UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

FELIPE PAIVA FONSECA

ANÁLISE CLINICOPATOLÓGICA DE 493 CASOS DE TUMORES DE GLÂNDULAS SALIVARES E CONSTRUÇÃO DE BLOCOS DE PARAFINA UTILIZANDO A TÉCNICA DE *TISSUE MICROARRAY*

Dissertação de Mestrado apresentada a Faculdade de Odontologia de Piracicaba da UNICAMP para obtenção do título de Mestre em Estomatopatologia, na Área de Patologia

Orientador: Prof. Dr. Pablo Agustin Vargas

Este exemplar corresponde à versão final da Dissertação defendida pelo aluno, e orientada pelo Prof.Dr. Pablo Agustin Vargas

Assinatura do Orientador

PIRACICABA, 2012

FICHA CATALOGRÁFICA ELABORADA POR
MARILENE GIRELLO – CRB8/6159 - BIBLIOTECA DA
FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP

F733a

Fonseca, Felipe Paiva, 1986-
Análise clinicopatológica de 493 casos de tumores de glândulas salivares e construção de blocos de parafina utilizando a técnica de tissue microarray / Felipe Paiva Fonseca. -- Piracicaba, SP : [s.n.], 2012.

Orientador: Pablo Agustin Vargas.
Dissertação (mestrado) - Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Neoplasias de cabeça e pescoço. 2. Doenças das glândulas salivares. 3. Análise em microsséries. I. Vargas, Pablo Agustin, 1973- II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para a Biblioteca Digital

Título em Inglês: Clinicopathologic analysis of 493 cases of salivary gland tumors and construction of paraffin blocks using the tissue microarray technique
Palavras-chave em Inglês:
Head and neck neoplasms
Salivary gland diseases
Microarray analysis
Área de concentração: Patologia
Titulação: Mestre em Estomatopatologia
Banca examinadora:
Pablo Agustin Vargas [Orientador]
Hélder Antônio Rebelo Pontes
Halbert Villalba
Data da defesa: 27-02-2012
Programa de Pós-Graduação: Estomatopatologia

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Dissertação de Mestrado, em sessão pública realizada em 27 de Fevereiro de 2012, considerou o candidato FELIPE PAIVA FONSECA aprovado.

Prof. Dr. PABLO AGUSTIN VARGAS

Prof. Dr. HELDER ANTONIO REBÊLO PONTES

Prof. Dr. HALBERT VILLALBA

Dedico este trabalho aos meus pais **Juares Figueiredo Fonseca** e **Clotilde de Freitas Paiva** que nunca mediram esforços para oferecer todas as condições necessárias para que pudesse alcançar meus objetivos, oferecendo apoio incondicional à minha trajetória profissional.

AGRADECIMENTO ESPECIAL

Ao meu orientador **Prof. Pablo Agustin Vargas**, tenho tantos agradecimentos que certamente uma sessão desta dissertação não seria suficiente para fazê-los em palavras, por isto me conforto com a certeza de que terei uma vida para expressá-los. Agradeço todos os ensinamentos e oportunidades que me foram dados, assim como sua atenção, compreensão e confiança, e ponho desta forma simples, que talvez não ressalte o merecido valor de sua dedicação, para que possa ressaltar ainda mais, a sua principal contribuição para minha formação, a qual se assenta não na sua excelência como pesquisador e professor, mas sim como de ser humano ao dedicar-se de maneira idônea e correta aos serviços públicos pelos quais é responsável, ao tratar seus alunos e colegas docentes com respeito e companheirismo, ao referir-se de maneira cavalheiresca a sua esposa e de forma respeitosa aos seus pais e irmãos, desta forma eu agradeço ao senhor sobretudo por poder tê-lo como exemplo de pessoa, o que faz do senhor não um grande orientador, mas um grande mestre, opinião esta certamente compartilhada com todos que o conhecem, em especial com seus alunos.

Apesar de não conhecer os caminhos pelos quais a vida me levará, onde quer que esteja levarei seus ensinamentos e repetirei suas atitudes, por acreditar que elas também engrandecerão a minha instituição e a todos que me cercam.

Muito obrigado por tudo e espero um dia poder retribuir ao menos uma parcela de tudo o que o senhor fez por mim, tendo consciência de que jamais estarei em posição de devolver-lhe tamanha dedicação.

AGRADECIMENTOS

À **Deus** e à **Nossa Senhora de Nazaré** que iluminaram meu caminho e me guiaram até esta Faculdade e aqui me protegeram dos males da vida.

Aos meus pais, **Juares Figueiredo Fonseca** e **Clotilde de Freitas Paiva**, por me ensinarem que a família é a base para o sucesso.

Aos meus irmãos, **Rafael Paiva** e **Valéria Fonseca**, por todo apoio e carinho e pela certeza de poder contar com ambos em qualquer momento.

À **Universidade Estadual de Campinas** na pessoa de seu magnífico reitor **Prof. Dr. Fernando Ferreira Costa** e à sua **Faculdade de Odontologia,** na pessoa de seu diretor **Prof. Dr. Jacks Jorge Júnior**, por oferecer-me suas estruturas e posições de destaque no cenário nacional e internacional para que pudesse usufruir de tais atributos e assim obter meu engrandecimento profissional.

Ao **Programa de Pós-Graduação em Estomatopatologia** na pessoa de seu coordenador **Prof. Dr. Ricardo Della Coletta** e muito bem representado por seus **professores e alunos**, agradeço a receptividade e o espaço a mim concedido para que pudesse desenvolver minhas atividades científicas e formar novas amizades.

O orgulho de ser aluno da Universidade Estadual de Campinas certamente é igualado pelo de ser aluno de um dos mais importantes patologistas orais do mundo. Ao **Prof. Dr. Oslei Paes de Almeida** só me resta, assim como dezenas já fizeram anteriormente, agradecer aos inúmeros ensinamentos e indescritível dedicação durante o curso. Realmente é impressionante sua sede por conhecimento e sua humildade em reconhecer que ainda há muito para aprender mesmo dominando muito dos conceitos da patologia e que tal aprendizado também pode ser encontrado em seus alunos. O sucesso de nosso programa reflete seu potencial e visão.

Ao **Prof. Dr. Márcio Ajudarte Lopes** tenho bastante a agradecer pela oportunidade de desenvolver atividades clínicas, trabalhos científicos e, sobretudo, por seus ensinamentos e conselhos não restritos a patologia, e que fazem de seus alunos, profissionais mais completos e

preparados para os desafios das vidas acadêmica e pessoal. Apesar de ter tido a oportunidade de conhecer um pouco do seu trabalho nestes dois anos, espero poder trabalhar ainda mais com o senhor para que eu possa obter o mesmo sucesso que o senhor e seus alunos obtêm ano após ano e que o colocam em posição de destaque no meio acadêmico.

Ao amigo de pós-graduação **Alan Roger dos Santos Silva** eu agradeço a educação e a receptividade com as quais me recebeu na faculdade e posteriormente na clínica de semiologia. Enquanto que ao **Prof. Dr. Alan Roger** agradeço todas as oportunidades oferecidas no desenvolvimento de trabalhos, projetos e pesquisas neste curto tempo de docência, assim como os incalculáveis ensinamentos transmitidos na prática clínica. Saiba que em ambas as situações, você será para mim um exemplo a ser seguido e desta forma sinto-me honrado por ter tido a oportunidade de ser seu colega de curso e logo em seguida seu aluno de pós-graduação.

Ao **Prof. Dr. Ricardo Della Coletta** agradeço toda a atenção que sempre me ofereceu durante o curso em todos os momentos em que solicitei sua ajuda. Agradeço também todos os ensinamentos repassados e fico na expectativa de ter a oportunidade de poder trabalhar de forma mais próxima com o senhor durante o doutorado.

Ao **Prof. Dr. Edgard Graner** apesar das poucas atividades desenvolvidas em conjunto, eu agradeço por poder tê-lo como um grande exemplo de pesquisador e professor, o que é refletido na admiração de todos seus alunos.

Ao **Prof. Dr. Jacks Jorge Júnior** agradeço sua educada recepção inicial na Faculdade e seus vários ensinamentos durante todo o curso. Desejo-lhe muita sorte na sua função de diretor e me ponho à disposição para ajudar-lhe no que for possível.

À **Profa. Ana Lúcia C. Rangel** eu não tenho palavras para agradecer todo o apoio dado ao desenvolvimento desta dissertação, sem dúvida este trabalho não teria como ser desenvolvido sem sua participação. Agradeço bastante também sua dedicação e atenção, e ainda mais sua educação com a qual sempre fui tratado. Espero poder trabalhar com você muitas vezes mais durante os próximos anos. De forma semelhante, agradeço imensamente ao **Dr. Alexandre**

Galvão Bueno por ter cedido seu precioso material para o desenvolvimento deste estudo e fico a disposição para poder retribuir a confiança depositada em nossa equipe.

Ao meu orientador de graduação e grande amigo **Prof. Dr. Hélder Antônio Rebelo Pontes** e **sua equipe** da Universidade Federal do Pará que durante o curso de graduação em odontologia me deram todas as condições para que hoje eu estivesse realizando o sonho de estudar em um dos mais importantes cursos de patologia oral. Agradeço também a confiança depositada em mim ao enviar os diversos casos oriundos de seu serviço ao longo dos últimos anos para serem diagnosticados em nossa faculdade e que ajudaram a ampliar meus conhecimentos em patologia. Ao senhor e à sua equipe ressalto nestas palavras minha eterna gratidão.

Ao **Prof. Dr. Erick Nelo Pedreira** pelos vários ensinamentos oferecidos durante os últimos anos e pela oportunidade de desenvolver diversos trabalhos em conjunto.

Aos funcionários do Departamento de Diagnóstico Oral (Patologia Oral), **Ana Paula** e **Luana Michele Ganhor Alescio,** do Departamento de Informática **José Marcos Tedesco Favarin** e aos técnicos dos laboratórios de Histopatologia, **Geovania Almeida, Valéria Alessandra Prado, João Carlos Gomes da Silva Júnior** e **Adriano Luís Martins** e de Imunoistoquímica, **Fabiana Cassarotti**, eu agradeço imensamente o apoio e a dedicação de vocês para o desenvolvimento deste trabalho e os ensinamentos repassados a mim durante o curso. Saibam que vocês representam peças fundamentais para a minha formação.

Aos amigos da clínica de semiologia, **Geane Soares**, **Aparecida Campion** e **Rogério de Andrade Elias,** eu agradeço todo o apoio e educação com os quais fui recebido e sempre fui tratado. O companheirismo de vocês faz com que minhas atividades clínicas se tornem cada vez mais prazerosas, e seus ensinamentos enriquecem meus conhecimentos.

Aos amigos de todas as horas **Marianne Vasconcelos, Camilla Borges, Andrea Araújo, Marcondes Sena** e **Bruno Benevenuto**, apesar de buscar palavras para descrever o que vocês representam para mim e a importância de vocês na minha formação, certamente não

poderei expressar da maneira que gostaria, por isso tenho que me contentar em agradecer todo o apoio, carinho e amizade de vocês e ressaltar que sem duvida ***tê-los como amigos foi minha maior vitória e minha maior conquista durante o mestrado.*** Espero poder estar junto com vocês por muitos e muitos anos. Muito obrigado por tudo!

À amiga **Ana Carolina P. Ribeiro** gostaria de agradecer por sua amizade verdadeira e por sua inestimável ajuda ao longo destes dois anos. Muito obrigado por ter me ensinado desde as etapas básicas de processamento de material até as obrigações de nossa rotina de histopatologia.

Ao amigo **Marcelo Takizawa** eu agradeço a dedicação com a qual me ajudou a desenvolver este trabalho, sem sua ajuda eu não teria conseguido concluí-lo. É uma grande satisfação tê-lo como amigo e co-autor desta dissertação. Você sempre será bem-vindo a Piracicaba.

