACC (Table 1). Interestingly, Arpino et al.[1] found 46% expression of ER in breast ACC, supporting that although it is rare in salivary gland tumors, ER expression is influenced by anatomical variations.

Our results support that estrogen do not participate in salivary gland MEC and ACC tumorigenesis. Consequently there is no rational for hormone therapy trials because probably it would not be an useful strategy in the management of these tumors.

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Table 1. Summary of the reports of estrogen receptor (ER) expression in salivary gland mucoepidermoid carcinoma (MEC) and adenoid cystic carcinoma (ACC).

Authors	Year	MEC		ACC	
		No. of cases	ER positive	No. of cases	ER positive
Dimery et al.[3]	1987	2	1 (50%)	4	3 (75%)
Lamey et al.[9]	1987	1	0	NP	NP
Wilson et al.[14]	1993	1	0	NP	NP
Miller et al.[10]	1994	NP	NP	5	0
Shick et al.[13]	1995	NP	NP	12	0
Gaffney et al.[6]	1995	6	0	7	0
Jeannon et al.[8]	1999	10	3 (30%)	6	0
Dori et al.[4]	2000	NP	NP	27	0
Nasser et al.[11]	2003	10	1 (10%)	10	0
Our study	2004	136	0	72	0

NP - not performed.

8. CONCLUSÃO

a. Estádio clínico avançado, padrão de crescimento sólido e expressão de p53 são fatores prognósticos significantes independentes em CAC da região de cabeça e pescoço.

b. Idade superior a 45 anos, período de queixa inferior a 18 meses, presença de parestesia, estádio T, N, M e presença de margens cirúrgicas comprometidas correlacionaram de forma significativa com pobre prognóstico em CAC.

c. CAC são tumores raros do seio maxilar, tipicamente apresentam em estádio clínico avançado e parece ter um comportamento mais agressivo que o carcinoma mucoepidermóide.

d. Em glândulas lacrimais, tumores epiteliais malignos são mais comuns que tumores benignos e CAC apresenta pobre prognóstico.

e. Tumores de glândulas sublinguais são raros.

f. Excisão cirúrgica ampla, esvaziamento cervical e radioterapia pós-operatória parecem ser eficientes no controle local de tumores sublinguais.

g. Estrógeno não participa do desenvolvimento tumoral de CAC e carcinoma mucoepidermóide.

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* De acordo com a norma da UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline

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ANEXO 1

ARCHIVES OF
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-----Mensagem original-----
De: archoto@jama-archives.org [mailto:archoto@jama-archives.org]
Enviada em: Sexta-feira, 11 de Fevereiro de 2005 17:03
Para: lp_kowalski@uol.com.br
Assunto: OTO05-0094 Receipt of New Paper by Archives of Otolaryngology

Dear Dr. Kowalski,

Thank you for submitting your manuscript entitled "PROGNOSTIC FACTORS IN HEAD AND NECK ADENOID CYSTIC CARCINOMA," received on February 11, 2005.Your manuscript has been assigned the following manuscript number: OTO05-0094. Please refer to the manuscript number in all subsequent communications.
We agree to consider this manuscript with the understanding that its content, figures, and tables have not been published or submitted elsewhere in print or electronic format in any language and will not be submitted elsewhere during the period of review by the Archives of Otolaryngology-Head & Neck Surgery.
Your manuscript will be reviewed by an editor here, and possibly by two or more peer reviewers. Every effort will be made to expedite the review process and to notify you of our decision as soon as possible. It is the policy of the Archives of Otolaryngology-Head & Neck Surgery not to return manuscripts that have been rejected (unless the author has specifically requested the manuscript's return). Only the original artwork, if submitted, will be returned.

Sincerely,
Michael E. Johns, MD
Editor

Submissions Being Processed for Author Danyel Elias da Cruz Perez, DDS, MSc

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
View Submission		ADENOID CYSTIC CARCINOMA AND MUCOEPIDERMOID CARCINOMA OF THE MAXILLARY SINUS: REPORT OF A 44-YEAR EXPERIENCE OF 25 CASES FROM A SINGLE INSTITUTION	02/04/2005	02/04/2005	Submitted to Journal

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Journal for the Sciences and Specialties of the Head and Neck
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HED-04-0496