Ao amigo **José Patrício de Oliveira Neto** são incontáveis os agradecimentos que tenho a fazer. Desde sua recepção em Piracicaba até sua partida para Recife foram muitas as lições que aprendi com você. Muito obrigado pela paciência e compreensão que você teve comigo durante dois anos de convivência diária.

Aos amigos de pós-graduação **Andréia Bufalino**, **Wilfredo Gonzalez**, **Adriele Ferreira Gouvêa**, **Ana Terezinha Mesquita**, **Luciana Yamamoto**, **Sabrina Nogueira de Moraes**, **Elizabete Bagordakis**, **Alicia Rumayor**, **Isadora Flores**, **José Ribamar Sabino**, **Karina Morais Faria**, **Rodrigo Neves**, **Daniel Berretta**, **Patrícia Feio**, **Débora Bastos**, **Fabiana Seguin**, **Michelle Agostini**, **Katya Pulido**, **Lara Alencar**, **Manoela Carrera**, **Mário Romañach**, **Marisol Martinez**, **Fernanda Viviane Mariano**, **Renato Hopp**, **Rogério Gondak**, **Rose Ortega**, **Tânia Benetti Soares**, **Victor Toral Rizo**, **Lívia Paranaíba**, **Andréia Aparecida da Silva** e **Marco Antônio Carvalho** que de diferentes formas me ajudaram a concluir este trabalho e que foram fundamentais para minha formação eu agradeço imensamente e espero poder ter a oportunidade de trabalhar com todos durante muito tempo.

"Tudo posso naquele que me fortalece"

(Filipenses 4:13)

RESUMO

Tumores de glândulas salivares correspondem à cerca de 3 a 6% de todos os tumores de cabeça e pescoço, apresentando uma ampla variação quanto à freqüência dos diferentes tipos histológicos e seus respectivos cursos clínicos. Desta forma, a determinação de novos marcadores moleculares que estejam relacionados com o comportamento biológico destas neoplasias se faz necessário e o uso da técnica de tissue microarray (TMA) ou micro-arranjo tecidual representa uma ferramenta altamente eficaz para a identificação destes marcadores. Sendo assim, o objetivo do presente estudo é avaliar as características clinicopatológicas de 493 neoplasias de glândulas salivares e descrever os princípios técnicos de construção de blocos de micro-arranjo tecidual, assim como suas vantagens e desvantagens para o estudo destes tumores. Para isto, os prontuários de um centro de patologia médica e de um centro de patologia oral compreendidos entre os anos de 2001 e 2011 foram revisados e os dados clinicopatológicos coletados, enquanto que a construção dos blocos de TMA foi realizada por meio de equipamento manual de arranjo tecidual em matriz, onde três áreas tumorais representativas foram selecionadas e incluídas no bloco receptor. Após a obtenção dos resultados, foi observado que o adenoma pleomórfico e o carcinoma mucoepidermóide representaram as neoplasias benigna e maligna de glândulas salivares mais freqüentes e após a construção de 12 blocos de TMA foi possível obter boa representatividade utilizando-se cilindros de 1,0, 2,0 ou 3,0 mm, especialmente em neoplasias sólidas. Portanto, a distribuição dos tumores de glândulas salivares na amostra estudada está de acordo com os achados relatados anteriormente na literatura e a técnica de TMA apresenta-se como uma metodologia de alto rendimento e baixo custo no estudo de tumores de glândulas salivares.

Palavras-chave: Micro-arranjo tecidual, Tumores de glândulas salivares, Epidemiologia.

ABSTRACT

Salivary gland tumors account for 3 to 6% of the head and neck tumors, with a broad variation in the incidence of their different histological subtypes and their respective clinical courses. For this reason, the determination of new molecular markers truly associated to the biological behavior of these neoplasias becomes necessary and the use of tissue microarray (TMA) technique represents a high-throughput laboratory tool for identifying such markers. The aim of the present study is to evaluate the clinic-pathological features of 493 salivary gland neoplasias and to describe the technical principles for construction of TMA blocks, as well as their advantages and disadvantages regarding the study of salivary gland tumors. For this, medical charts of a general pathology service and of an oral pathology service from 2001 to 2011 were reviewed and the clinic-pathological data acquired, whereas TMA blocks were constructed using a manual tissue arrayer by selecting three representative neoplastic areas to be included in the recipient block. Following the acquisition of the results, it was observed that pleomorphic adenoma and mucoepidermoid carcinoma represented the most frequent benign and malignant neoplasias, respectively, and that after the building of 12 TMA blocks it was possible to obtain high representative cores by using 1.0, 2.0 and 3.0 mm cylinders, especially in solid neoplasias. Hence, the distribution of salivary gland tumors in the sample studied is in agreement with the findings reported previously in the literature and the TMA technique presents as a high-throughput and low-cost methodology in salivary gland tumors study.

Key Words: Tissue microarray, Salivary gland tumors, Epidemiology.

SUMÁRIO

1 INTRODUÇÃO

Glândulas salivares estão distribuídas por todo trato aerodigestivo superior. As glândulas salivares maiores são representadas pelas glândulas parótida, submandibular e sublingual, enquanto que aproximadamente 450 a 750 glândulas salivares menores estão presentes em uma variedade de sítios anatômicos como lábios, mucosa jugal, palato, língua, orofaringe, seios paranasais e espaço parafaríngeo. As glândulas salivares normais são compostas por um complexo sistema de ductos e ácinos que de acordo com a Organização Mundial da Saúde podem dar origem a 34 tipos histológicos de neoplasias que apresentam caracteristicamente uma ampla heterogeneidade morfológica. Tumores de glândulas salivares representam um grupo incomum de neoplasias que correspondem à cerca de 3 a 6% de todos os tumores de cabeça e pescoço, com uma incidência global estimada em 0,4 a 13,5 casos para cada 100.000 pessoas anualmente, apesar de diferentes estudos terem apresentado variações quanto à freqüência de cada tipo histológico (Barnes et al., 2005; Ito et al., 2005; Jones et al., 2008; Tian et al., 2010; Mahmood et al., 2011).

Neoplasias malignas estão presentes em cerca de 15 a 32% dos tumores da glândula parótida, 37 a 45% dos tumores da glândula submandibular e 70 a 90% da glândula sublingual (Neville et al., 2002). Essas neoplasias possuem uma ampla variação quanto ao seu comportamento biológico, apresentando-se de forma indolente em aproximadamente 40% dos casos, causando tumefações de crescimento lento e assintomático. Em contraste, diversos casos apresentam um comportamento agressivo, com a presença de paralisia facial e uma franca evolução maligna, caracterizada pelo crescimento rápido da lesão, dor e ulcerações (Guzzo et al., 2010).

Quando as glândulas salivares menores são acometidas, 40 a 50% dos casos ocorrem no palato e aproximadamente 30% dos casos refere-se à neoplasias malignas, normalmente causando uma tumefação submucosa assintomática, por vezes com ulceração central. Se a laringe está envolvida o tumor pode causar dispnéia e disfagia, enquanto lesões da cavidade nasal e nasofaringe podem apresentar dor facial, sangramento e obstrução nasal (Neville et al., 2002).

A agressividade das neoplasias de glândulas salivares está associada com o grau histopatológico de malignidade, com o índice de recidivas e a capacidade de disseminação. A disseminação linfática é menos freqüente do que a observada no carcinoma epidermóide bucal; porém, pode ser freqüente em alguns subtipos histológicos, podendo chegar a 54% de freqüência (Guzzo et al., 2010; Mariano et al., 2011). Por outro lado, metástases à distância mais comumente para os pulmões (80%), ossos (15%) e fígado (5%) ocorrem em cerca de 17% dos casos de carcinomas de glândula parótida, em 37% daqueles afetando a glândula submandibular e em 24% nos casos de glândulas menores, representando a principal causa de morte decorrente de neoplasias de glândulas salivares. Carcinoma adenóide cístico, adenocarcinoma sem outra especificação, carcinoma ex-adenoma

pleomórfico, carcinoma mucoepidermóide de alto grau, carcinoma de pequenas células e carcinoma ductal exibem o maior índice de metástases à distância (Luukkaa et al., 2009; Loh et al., 2009; Mariano et al., 2011).

A sobrevida dos pacientes afetados por neoplasias de glândulas salivares malignas é bastante variável. Alguns estudos têm demonstrado uma taxa de sobrevida geral de aproximadamente 78% e 70% após 5 e 10 anos, respectivamente (Bjorndal et al., 2011). Pacientes acometidos por adenocarcinoma polimorfo de baixo grau apresentam um índice de sobrevida de 5 anos elevado, em torno de 95 a 100%, assim como aqueles afetados pelo carcinoma de células acinares cuja sobrevida gira em torno de 75 a 96%, enquanto que pacientes afetados pelo carcinoma mucoepidermóide possuem uma sobrevida de 5 anos média variando de 43 a 92% dependendo do seu grau histológico (Guzzo et al., 2010; Seethala, 2011). De forma interessante, o carcinoma adenóide cístico apresenta uma boa taxa de sobrevida após 5 anos (75 a 80%), porém um índice após 15 anos bastante reservado (35%); devido, sobretudo, a sua capacidade de originar metástases tardias (Seethala, 2011). O estadiamento tumoral, a gradação, o envolvimento neural, a extensão tumoral para fora do tecido glandular e o envolvimento de nódulos linfáticos cervicais parecem ser fatores importantes para a avaliação da sobrevida dos pacientes afetados, sendo capazes de influenciar os resultados do tratamento preconizado (Lloyd et al., 2010). Além disso, a idade do paciente, o sítio anatômico acometido e as margens cirúrgicas livres de lesão também devem ser considerados como determinantes prognósticos para o controle loco-regional em neoplasias glandulares (Koul et al., 2007; Loh et al., 2009; Mücke et al., 2009; Speight et al., 2009). Outros fatores como o padrão histológico predominante, a presença de invasão angiolinfática, necrose tumoral e composição mioepitelial também podem influir na taxa de sobrevida de alguns tumores (Rapidis et al., 2007; Speight et al., 2009; Guzzo et al., 2010).

O protocolo terapêutico para carcinomas de glândulas salivares maiores e menores passíveis de ressecção é a excisão cirúrgica. A utilização de radioterapia como modalidade terapêutica primária deve ser considerada em casos onde o paciente se recusa a ser submetido ao tratamento cirúrgico padrão ou em casos inoperáveis ou ainda como terapia adjuvante à cirurgia para neoplasias de alto grau (Mahmood et al., 2011; Seethala, 2011). Para tumores em glândulas maiores ou menores a quimioterapia tem um papel apenas discreto, sendo utilizada como alternativa paliativa para doenças inoperáveis, para pacientes que se recusam a submeter-se à radioterapia, e para pacientes apresentando metástases (Koul et al., 2007; Mücke et al., 2009; Lloyd et al., 2010; Seethala, 2011).

Dentre as seqüelas oriundas do tratamento empregado, a morbidade do nervo facial é comumente observada após o tratamento de neoplasias de glândulas salivares em parótida, especialmente em casos no quais o nervo está diretamente envolvido pela neoplasia. Seqüelas pós-operatórias adicionais incluem fistula salivar, neuromas do grande nervo auricular, síndrome de

Frey e sensação de anestesia na pele periauricular. As seqüelas decorrentes da radioterapia incluem eritema cutâneo, mucosite e disfagia, além de descamação e úlceras mucosas. Efeitos colaterais tardios incluem telangiectasia, alteração de paladar permanente, fibrose subcutânea, xerostomia e otite média ou interna associada com perda parcial de audição e dor (Guzzo et al., 2010).

Tendo em vista o pouco conhecimento a cerca dos eventos moleculares que cuminam com o surgimento das diferentes neoplasias de glândulas salivares, assim como o desconhecimento de marcadores que favoreçam uma melhor determinação do comportamento clínico de cada tumor, a identificação de novos marcadores moleculares se torna imperativa para que haja um melhor entendimento destas neoplasias e uma melhor abordagem terapêutica aos indivíduos acometidos.

Após a descoberta do genoma humano e o desenvolvimento de novas técnicas laboratoriais como o microarranjo de DNA e a proteômica, que permitiram a análise de milhares de genes e de proteínas de forma mais simplificada, o desenvolvimento de estudos de validação de proteínas tornou-se amplamente necessário para a identificação de moléculas que realmente estejam relacionadas ao comportamento clínico neoplásico (Nocito et al., 2001; Radhakrishnan et al., 2008). Nesta linha de pensamento, a técnica de microarranjo tecidual em matriz, ou *tissue microarray* (TMA), descrita em 1998 por Kononen et al. tem apresentado uma ampla aceitação por parte da literatura mundial. Trata-se da construção de um bloco de parafina contendo fragmentos cilíndricos de amostras teciduais ou tumorais obtidos de dezenas ou centenas de blocos de parafina originais. Os cilindros teciduais são dispostos no bloco receptor seguindo uma ordem predeterminada. Desta forma, um bloco de parafina contendo amostra de centenas de tumores de modo ordenado representa uma poderosa ferramenta para a patologia investigativa aplicada. Com o uso de diferentes metodologias laboratoriais, como a imunoistoquímica, hibridização *in situ*, análise de ploidia de DNA por imagem, morfometria nuclear e FISH, em uma lâmina obtida do bloco de TMA, é possível conhecer a expressão de um determinado marcador ou uma determinada amplificação ou translocação cromossômica em centenas de amostras ao custo de uma única reação (Kallioniemi et al., 2001; Marek et al., 2002; Andrade et al., 2007).

Dentre as várias aplicações do método seu uso se destaca na avaliação de novos anticorpos imunoistoquímicos, na pesquisa em larga escala de fatores prognósticos ou preditivos de diferentes neoplasias, na uniformização das reações em grandes estudos retrospectivos e na validação ao nível protéico da hiperexpressão ou hipoexpressão gênica (Camp et al., 2001; Marek et al., 2002; Andrade et al., 2007). Estudos utilizando a técnica de *tissue microarray* têm sido desenvolvidos na investigação imunoistoquímica de uma serie de neoplasias humanas, como tumores de cabeça e pescoço, linfomas, câncer de mama, câncer de pulmão, câncer de próstata, dentre outros (Ananthanarayanan et al. 2011).

O uso do bloco de TMA apresenta múltiplas vantagens em relação ao corte tradicional, como a grande economia de reagentes e de tempo para a realização das reações, a padronização destas reações e uma maior facilidade em sua interpretação, e a possibilidade de uso em diferentes linhas de pesquisa, com sua utilização em mais de um projeto. Entretanto, a maior preocupação quanto ao uso do TMA refere-se à representação da lesão, considerando a pequena amostra estudada. A fidelidade dos resultados em relação ao uso dos cortes convencionais foi objeto de estudo por ocasião da validação do método e está demonstrada em vários trabalhos da literatura que obtiveram alta concordância nos resultados com os cortes convencionais, além de um custo em média sete vezes menor; recomendando, portanto, o uso de TMA na patologia investigativa, o que pode ser representado por meio da crescente utilização do método nos artigos publicados anualmente (Rimm et al., 2001; Andrade et al., 2007).

Apesar de representar uma importante ferramenta para o melhor entendimento da patogênese das neoplasias de glândulas salivares, poucos estudos foram desenvolvidos aplicando-se a técnica de TMA na avaliação de marcadores moleculares que possam estar relacionados com o comportamento biológico destes tumores (Freier et al., 2005; Vargas et al., 2008). Desta forma, o desenvolvimento do presente estudo tem como proposta a caracterização clínico-patológica de uma ampla amostra de neoplasias glandulares, com o subseqüente arranjo destes tumores em blocos de TMA. Além disso, pontos técnicos importantes para que obtenhamos o máximo aproveitamento desta técnica, assim como suas vantagens e desvantagens em relação às peculiaridades encontradas em neoplasias glandulares são adequadamente abordados ao longo do estudo.

CAPÍTULO 1

Artigo submetido para publicação no periódico *Oral Surgery Oral Medicine Oral Pathology and Oral Radiology*

CLINICOPATHOLOGIC ANALYSIS OF 493 CASES OF SALIVARY GLAND TUMORS IN A SOUTHERN BRAZILIAN POPULATION

Felipe Paiva Fonseca - **DDS**[1], Marianne de Vasconcelos Carvalho – **DDS, MSc**[1], Oslei Paes de Almeida – **DDS, PhD**[1], Ana Lúcia Carrinho Ayroza Rangel – **DDS, PhD**[2], Marcelo Caname Hirata Takizawa – **DDS**[2], Alexandre Galvão Bueno – **MD**[3] and Pablo Agustin Vargas – **DDS, PhD**[1]

01. Department of Oral Diagnosis, Piracicaba Dental School, State University of Campinas – São Paulo – Brazil.
02. Department of Pathology, State University of Western Paraná – Paraná – Brazil.
03. Surgical Pathologist at Anatom Laboratory – Paraná – Brazil.

Corresponding author:

Prof. Dr. Pablo Agustin Vargas

Department of Oral Diagnosis (Oral Pathology) – Piracicaba Dental School – State University of Campinas. Limeira Street, No. 901 ZipCode: 13414-903 Piracicaba - São Paulo – Brazil. Tel.: +55 19 32015315 E-mail: pavargas@fop.unicamp.br

Abstract Words: 150 **Manuscript Words:** 3761 **Figures:** 5 **Tables:** 7

The authors state that they have no potential conflict of interest that could bias the results obtained in the current study. The study was supported by grants from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Abstract

Objective: To determine the distribution and demographic features of salivary gland tumors (SGTs) in a large Brazilian population.

Study Design: 493 cases of SGTs diagnosed between 2001 and 2011 from a surgical pathology service and an oral pathology service, were reviewed in respect to their clinicopathologic features.

Results: 369 tumors were benign and 124 malignant. The mean age of patients with benign tumors was 46.3 years and with malignancies 54.0 years. The parotid gland was the most common location (42.3%). Pleomorphic adenoma (PA) and Warthin tumor were the most common benign neoplasias, whereas mucoepidermoid carcinoma (MEC) and adenocarcinoma NOS were the most frequent malignancies.

Conclusion: The present data confirmed that PA and MEC are the most common benign and malignant SGTs. However, it is important to consider differences in the frequency of neoplasias from medical and dental services, as they reflect tumors predominantly found in minor and major salivary glands.

Keywords: Salivary gland tumors; Epidemiology; Pleomorphic adenoma.

Introduction

Salivary gland tumors consist of a group of heterogeneous lesions with complex clinicopathologic characteristics and distinct biological behavior, that correspond to approximately 3% to 10% of the neoplasms of the head and neck region[1,2,3]. According to the World Health Organization (WHO), the global annual incidence, when all salivary gland tumors are considered, varies from 0.4 to 13.5 cases per 100,000 inhabitants, which accounts for only 0.3% of all

malignancies in the United States[4]. However, reports from numerous regions of the world have shown differences in the incidence and frequency of tumor types, indicating a geographic variation in the frequency of these neoplasias[5,6] **(Fig. 1)**.

Although many retrospective studies regarding the incidence of salivary gland tumors have been reported, the epidemiology of these neoplasms is not well established since these studies are frequently restricted to a specific population[7,8], an anatomical location[9] or specific tumor type[10]. In addition, differences can be found in the frequency of salivary gland tumors derived from surgical pathology centers and those from oral pathology laboratories. Therefore, the objective of the current study is to retrospectively review the characteristics of 493 salivary gland tumors retrieved from one surgical pathology service and one oral pathology laboratory, located in the Southern Brazil, and to evaluate the clinicopathological differences between these two samples.

Material and Methods

The files of a private surgical pathology service in Cascavel, Paraná state, and of the Department of Oral Pathology of the Piracicaba Dental School, were retrospectively reviewed. During an 11-year period, between January 2001 and December 2011 (surgical pathology 2001 – 2009 and oral pathology 2002 – 2011), 493 cases of salivary gland tumors were retrieved from both archives. Clinical data concerning age, gender and tumor location, were obtained from clinical charts. Microscopical slides of all cases were reviewed by three oral pathologists and, where necessary, new sections were prepared and stained with hematoxylin and eosin, periodic acid-Schiff, or mucicarmine. All cases were classified according to the 2005 WHO's Histological Typing of Salivary Gland Tumors[4].

The current study was approved by the Ethical Committee of the Piracicaba Dental School – State University of Campinas (Protocol 141/2011).

Results

General overview

Considering the total of 493 cases, 369 (74.8%) were benign and 124 (25.1%) malignant (a ratio of 2.9:1), distributed among seven benign and eight malignant histological subtypes, accounting for 5.0% of all benign and malignant head and neck neoplasias detailed by both centers **(Table 1)**. The overall male to female ratio was 0.8:1 (220 male versus 273 female), while in the benign cases this proportion was 0.7:1 (156 male versus 213 female) and in the malignant cases 1.1:1 (64 males versus 60 female) **(Table 1)**. Most tumors occurred in patients of 31 - 70 years old, with an average age of 48.2 years (range 8 - 88 years). The mean age of the patients with benign tumors was 46.3 years, and with malignancies 54.0 years **(Fig. 2)**. The exact distribution of each salivary gland tumor, according to the age of the patients is depicted in **Table 2.**

The parotid gland was the most commonly affected location, with a frequency of 42.3%, followed by the palate (19.2%), lips (7.7%) and the submandibular gland (6.8%). No tumors of the sub-lingual gland were found. Benign tumors predominated in the parotid glands, followed by the palate and lips (37.3%, 11.9% and 6.0%, respectively), whereas malignancies were more frequent in the palate, parotid gland, cheek and submandibular gland (7.3%, 5.0%, 2.0% and 1.8%, respectively) **(Fig. 3 and Table 3)**.

Among the 369 benign salivary gland tumors, 314 were pleomorphic adenomas (63.6% of total, or 85.0% of the benign) and 36 Warthin tumors (7.3% of total, or 9.7% of the benign), and these represented the most common benign neoplasias. Concerning malignant tumors, there were 39 mucoepidermoid carcinomas (7.9% of total, or 31.4% of the malignant), 33 adenocarcinomas NOS (6.6% of total, or 26.6% of the malignant) and 22 adenoid cystic carcinomas (4.4% of total, or 17.7% of the malignant), which represented the most frequent, the second most frequent and the third most frequent malignancies respectively.

Dental hospital sample

In a 10-year period (2002 - 2011), there were 161 salivary gland tumors diagnosed at the oral pathology department of the Piracicaba Dental School, which corresponded to 6.4% of all head and neck tumors diagnosed by this department, of which 88 were benign (54.1%) and 73 (45.9%) were malignant, with a benign-malignant ratio of 1.2:1, representing four benign subtypes and seven malignant subtypes **(Table 4)**.

Most tumors occurred in patients of 31 - 50 years old (mean age 48.9 years, range 8 – 88 years) and the male to female ratio was 0.7:1. The minor glands in the palate were the most common site (86 cases, or 53.4%), followed by the lips (33 cases, or 20.4%) and the cheek (15 cases or 9.3%) **(Fig. 4)**. Pleomorphic adenoma was the most frequent histological type (73 cases, or 45.3%), followed by canalicular adenoma (nine cases, or 5.5%). Regarding the most common malignant tumors, there were 35 mucoepidermoid carcinomas (21.7%), 14 polymorphous low-grade adenocarcinomas (8.6%) and 12 adenoid cystic carcinomas (7.4%).

Private Surgical Pathology Sample

In a 9-year period (2001 - 2009), 332 salivary gland tumors were found in a private surgical pathology center located in the city of Cascavel, Paraná state, accounting for 4.6% of the head and neck neoplasias seen at this center, of which 281 were benign (84.6%) and 51 (15.3%) were malignant, with a benign-malignant ratio of 5.5:1, distributed between five benign and six malignant subtypes **(Table 5)**.