Epithelial lacrimal gland tumors: a clinicopathological study of eighteen cases

29 Nov 2004

Reviews Inbound
Mariann Crapanzano
mcrapanz@mdanderson.org

ANEXO 2

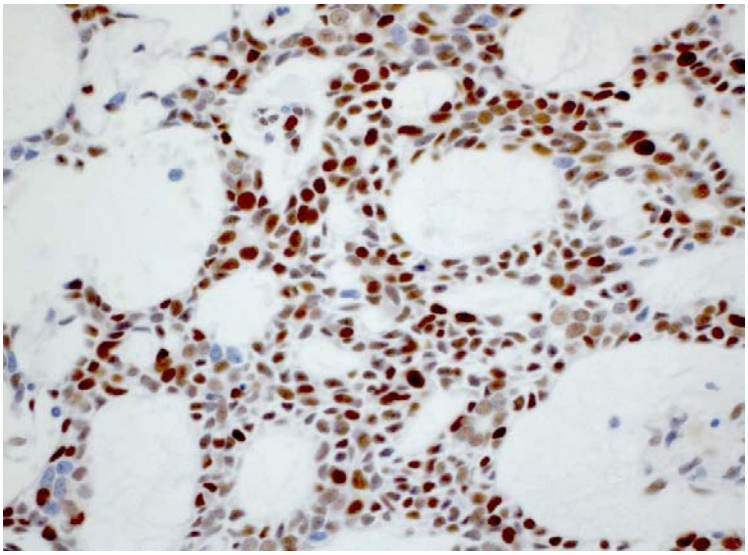

Figura 1- Forte expressão de PCNA em CAC cribriforme

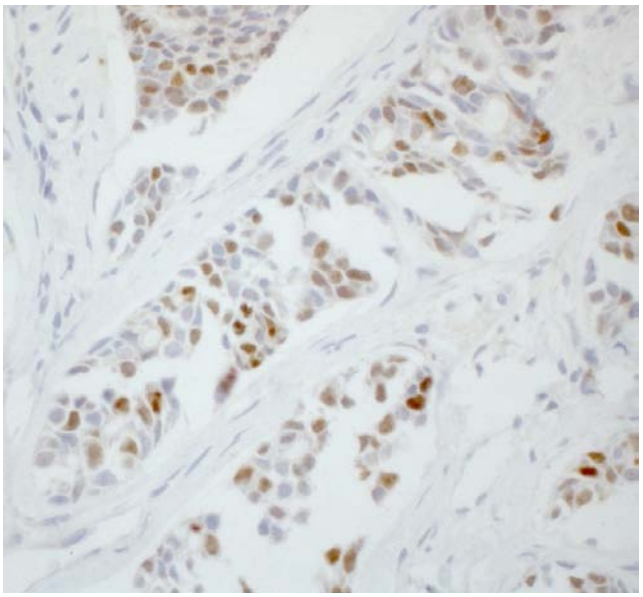

Figura 2- Expressão de Ki-67 em CAC

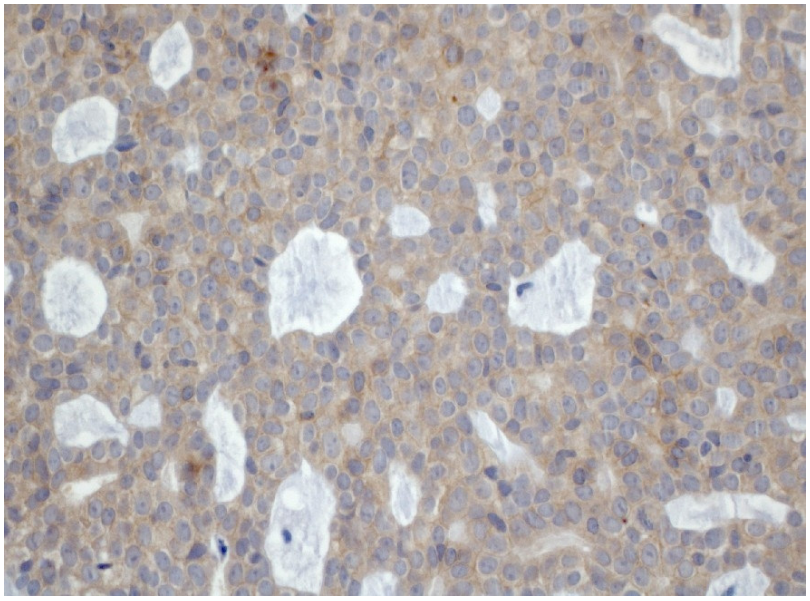