Patients from 31 to 70 years old were the most affected, with a mean age of 47.7 years (range 8 – 86 years) and a male to female ratio of 0.8:1. The parotid gland was by far the most affected site (62.6%), followed by the submandibular gland (10.5%) and the minor glands in the palate (2.7%) **(Fig. 5)**. Pleomorphic adenoma was the most frequent histological type (241 cases, or 72.5%), followed by Warthin tumor (36 cases, or 10.8%). Adenocarcinoma NOS was the most common malignant tumor (27 cases, or 8.1%), followed by adenoid cystic carcinoma (10 cases, or 3.0%) and carcinoma ex-pleomorphic adenoma (6 cases, or 1.8%).

Major versus Minor salivary glands

Examining the distribution of salivary gland tumors affecting major and minor salivary glands, 243 neoplasias were located in the major glands and 156 in minor glands (1.5:1 ratio), (in 94 cases the location was not specified) **(Table 3)**. There were only two cases affecting major glands in the sample derived from the oral pathology center (1.2%), both found in the parotid gland, whereas 86.9% of the tumors affected minor glands, and in 11.8% of the cases the location was not specified. On the other hand, tumors of the major glands accounted for 72.5% of the neoplasias diagnosed in the general pathology center (62.3% affecting the parotid and 10.2% the submandibular glands), whereas 4.8% of the tumors affected minor glands and in 22.5% of the cases the location was not specified. In major glands there was a significantly higher benign to malignant ratio than the observed in minor glands (6.1:1 versus 1.6:1, respectively).

Pleomorphic adenoma was the most common benign tumor in both major and minor glands [72.8% (177 cases out of 243) and 51.9% (81 cases out of 156), respectively], followed by Warthin tumor in major glands (12.3% of the 243 major glands tumors) and canalicular adenoma in minor glands (7.0% of the 156 cases affecting minor glands). Regarding malignancies, adenocarcinoma NOS and adenoid cystic carcinoma were the most frequent neoplasias of the major glands (8.2% and 1.2%, respectively), whereas MEC and LGPA represented the most common malignancies in minor glands (17.3% and 8.3%, respectively).

Discussion

Salivary gland tumors are a large and diverse group of lesions, with a complex histological classification due to their morphological heterogeneity. There are numerous epidemiological studies of salivary gland tumors in different countries, with varied results. The variety of results could be a consequence of various factors that might include: the origin of the study (medical or dental

centers), divergences in the histological classification, restriction to a specific population, anatomical location or tumor types[6,7,11-33] **(Table 6 and 7)**. Herein, we evaluated 493 cases of salivary gland tumors from one medical and one oral pathology laboratory located in southern Brazil, aiming to investigate the distribution and the clinicopathologic features of these neoplasias in a large representative sample, and identify possible differences between the populations of both services. Although there was some variability, particularly regarding the palate as the most affected site by malignancies, the results obtained revealed that the tumor types and their demographic features are in agreement with numerous reports, including those previously reported in Brazil[1,3,5,34].

According to the WHO, female patients are slightly more affected than males, although some variation can be found when analyzing specific tumor types[4]. In the current study the male-female ratio was 0.8:1, which is in accordance with the majority of studies, including Brazilian reports[1,3,5,12,14,24,27,34], although several reports have shown an increased frequency in male patients[15,35,36]. It should be considered that benign tumors presented a male-female ratio of 0.7:1, whereas malignant neoplasias revealed a ratio of 1.1:1, indicating that benign tumors were slightly more predominant in females, whereas malignancies more often occurred in males, which is in accordance with previous studies[3,5,14,26], but this is in contrast to that previously reported in Mexico[21], where female patients were more frequently affected by malignancies than male patients.

The age distribution in the present survey varied from 8 to 88 years old, with a mean age of 48.2 years, similar to other studies[3,12,21]. As reported previously[3,15], it was observed that for malignant neoplasias the mean age of the patients is about a decade higher than for benign tumors. However, Jansisyanont et al (2002) reported that patients affected by malignant tumors were on average six years younger than those affected by benign neoplasias[25]. In summary, it appears that there is a broad overlap in risk ages for patients with benign and malignant tumors, and therefore, this clinical feature cannot be considered a reliable parameter for diagnosis of malignancies.

In the majority of the studies, and in those derived from medical centers, the parotid gland is by far the most commonly affected location, with 64% to 80% of all primary salivary gland tumors

occurring at this site, more specifically in the superficial lobe[37]. In the present study the parotid gland was also the most affected site (42.3%), followed by minor glands of the palate, lips and the submandibular gland, which is in accordance to the observations of previous studies[6,36]. The parotid gland was the most common location for benign tumors, whereas the palate was the most common site for malignancies. Although some studies conclude that minor glands, especially of the palate, are proportionally more affected by malignancies than major glands[16,20], the great majority of studies report the parotid gland as the most affected site by both benign and malignant neoplasias[6,14,15]. Also, in contrast to what was observed in the present survey, in many studies the submandibular gland has been found to be the second most affected site, and this has resulted in the samples studied mainly being formed of major glands[1,3,15,16,38].

In the present study, 74.8% of the cases were benign and 25.1% were malignant, confirming the predominance of benign salivary gland tumors[3,6,13,27,38,39]. All epidemiological studies clearly show that PA is by far the most common salivary gland neoplasia, both in major and minor glands[13,36]. In this survey, PA corresponded to 63.6% of all tumors, accounting for 72.8% of the major, and 52.6% of the minor glands, and mainly affecting female patients in the fourth decade of life. The second most common benign tumor was Warthin tumor that accounted for 7.3% of all tumors and 9.7% of benign. As Warthin tumor occur almost exclusively in the parotid, in studies considering only tumors of the minor salivary glands, it is rarely reported[6,9].

In accordance with the majority of the studies, mucoepidermoid carcinoma was the most frequent malignant neoplasia found in the present survey (7.9% of all tumors), corresponding to 1.2% and 17.3% of the major and minor glands neoplasias respectively, and mainly diagnosed in female patients in the fifth decade of life[5,6,12,40]. However, unlike some previous studies, in our series adenocarcinoma NOS was the second most frequent malignant tumor, accounting for 6.6% of all tumors, and for 26.6% of malignant tumors[1,36]. This neoplasia mostly affected the parotid gland of male patients in the sixth and seventh decades of life. Adenoid cystic carcinoma was the third most common malignant tumor, accounting for 4.4% of all tumors. This incidence is lower than

those observed in many other studies, where it represents the most, or the second most, frequent malignant neoplasia[13,16,18,36,39,41]. In our survey, adenoid cystic carcinoma usually affected the submandibular and the palate glands, with equal sex distribution and predominance for the seventh decade of life.

The main difference observed between samples derived from surgical and oral pathology services was related to the distribution of tumors preferentially affecting the major or minor salivary glands. In the current study, most cases from the surgical pathology center affected major glands (72.5%), particularly the parotid that accounted for 62.3% of the 332 cases. On the other hand, intraoral minor salivary glands represented the most common site in the oral pathology service, accounting for 86.9% of the 161 cases. These findings are in agreement with almost all international series previously reported[6,26]. It is apparent that most epidemiological studies suffer this bias, as can be seen with other diseases such as odontogenic tumors (ameloblastoma versus odontoma), and oral cancer (studies that include cases from lip and oropharynx versus those that do not). Thus, in spite of the overall distribution of SGTs in the current study diverged from those conducted in dental hospitals, regarding only neoplasias affecting intra-oral minor glands the present results are in agreement with the majority of the previous studies from oral pathology services, that have observed MEC and ACC, or LGPA, as the most frequent malignancies, and PA and canalicular adenoma as the most common benign neoplasias[9,25,26,30,33,39], as well as minor glands of the palate being the most frequently affected site[29,31]. These results, therefore, highlight the importance of adequately characterizing the origin of the population studied. In addition, the benign-malignant ratio proved to be higher in the medical sample than in the dental one, mainly due to the higher incidence of pleomorphic adenoma in the former, which has been reported in almost every previous study[6,16]. By contrast, no significant difference could be found in gender distribution and in the mean age of the patients between the two centers.

In conclusion, demographic data from retrospective studies may be helpful to increase understanding of the biological and clinical characteristics of salivary gland tumors, and according

to the results obtained in the current study, it has been confirmed that male patients are slightly more frequently affected by malignancies than female patients, and that these malignant tumors mainly occur in patients about a decade older than those affected by benign neoplasias, although risk age may overlap between both groups. Nevertheless, to better analyze and understand the frequency and distribution of salivary gland tumors subtypes, it is important to consider whether the sample studied is mainly derived from a medical or dental hospital, since tumors with higher incidence in the minor glands predominate in oral pathology laboratories, and those with higher incidence in major glands are more frequently diagnosed in surgical pathology services.

References

1. Vargas PA, Gerhard R, Araújo Filho VJF, Castro IV. Salivary gland tumors in a Brazilian Population: A retrospective study of 124 cases. Rev Hosp Clín Fac Med S Paulo 2002; 57(6): 271-276.
2. Ansari MH. Salivary gland tumors in an Iranian population: A Retrospective Study of 130 Cases. J Oral Maxillofac Surg 2007; 65: 2187-2194.
3. Oliveira FA, Duarte ECB, Taveira CT, Máximo AA, Aquino EC, Alencar RC, et al. Salivary gland tumor: A review of 599 cases in a brazilian population. Head Neck Pathol 2009; 3: 271-275.
4. Barnes L, Eveson JW, Reichart PA, Sidranskiy D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. 1st Ed. Lyon, France: IARC Press: 2005; p. 211-281.
5. Ito FA, Ito K, Vargas PA, Almeida OP, Lopes MA. Salivary gland tumors in a Brazilian population: a retrospective study of 496 cases. Int J Oral Maxillofac Surg 2005; 34: 533–536.

6. Luksic I, Virag M, Manoilovic S, Macan D. Salivary gland tumours: 25 years of experience from a single institution in Croatia. J Craniomaxillofac Surg.

7. Ellies M, Schaffranietz F, Arglebe C, Laskawi R. Tumors of the salivary glands in childhood and adolescence. J Oral Maxillofac Surg 2006; 64: 1049-1058.

8. Muenscher A, Diegel T, Jaehne M, Ussmüller J, Koops S, Sanchez-Hanke M. Benign and malignant salivary gland diseases in children: A retrospective study of 549 cases from the Salivary Gland Registry, Hamburg. Auris Nasus Larynx 2009; 36: 326-333.

9. Pires FR, Pringle GA, Almeida OP, Chen SY. Intra-oral minor salivary gland tumors: A clinicopathological study of 546 cases. Oral Oncol 2007; 43: 463-470.

10. Kokemueller H, Swennen G, Brueggemann N, Brachvogel P, Eckardt A, Hausamen JE. Epithelial malignancies of the salivary glands: clinical experience of a single institution - a review. Int J Oral Maxillofac Surg 2004; 33: 423–432.

11. Wang D, Li Y, He H, Liu L, Wu L, He Z. Intraoral minor salivary gland tumors in a Chinese population: a retrospective study on 737 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104: 94-100.

12. Long-jiang L, Yi L, Yu-ming W, Hua L, Hong-wei Z. Clinical analysis of salivary gland tumor cases in West China in past 50 years. Oral Oncol 2008; 44: 187-192.

13. Jones AV, Craig GT, Speight PM, Franklin CD. The range and demographics of salivary gland tumours diagnosed in a UK population. Oral Oncol 2008; 44(4): 407-417.

14. Chidzonga MM, Lopez Perez VM, Alvarez ALP. Salivary gland tumours in Zimbabwe: report of 282 cases. Int J Oral Maxillofac Surg 1995; 24: 293-297.

15. Kara MI, Göze F, Ezirganli Ş, Polat S, Muderris S, Elagoz S. Neoplasms of the salivary glands in a Turkish adult population. Med Oral Patol Oral Cir Bucal 2010; 15(6): e880-e885.

16. Al-Khateeb TH, Ababneh KT. Salivary tumors in north Jordanians: A descriptive study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103: e53-e59.
17. Ochicha O, Malami S, Mohammed A, Atanda A. A histopathologic study of salivary gland tumors in Kano, northern Nigeria. Indian J Pathol Microbiol 2009; 52(4): 473-476.
18. Renehan A, Cleave N, Hancock D, Smith P, McGurk M. Long-term follow-up of over 1000 patients with salivary gland tumours treated in a single centre. Br J Surg 1996; 83: 1750-1754.
19. Saku T, Hayashi Y, Takahara O, Matsuura H, Tokunaga M, Tokuoka S, et al. Salivary gland tumors among atomic bomb survivors, 1950–1987. Cancer 1997; 79: 1465-1475.
20. Shishegar M, Ashraf MJ, Azarpira N, Khademi B, Hashemi B, Ashrafi. Salivary gland tumors in maxillofacial region: A retrospective study of 130 cases in a southern Iranian population. Pathol Res Int 2011; 2011: 1-5.
21. Mejía-Velázquez CP, Durán-Padilla MA, Gómez-Apo E, Quezada-Rivera D, Gaitán-Cepeda LA. Tumors of the salivary gland in mexicans. A retrospective study of 360 cases. Med Oral Patol Oral Cir Bucal.
22. Rivera-Bastidas H, Ocanto RA, Acevedo AM. Intraoral minor salivary gland tumors; a retrospective study of 62 cases in a Venezuelan population. J Oral Pathol Med 1996; 25: 1-4.
23. Dhanuthai K, Boonadulyarat M, Jaengjongdee T, Jiruedee K. A clinico-pathologic study of 311 intra-oral salivary gland tumors in Thais. J Oral Pathol Med 2009; 38: 495–500.
24. Jaber MA. Intraoral minor salivary gland tumors: a review of 75 cases in a Libyan population. Int J Oral Maxillofac Surg 2006; 35: 150–154.
25. Jansisyanont P, Blanchaert Jr RH, Ord RA. Intraoral minor salivary gland neoplasm: a single institution experience of 80 cases. Int J Oral Maxillofac Surg 2002; 31: 257–261.
26. Lopes MA, Kowalski LP, Santos GC, Almeida OP: A clinicopathologic study of 196 intraoral minor salivary gland tumours. J Oral Pathol Med 1999; 28: 264–267.

27. Loyola AM, Araújo VC, Sousa SOM, Araújo NS. Minor salivary gland tumours. A retrospective study of 164 cases in a Brazilian population. Oral Oncol, Eur J Cancer 1995; 31B(3): 197-201.

28. Poomsawat S, Punyasingh J, Weerapradist W. A retrospective study of 60 cases of salivary gland tumors in a Thai population. Quintessence Int 2004; 35: 577-581.

29. Toida M, Shimokawa K, Makita H, Kato K, Kobayashi A, Kusunoki Y, et al. Intraoral minor salivary gland tumors: A clinicopathological study of 82 cases. Int J Oral Maxillofac Surg 2005; 34: 528–532.

30. Van Heerden WFP, Raubenheimer EJ. Intraoral salivary gland neoplasms: A retrospective study of seventy cases in an African population. Oral Surg Oral Med Oral Pathol 1991; 71: 579-582.

31. Vani NV, Ponniah I. The frequency and distribution pattern of minor salivary gland tumors in a government dental teaching hospital, Chennai, India. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 111: e32-e39.

32. Waldron CA, El-Mofty SK, Gnepp DR. Tumors of the intraoral minor salivary glands: A demographic and histologic study of 426 cases. Oral Surg Oral Med Oral Pathol 1988; 66: 323-333.

33. Yih WY, Kratochvil FJ, Stewart JCB. Intraoral minor salivary gland neoplasms: Review of 213 cases. J Oral Maxillofac Surg 2005; 63: 805-810.

34. Lima SS, Soares AF, Amorim RFB, Freitas RA. Epidemiologic profile of salivary gland neoplasms: analysis of 245 cases. Rev Bras Otorrinolaringol 2005; 71(3): 335-340.

35. Otoh EC, Johnson NW, Olasoji H, Danfillo IS, Adeleke OA. Salivary gland neoplasms in Maiduguri, north-eastern Nigeria. Oral Dis 2005; 11: 386-391.

36. Tian Z, Li L, Wang L, Hu Y, Li J. Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6982 cases in an eastern Chinese population. Int J Oral Maxillofac Surg 2010; 39: 235-242.

37. Subhashraj K. Salivary gland tumors: a single institution experience in India. Br J Oral Maxillofac Surg 2008; 46: 635-638.
38. Satko I, Stanko P, Longauerova I. Salivary gland tumours treated in the stomatological clinics in Bratislava. J CranioMaxillofac Surg 2000; 28: 56-61.
39. Buchner A, Merrell PW, Carpenter WM. Relative frequency of intra-oral minor salivary gland tumors: a study of 380 cases from northern California and comparison to reports from other parts of the world. J Oral Pathol Med 2007; 36: 207-214.
40. Tilakaratne WM, Jayasooriya PR. Epithelial salivary tumors in Sri Lanka: A retrospective study of 713 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108: 90-98.
41. Vuhahula EAM. Salivary gland tumors in Uganda: clinical pathological study. African Health Scienc 2004; 4(1): 15-23.

Figure legends

Figure 1. Heterogeneous distribution of the most frequent salivary gland tumors (pleomorphic adenoma, mucoepidermoid carcinoma and adenoid cystic carcinoma) among different geographic regions of the world.

Figure 2. Distribution of 493 benign and malignant salivary gland tumors diagnosed in Southern Brazil according to the patient's age.

Figure 3. Distribution of 493 benign and malignant salivary gland tumors diagnosed in Southern Brazil according to the primary site of involvement.

Figure 4. Distribution of 161 salivary gland tumors diagnosed by the Department of Oral Pathology of the Piracicaba Dental School according to the primary site of involvement.

Figure 5. Distribution of 332 salivary gland tumors diagnosed by a private Surgical Pathology Center in Cascavel (Paraná state) according to the primary site of involvement.