Figura 3- Expressão membranosa e citoplasmática de c-erbB-2 em CAC

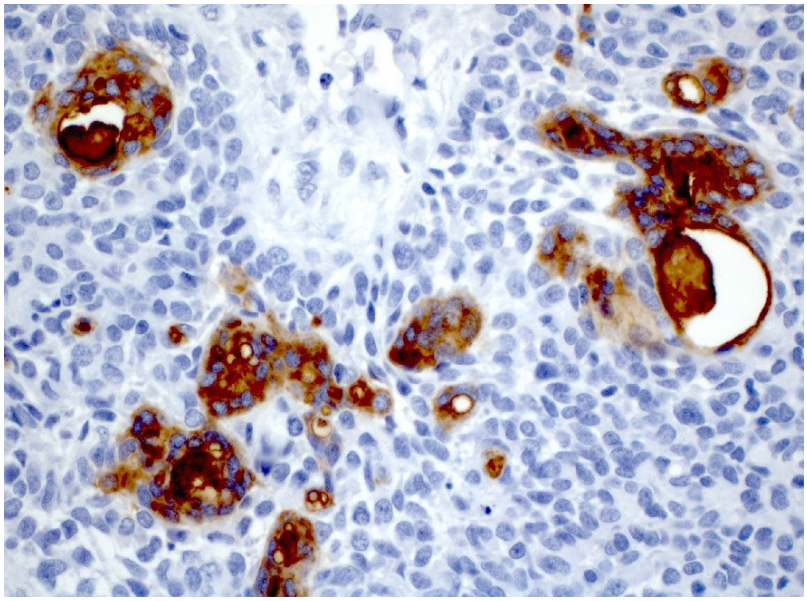

Figura 4 – Expressão de CEA em áreas ductais de CAC sólido

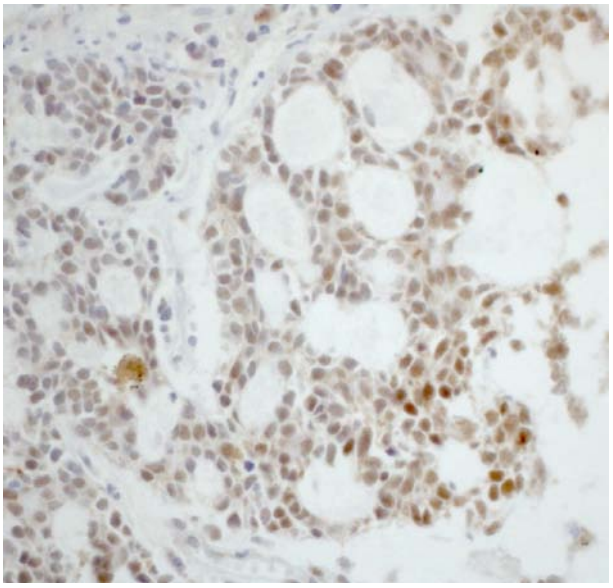

Figura 5 – Expessão de proteína p53 em CAC cribriforme

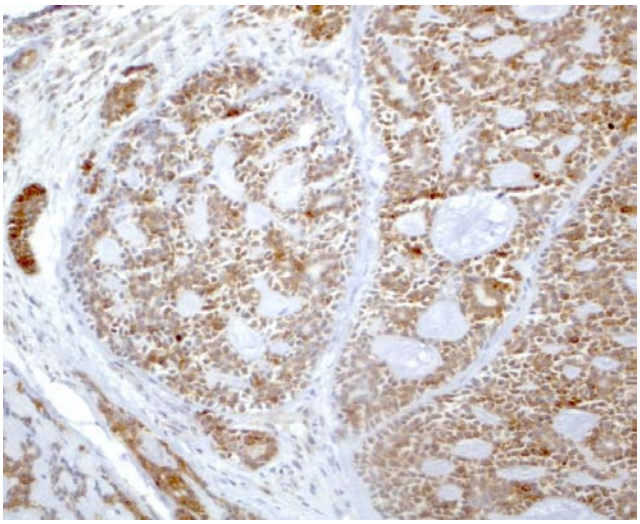

Figura 6 – Forte expressão de bcl-2 em CAC cribriforme