Figure 1

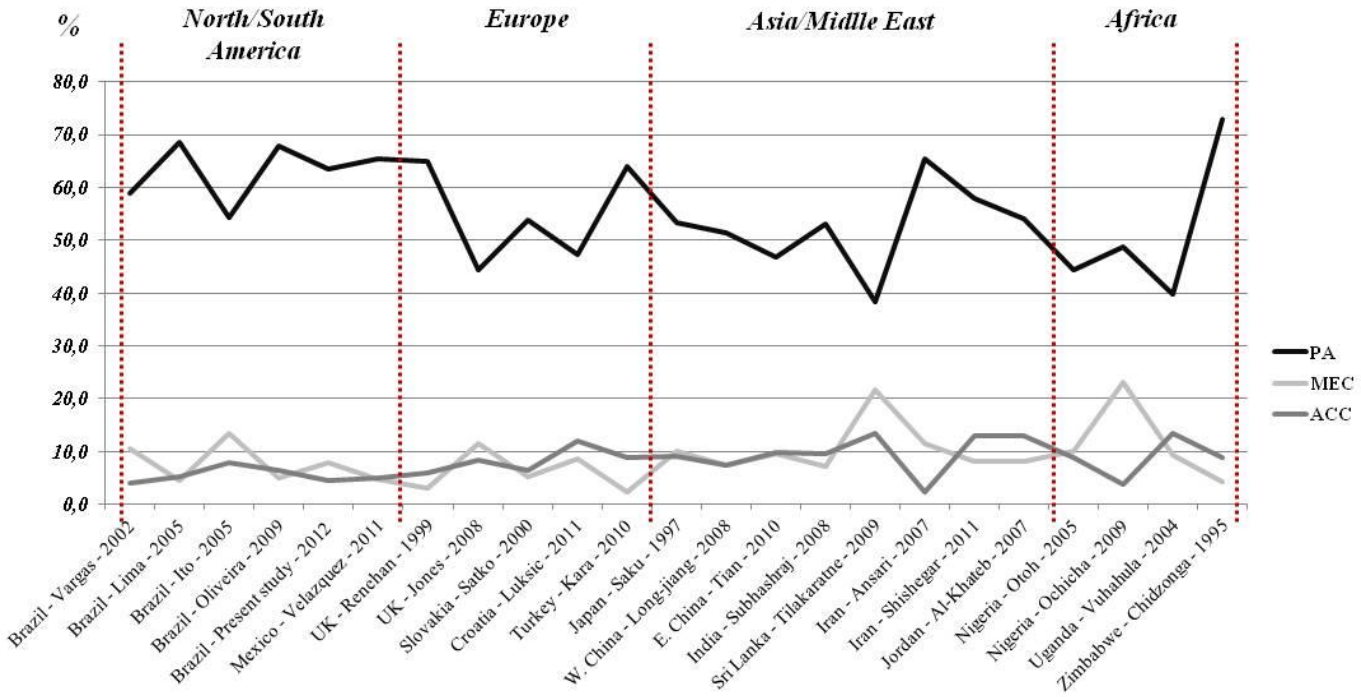


Figure 2

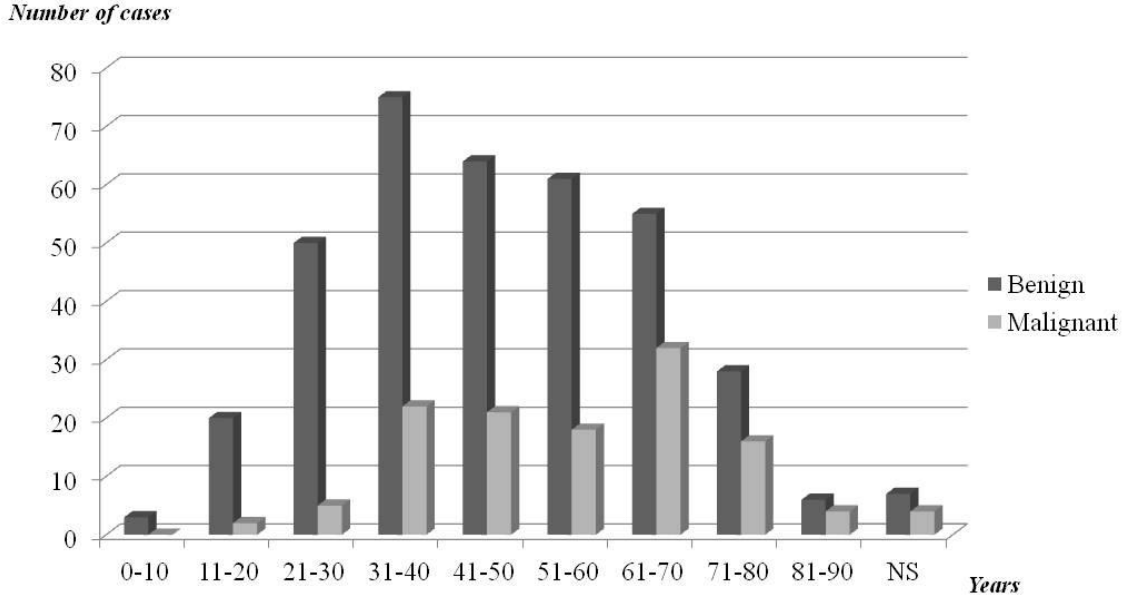


Figure 3

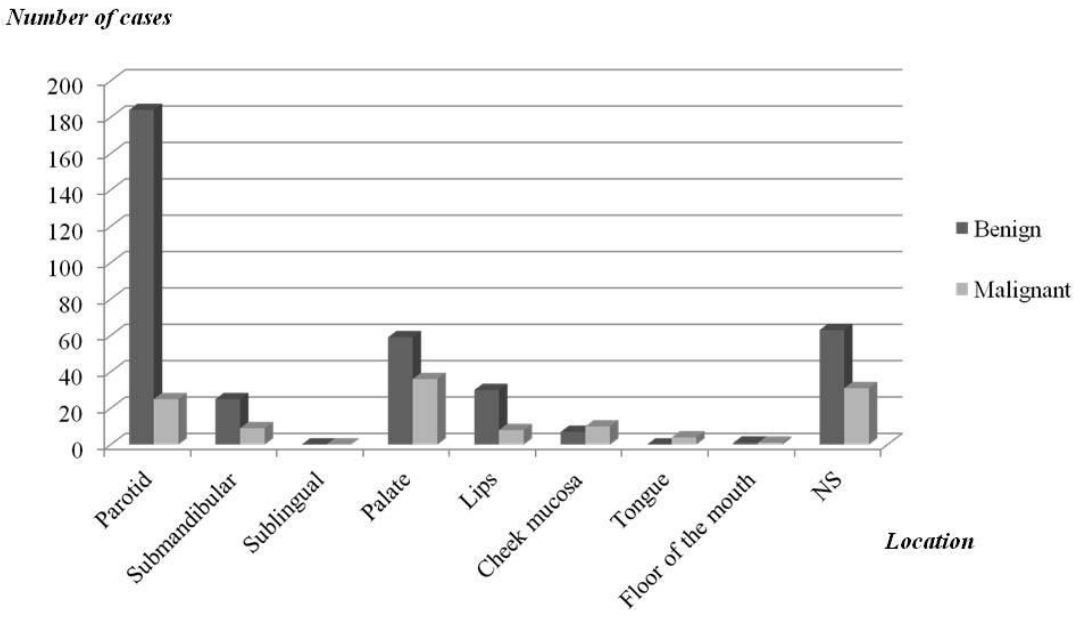


Figure 4

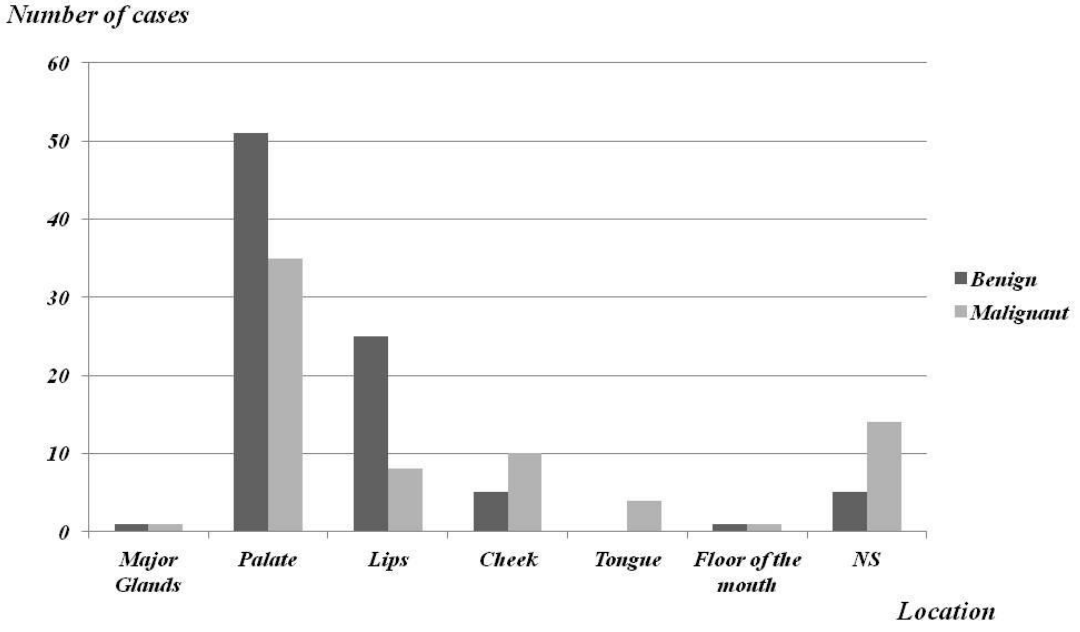


Figure 5

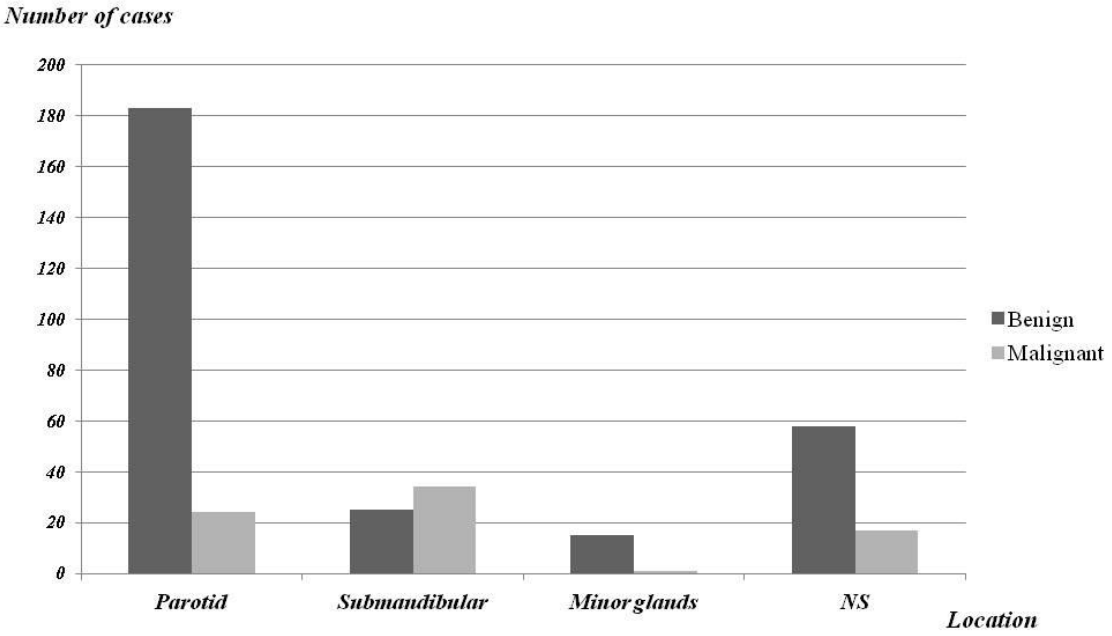

Number of cases
200
180
160
140
120
100
80
60
40
20
0
Parotid
Submandibular
Minor glands
NS
Location
Benign
Malignant

Table 1. Histological and gender distribution of 493 salivary gland tumors.

	n=493	*%*	***% in the group (Benign or malignant)***	**Gender**			
				Male		**Female**	
				n	*%*	**n**	*%*
Benign tumors							
Pleomorphic adenoma	314	*63.6*	*85.0*	123	*24.9*	*191*	*38.7*
Warthin tumor	36	*7.3*	*9.7*	27	*5.4*	*9*	*1.8*
Canalicular adenoma	11	*2.2*	*2.9*	5	*1.0*	*6*	*1.2*
Cystadenoma	3	*0.6*	*0.8*	0	*0.0*	*3*	*0.6*
Myoepithelioma	3	*0.6*	*0.8*	0	*0.0*	*3*	*0.6*
Basal cell adenoma	1	*0.2*	*0.2*	0	*0.0*	*1*	*0.2*
Oncocytoma	1	*0.2*	*0.2*	1	*0.2*	*0*	*0.0*
Total	369	*74.7*	*100*	156	*31.5*	*213*	*43.1*
Malignant tumors							
Mucoepidermoid Carcinoma	39	*7.9*	*31.4*	16	*3.2*	*23*	*4.6*
Adenocarcinoma, NOS	33	*6.6*	*26.6*	23	*4.6*	*10*	*2.0*
Adenoid Cystic Carcinoma	22	*4.4*	*17.7*	11	*2.2*	*11*	*2.2*
PLGA	14	*2.8*	*11.2*	5	*1.0*	*9*	*1.8*
CExAP	8	*1.6*	*6.4*	5	*1.0*	*3*	*0.6*
Acinic Cells Carcinoma	5	*1.0*	*4.0*	3	*0.6*	*2*	*0.4*
EMC	2	*0.4*	*1.6*	1	*0.2*	*1*	*0.2*
Myoepithelial carcinoma	1	*0.2*	*0.8*	0	*0.0*	*1*	*0.2*
Total	124	*25.1*	*100*	64	*13.1*	*60*	*12.1*

PLGA: Polymorphous Low-Grade Adenocarcinoma; NOS: Not otherwise specified; EMC: Epithelial-Myoepithelial Carcinoma; CExAP: Carcinoma Ex-Adenoma Pleomorphic.

Table 2. Distribution of 493 benign and malignant salivary gland tumors according to the age.

	0-10		11-20		21-30		31-40		41-50		51-60		61-70		71-80		81-90		NS	
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Benign tumors																				
Pleomorphic adenoma	3	*0.6*	20	*4.0*	49	*9.9*	74	*15.0*	55	*11.1*	47	*9.5*	40	*8.1*	15	*3.0*	5	*1.0*	6	*1.2*
Warthin tumor	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	6	*1.2*	9	*1.8*	13	*2.6*	5	*1.0*	1	*0.2*	1	*0.2*
Canalicular adenoma	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	3	*0.4*	0	*0.0*	7	*1.4*	0	*0.0*	0	*0.0*
Basal cell adenoma	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.4*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Cystadenoma	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	1	*0.2*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*
Myoepithelioma	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	1	*0.2*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*
Oncocytoma	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*
Sub-total	3	*0.6*	20	*4.0*	50	*10.1*	75	*15.2*	64	*12.9*	61	*12.3*	55	*11.1*	28	*5.6*	6	*1.2*	7	*1.4*
Malignant tumors																				
Mucoepidermoid Carcinoma	0	*0.0*	2	*0.4*	4	*0.8*	7	*1.4*	11	*2.2*	3	*0.6*	6	*1.2*	6	*1.2*	0	*0.0*	0	*0.0*
Adenocarcinoma, NOS	0	*0.0*	0	*0.0*	1	*0.2*	3	*0.6*	2	*0.4*	9	*1.8*	7	*1.4*	5	*1.0*	3	*0.6*	3	*0.6*
Adenoid Cystic Carcinoma	0	*0.0*	0	*0.0*	0	*0.0*	6	*1.2*	2	*0.4*	2	*0.4*	7	*1.4*	3	*0.6*	1	*0.2*	1	*0.2*
PLGA	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	3	*0.6*	2	*0.4*	6	*1.2*	2	*0.4*	0	*0.0*	0	*0.0*
CExAP	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	2	*0.4*	1	*0.2*	5	*1.0*	0	*0.0*	0	*0.0*	0	*0.0*
Acinic Cells Carcinoma	0	*0.0*	0	*0.0*	0	*0.0*	3	*0.6*	1	*0.2*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
EMC	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*
Myoepithelial carcinoma	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Sub-total	0	*0.0*	2	*0.4*	5	*1.0*	22	*4.4*	21	*4.2*	18	*3.6*	32	*6.4*	16	*3.2*	4	*0.8*	4	*0.8*
Total	3	*0.6*	22	*4.4*	55	*11.1*	97	*19.6*	85	*17.2*	79	*16.0*	87	*17.6*	44	*8.9*	10	*2.0*	11	*2.2*

PLGA: Polymorphous Low-Grade Adenocarcinoma; CExPA: Carcinoma Ex-Pleomorphic Adenoma; EMC: Epithelial-Myoepithelial Carcinoma

Table 3. Distribution of the 493 salivary gland tumors according to the location (major and minor salivary glands).

	Major Salivary Glands						Minor Salivary Glands										NS	
	Parotid		Submandibular		Sublingual		Palate		Lips		Cheek mucosa		Tongue		Floor of the mouth			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Benign tumors																		
Pleomorphic adenoma	153	*31.0*	24	*4.8*	0	*0.0*	54	*10.9*	20	*4.0*	7	*1.4*	0	*0.0*	0	*0.0*	56	*11.3*
Warthin tumor	29	*5.8*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	6	*1.2*
Canalicular adenoma	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	10	*1.8*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Cystadenoma	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	1	*0.2*
Myoepithelioma	0	*0.0*	0	*0.0*	0	*0.0*	3	*0.6*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Basal cell adenoma	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Oncocytoma	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*
Sub-total	184	*37.3*	25	*5.0*	0	*0.0*	59	*11.9*	30	*6.0*	7	*1.4*	0	*0.0*	1	*0.0*	63	*12.7*
Malignant tumors																		
Mucoepidermoid	3	*0.6*	0	*0.0*	0	*0.0*	21	*4.2*	1	*0.2*	2	*0.4*	2	*0.4*	1	*0.2*	9	*1.8*
Carcinoma																		
Adenocarcinoma, NOS	17	*3.4*	3	*0.6*	0	*0.0*	2	*0.4*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	10	*2.0*
Adenoid Cystic	2	*0.4*	4	*0.8*	0	*0.0*	4	*0.8*	1	*0.2*	3	*0.6*	2	*0.4*	0	*0.0*	6	*1.2*
Carcinoma																		
PLGA	0	*0.0*	0	*0.0*	0	*0.0*	7	*1.4*	4	*0.8*	2	*0.4*	0	*0.0*	0	*0.0*	1	*0.2*
CExAP	1	*0.2*	2	*0.4*	0	*0.0*	1	*0.2*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*	3	*0.6*
Acinic Cells Carcinoma	2	*0.4*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	1	*0.2*	0	*0.0*	0	*0.0*	1	*0.2*
EMC	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*	0	*0.0*	1	*0.2*	0	*0.0*	0	*0.0*	0	*0.0*
Myoepithelial carcinoma	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	0	*0.0*	1	*0.2*
Sub-total	25	*5.0*	9	*1.8*	0	*0.0*	36	*7.3*	8	*1.6*	10	*2.0*	4	*0.8*	1	*0.0*	31	*6.2*
Total	209	*42.3*	34	*6.8*	0	*0.0*	95	*19.2*	38	*7.7*	17	*3.4*	4	*0.8*	2	*0.4*	94	*19.0*

PLGA: Polymorphous Low-Grade Adenocarcinoma; CExPA: Carcinoma Ex-Pleomorphic Adenoma; EMC: Epithelial-Myoepithelial Carcinoma

Table 4. Histological and gender distribution of 161 salivary gland tumors diagnosed at the Piracicaba Dental School, São Paulo State, Brazil.

	n = 161	%	% in the group (Benign or malignant)	Gender			
				Male		Female	
				n	%	n	%
Benign tumors							
Pleomorphic adenoma	73	*45.3*	*82.9*	33	*20.5*	40	*24.8*
Canalicular adenoma	9	*5.5*	*10.2*	4	*2.5*	5	*3.1*
Cistadenoma	3	*1.8*	*3.4*	0	*0.0*	3	*1.9*
Myoepithelioma	3	*1.8*	*3.4*	0	*0.0*	3	*1.9*
Total	88	*54.6*	*100*	37	*23.0*	51	*31.7*
Malignant tumors							
Mucoepidermoid Carcinoma	35	*21.7*	*47.9*	15	*9.3*	20	*12.4*
PLGA	14	*8.6*	*19.1*	5	*3.1*	9	*5.6*
Adenoid Cystic Carcinoma	12	*7.4*	*16.4*	5	*3.1*	7	*4.3*
Adenocarcinoma, NOS	6	*3.7*	*8.2*	2	*1.2*	4	*2.5*
Acinic Cells Carcinoma	2	*1.2*	*2.7*	1	*0.6*	1	*0.6*
EMC	2	*1.2*	*2.7*	1	*0.6*	1	*0.6*
CExAP	2	*1.2*	*2.7*	2	*1.2*	0	*0.0*
Total	73	*45.3*	*100*	31	*19.2*	42	*26.0*

PLGA: Polymorphous Low-Grade Adenocarcinoma; NOS: Not otherwise specified; EMC: Epithelial-Myoepithelial Carcinoma; CExAP: Carcinoma Ex-Adenoma Pleomorphic.

Table 5. Histological and gender distribution of the 332 salivary gland tumors diagnosed at the Private Surgical Pathology Center *(Anatom Laboratory)*, Cascavel - Paraná State, Brazil.

	n = 332	**%**	***% in the group (Benign or malignant)***	**Gender**			
				Male		**Female**	
				n	**%**	**n**	**%**
Benign tumors							
Pleomorphic adenoma	241	*72.5*	*85.7*	91	*27.4*	150	*45.1*
Warthin tumor	36	*10.8*	*12.8*	27	*8.1*	9	*2.7*
Canalicular adenoma	2	*0.6*	*0.7*	0	*0.0*	2	*0.6*
Basal cell adenoma	1	*0.3*	*0.3*	0	*0.0*	1	*0.3*
Oncocitoma	1	*0.3*	*0.3*	1	*0.3*	0	*0.0*
Total	281	*84.6*	*100.0*	119	*35.7*	162	*48.7*
Malignant tumors							
Adenocarcinoma, NOS	27	*8.1*	*52.9*	21	*6.3*	6	*1.8*
Adenoid Cystic Carcinoma	10	*3.0*	*19.6*	6	*1.8*	4	*1.2*
CExAP	6	*1.8*	*11.7*	3	*0.9*	3	*0.9*
Mucoepidermoid Carcinoma	4	*1.2*	*7.8*	1	*0.3*	3	*0.9*
Acinic Cells Carcinoma	3	*0.9*	*5.8*	2	*0.6*	1	*0.3*
Myoepithelial carcinoma	1	*0.3*	*1.9*	0	*0.0*	1	*0.3*
Total	51	*15.3*	*100.0*	33	*9.9*	18	*5.4*

NOS: Not otherwise specified; CExAP: Carcinoma Ex-Adenoma Pleomorphic.

Table 6. Incidence of major and minor salivary gland tumors in different continents.

Authors	Country	*n*	Benign Tumors				Malignant tumors						
			Pleomorphic Adenoma	Warthin Tumor	Canalicular Adenoma	Basal cell Adenoma	MEC	ACC	PLGA	AcCC	CExPA	Adenoc. NOS	EMC
Present study., 2012	Brazil	493	*63.6*	*7.3*	*2.2*	*0.2*	*7.9*	*4.4*	*2.8*	*1.0*	*1.6*	*6.6*	*0.4*
Velázquez et al., 2011	Mexico	360	*65.5*	*5.5*	*0.2*	*2.2*	*4.7*	*5.0*	*0.5*	*2.5*	*1.9*	*0.0*	*0.0*
Luksic et al., 2011	Croatia	779	*47.2*	*11.1*	*0.0*	*0.7*	*8.6*	*12.0*	*0.5*	*3.0*	*4.2*	*2.1*	*0.0*
Shishegar et al., 2011	Iran	392	*58.0*	*6.0*	*0.0*	*0.7*	*8.0*	*13.0*	*0.6*	*1.5*	*0.6*	*1.0*	*1.0*
Tian et al., 2010	China	6982	*46.9*	*13.8*	*0.04*	*3.7*	*9.6*	*9.8*	*0.4*	*2.5*	*2.6*	*1.9*	*0.5*
Kara et al., 2010	Turkey	125	*64.0*	*4.8*	*0.0*	*0.8*	*2.4*	*8.8*	*0.8*	*0.8*	*1.6*	*4.0*	*0.0*
Tilakaratne et al., 2009	Sri Lanka	713	*38.4*	*4.1*	*0.3*	*1.4*	*21.6*	*13.5*	*4.2*	*2.5*	*3.2*	*3.6*	*0.6*
Ochicha et al., 2009	Nigeria	78	*48.7*	*0.0*	*0.0*	*2.0*	*23.1*	*3.8*	*5.1*	*1.3*	*0.0*	*0.0*	*0.0*
Oliveira et al., 2009	Brazil	599	*67.8*	*6.3*	*0.0*	*0.0*	*5.0*	*6.5*	*0.0*	*0.0*	*0.0*	*6.2*	*0.0*
Long-jiang et a., 2008	China	3461	*51.3*	*4.4*	*0.4*	*1.7*	*7.5*	*7.3*	*1.3*	*2.4*	*4.2*	*6.6*	*0.4*
Subhashraj, 2008	India	684	*53.1*	*3.1*	*0.4*	*1.6*	*7.2*	*9.6*	*0.0*	*2.8*	*3.5*	*4.5*	*0.4*
Jones et al., 2008	UK	741	*44.4*	*4.6*	*4.7*	*5.0*	*11.5*	*8.4*	*3.8*	*2.6*	*3.2*	*1.8*	*0.8*
Al-Khateb et al., 2007	Jordan	102	*54.0*	*4.0*	*0.0*	*0.0*	*8.0*	*13.0*	*0.0*	*4.0*	*1.0*	*1.0*	*0.0*
Ansari, 2007	Iran	130	*65.4*	*0.0*	*1.8†*		*11.5*	*2.3*	*0.0*	*0.0*	*0.7*	*4.6*	*0.0*
Ito et al., 2005	Brazil	496	*54.2*	*8.5*	*0.4*	*0.6*	*13.5*	*7.9*	*1.8*	*1.8*	*0.6*	*1.4*	*1.0*
Lima et al., 2005	Brazil	245	*68.5*	*6.9*	*0.8*	*0.0*	*4.4*	*5.3*	*0.0*	*4.9*	*3.6*	*3.6*	*0.0*
Otoh et al., 2005	Nigeria	79	*44.3*	*2.5*	*3.8†*		*10.1*	*8.9*	*0.0*	*2.5*	*5.1*	*2.5*	*0.0*
Vuhahula, 2004	Uganda	268	*39.9*	*0.0*	*0.0*	*3.4*	*9.3*	*13.4*	*3.7*	*6.0*	*2.2*	*3.7*	*2.2*
Vargas et al., 2002	Brazil	124	*59.0*	*10.5*	*0.0*	*0.8*	*10.5*	*4.0*	*0.0*	*0.8*	*2.4*	*0.8*	*0.8*
Satko et al., 2000	Slovakia	1021	*53.9*	*9.7*	*0.0*	*2.5*	*5.2*	*6.4*	*0.0*	*3.9*	*0.8*	*3.5*	*0.0*
Renehan et al., 1999	UK	1194	*65.0*	*13.0*	*0.0*	*1.0*	*3.0*	*6.0*	*0.0*	*2.0*	*2.0*	*3.0**	*0.08*
Saku et al., 1997	Japan	120	*53.3*	*13.3*	*0.8*	*2.5*	*10.0*	*9.1*	*0.8*	*0.0*	*4.1*	*2.5*	*0.0*
Chidzonga et al., 1995	Zimbabwe	282	*73.0*	*7.0*	*0.0*	*0.0*	*4.2*	*8.9*	*0.0*	*0.0*	*0.4*	*0.0*	*0.0*

MEC: Mucoepidermoid carcinoma; ACC: Adenoid cystic carcinoma; PLGA: Polymorphous low-grade adenocarcinoma; AcCC: Acinic cell carcinoma; CExPA: Carcinoma ex-pleomorphic adenoma; Adenoc. NOS: Adenocarcinoma not otherwise specified; EMC: Epithelial-myoepithelial carcinoma; * Included basal cell adenocarcinoma, papillary cystadenocarcinoma, mucinous adenocarcinoma and adenocarcinoma, not otherwise specified. † Basal cell adenoma and canalicular adenoma were evaluated as monomorphic adenoma in these studies.

Table 7. Incidence of intra-oral minor salivary gland tumors in different continents.

Authors	Country	*n*	Benign Tumors				Malignant tumors						
			Pleomorphic Adenoma	Warthin Tumor	Canalicular Adenoma	Basal cell Adenoma	MEC	ACC	PLGA	AcCC	CExPA	Adenoc NOS	EMC
Present study., 2012	Brazil	156	*51.9*	*0.0*	*7.0*	*0.0*	*17.3*	*6.4*	*8.4*	*1.2*	*1.2*	*1.9*	*1.2*
Vani et al. 2011	India	185	*22.1*	*0.0*	*0.0*	*1.0*	*34.0*	*14.5*	*9.7*	*0.0*	*0.5*	*7.5*	*0.0*
Dhanuthai et al., 2009	Thailand	311	*42.7*	*0.0*	*0.3*	*2.8*	*22.8*	*18.3*	*0.6*	*0.0*	*0.6*	*9.3*	*0.3*
Buchner et al., 2007	USA	380	*39.2*	*0.0*	*6.1*	*1.6*	*21.8*	*6.3*	*7.1*	*1.6*	*0.5*	*2.1*	*0.0*
Pires et al., 2007	USA	546	*33.2*	*0.0*	*9.2*	*0.0*	*22.9*	*6.4*	*5.1*	*3.8*	*0.4*	*3.8*	*0.4*
Wang et al., 2007	China	737	*37.3*	*0.1*	*0.0*	*0.5*	*12.4*	*19.4*	*4.6*	*0.9*	*3.0*	*5.6*	*1.4*
Jaber et al., 2006	Libya	75	*30.6*	*0.0*	*1.3*	*2.6*	*25.3*	*17.3*	*4.0*	*0.0*	*2.6*	*10.6*	*0.0*
Toida et al., 2005	Japan	82	*65.8*	*0.0*	*0.0*	*0.0*	*9.7*	*12.1*	*0.0*	*3.6*	*2.4*	*2.4*	*0.0*
Yih et al., 2005	USA	213	*43.6*	*0.0*	*11.7*	*0.0*	*21.1*	*10.3*	*8.4*	*0.5*	*0.9*	*1.9*	*0.0*
Poomsawat et a., 2004	Thailand	60	*30.0*	*0.0*	*0.0*	*0.0*	*43.3*	*15.0*	*1.7*	*0.0*	*5.0*	*1.7*	*0.0*
Jansisyanont et al., 2002	USA	80	*21.3*	*0.0*	*1.2*	*1.2*	*41.3*	*8.8*	*11.3*	*3.8*	*0.0*	*3.8*	*0.0*
Lopes et al., 1999	Brazil	196	*33.1*	*0.0*	*0.0*	*1.5*	*38.7*	*17.3*	*1.5*	*0.5*	*0.0*	*4.5*	*0.0*
Bastidas et al., 1996	Venezuela	62	*38.7*	*0.0*	*0.0*	*3.2*	*29.0*	*9.7*	*0.0*	*0.0*	*0.0*	*3.2*	*0.0*
Loyola et al., 1995	Brazil	164	*53.0*	*0.0*	*0.0*	*1.0*	*17.0*	*13.0*	*2.0*	*4.0*	*0.0*	*1.0*	*1.0*
Van Heerden et al., 1991	S. Africa	70	*48.5*	*0.0*	*0.0*	*0.0*	*15.2*	*12.8*	*15.7*	*0.0*	*7.1*	*1.4*	*1.4*
Waldron et al., 1988	USA	426	*40.8*	*0.0*	*10.7†*		*15.2*	*9.3*	*11.0**	*3.5*	*1.4*	*0.0*	*0.0*

MEC: Mucoepidermoid carcinoma; ACC: Adenoid cystic carcinoma; PLGA: Polymorphous low-grade adenocarcinoma; AcCC: Acinic cell carcinoma; CExPA: Carcinoma ex-pleomorphic adenoma; Adenoc. NOS: Adenocarcinoma not otherwise specified; EMC: Epithelial-myoepithelial carcinoma; * The authors included the so-called lobular, polymorphous, terminal duct, trabecular carcinomas; † Basal cell adenoma and canalicular adenoma were evaluated as monomorphic adenoma in this study.

CAPÍTULO 2

Artigo submetido para publicação no periódico *Medicina Oral Patología Oral Cirurgia Bucal.*

TISSUE MICROARRAY CONSTRUCTION FOR SALIVARY GLAND TUMORS STUDY

TMA IN SALIVARY GLAND TUMORS

Felipe Paiva Fonseca – **DDS**[1], Oslei Paes de Almeida – **DDS, PhD**[1], Ana Lúcia Carrinho Ayroza Rangel – **DDS, PhD**[2] and Pablo Agustin Vargas – **DDS, PhD**[1]

01. Piracicaba Dental School, State University of Campinas – São Paulo –Brazil.

02. Department of Pathology, State University of Western Paraná – Paraná – Brazil.

Corresponding Author:

Prof. Dr. Pablo Agustin Vargas

Piracicaba Dental School – State University of Campinas (UNICAMP)

Department of Oral Diagnosis - Oral Pathology Division

Av. Limeira, 901 ZipCode: 13414-903 Piracicaba - São Paulo State - Brazil

Tel.: +55 19 32015315; E-mail: pavargas@fop.unicamp.br

Summary

Objective: To describe and discuss the design, building and usefulness of tissue microarray (TMA) blocks for the study of salivary gland tumors (SGTs). **Study Design:** 238 formalin-fixed, paraffin-embedded SGTs were arranged in blocks of TMA using a manual tissue arrayer. Three representative cores of 1.0, 2.0 or 3.0mm were taken from each original block and their

characteristics were analyzed and described. **Results:** It was created 12 TMA blocks that presented highly representative neoplastic cylinders. However, those neoplasias rich in cystic spaces such as mucoepidermoid carcinoma and Warthin tumor presented more difficulties to be sampled, as the neoplastic tissue available was scarce. Tissue damage and loss during TMA construction was estimated as 3.7%. **Conclusion:** Representative areas of SGTs, with relatively small loss of tissue, can be obtained with the construction of TMA blocks for molecular studies. However, tumors rich in cystic spaces present more difficulties to be adequately sampled.

Key-words: Tissue microarray; TMA; Salivary gland tumors; Immunohistochemistry.

Introduction

Salivary gland tumors (SGT) are uncommon neoplasms that account for 6% of all head and neck neoplasias and 0.3% of all cancers. The incidence of SGT per 100.000 persons is reportedly as 0.9 in women and 1.5 in men, with varied biological behavior and clinical outcome(1,2). The development and progression of SGTs, like other neoplasias result from multiple genetic alterations and molecular studies may help to understand the mechanisms involved in their tumorigenesis.

The development of cDNA microarray and proteomic techniques, allowed the analysis of thousands of genes or proteins in one single experiment, facilitating the identification of molecules with potential clinical applications(3,4). In line with these new approaches, in 1998 Kononen *et al*(5) promulgated the idea of translating the convenience of DNA microarrays to tissues. This new methodology allowed simultaneous screening of dozens of tumor specimens at once, being popularly called as tissue microarray (TMA)(6).

TMA technology has the potential to significantly accelerate *in situ* studies of tissue specimens, to explore associations between molecular changes and clinic-pathological informations and to ensure preservation of unique and precious research materials. Briefly, tiny tissue cylinders

are acquired from hundreds of different primary tumor blocks and arranged in a matrix configuration within a recipient paraffin block. Sections from such TMA blocks can then be used for simultaneous *in situ* analyses of up to 1000 tissue specimens either at the DNA, RNA or protein levels; providing maximal use of limited tissue resources(3,6,7,8).

The use of this high-throughput technique significantly facilitates the identification of new molecular markers that could predict the clinical behavior of tumors, helping to better understand their pathogenesis and biological characteristics. However, very few studies have applied this technology in the evaluation of SGTs, and their known morphological heterogeneity could theoretically affect the validity of the results obtained. Therefore, the objective of this article is to present and discuss the design and building of TMA blocks of 238 SGTs, considering its relevant technical points.

Material and methods

From January 2001 to December 2011, 493 cases of SGTs were retrieved from the archives of the Oral Pathology Department of the Piracicaba Dental School (161 cases) and from a Surgical Pathology laboratory of the Brazilian Southern state of Paraná (332 cases). Histological preparations stained with H&E were reviewed by three oral pathologists and, when necessary, new cuts were performed and stained with periodic acid-Schiff and mucicarmine. All cases were classified according to the 2005 WHO's Histological Typing of Salivary Gland Tumors(9). Those primary SGTs that affected major or minor salivary glands were included in the study. Cases without enough tissue available for TMA construction were excluded from the study. After this selection, 238 formalin-fixed, paraffin-embedded primary SGTs remained available for being arrayed.

Representative tumor areas were selected and marked on H&E-stained sections using an objective marker (1.8 mm; Nikon Corporation, Tokyo, Japan). The slide was then overlaid on the original paraffin block to determine the corresponding area to be used. TMA were constructed using

a manual tissue arrayer (Sakura Co.; Japan). Three representative cylindrical cores of 1.0, 2.0 or 3.0 mm diameter were taken from each original tissue block and then arrayed sequentially into a recipient ready-to-use paraffin block (Sakura Co.; Japan). Two cores of normal parotid gland tissue and one of oral squamous cell carcinoma were inserted into the left upper corner of each recipient block as controls for future immunohistochemical reactions and for orientation when examining the slides. A map specifying the exact position of each case was made, to facilitate the interpretation of the histological and immunohistochemical results.

The current study has been approved by the Ethical Committee of the Piracicaba Dental School – State University of Campinas (Protocol 141/2011).

Results

Among the 238 SGTs used in the construction of the TMA blocks, there were 200 benign and 38 malignant tumors **(Table 1)**. 72.7% of the cases involved major salivary glands (173 cases), whereas 27.3% affected intra-oral minor glands (65 cases). It was built 12 TMA blocks; from these, it was created 8 blocks of pleomorphic adenoma (6 of 2.0mm cores, 1 of 1.0mm cores and 1 of 3.0mm cores), 1 block of Warthin tumor (2.0mm cores) and 1 block containing pleomorphic adenoma, Warthin tumor and canalicular adenoma (2.0mm cores). The last 2 blocks were composed by 2.0mm cores of malignant tumors that included mucoepidermoid carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, polymorphous low-grade adenocarcinoma, epithelial-myoepithelial carcinoma, myoepithelial carcinoma, carcinoma ex-pleomorphic adenoma and adenocarcinoma not otherwise specified **(Fig. 1)**.

Most of the time and efforts to construct the blocks were actually spent in the search, organization and pathological review of the tissue specimens to be included in the arrays, whereas the TMA building itself usually took from 1 to 2 hours for each block depending on the diameter of the cylinders used.

As expected, the use of larger needles caused more damage to the original tissue blocks. Hence, while those blocks that provided 1.0mm cores could be used in other projects, most of those

specimens that provided 3.0mm cores could not be used again. In addition, larger needles substantially reduced the number of specimens that could be arrayed. TMA blocks composed by 2.0mm cylinders allowed as many as 60 specimens to be arrayed into a ready-to-use recipient block of approximately 45x20mm, what corresponded to 19 different cases plus controls. Those composed by 1.0mm cylinders allowed 120 specimens, representing 39 cases; whereas those TMAs composed by 3.0mm cylinders allowed only 30 specimens or 9 different cases, always using triplicate arrangement.

In approximately 5% of the cases there was a slight difference between the area selected in the H&E-stained slide to be inserted in the TMA block and the one that in fact was inserted. It was considered that the rate of tissue loss attributable to tissue damage during TMA construction was about 3.7%.

Most of the tumors were PA, and using three cores, highly representative areas of the tumor were obtained using either 1.0, 2.0 or 3.0 mm punches **(Fig. 2)**. In tumors with a more homogenous morphological pattern, as canalicular adenoma and myoepithelial carcinoma, the representativity of the TMA was even higher. However, tumors rich in cystic spaces as low-grade mucoepidermoid carcinoma and Warthin tumor the tissue samples were not considered adequate, since only few neoplastic tissue was available in the TMA blocks **(Fig. 3)**.

Discussion

Tissue microarrays (TMAs) were developed by Kononen *et al.* (1998)(5) and are now widely accepted as a fast and cost-effective tool that facilitates the analysis of molecular alterations in thousands of tissue specimens by acquiring cylindrical cores of formalin-fixed, paraffin-embedded tissue specimens and arraying them into a recipient block. These TMA blocks can be used for any *in situ* tissue analysis, including IHC, *in situ* hybridization, DNA ploidy, nuclear morphometry, and FISH(10).

During the last decade, numerous studies have validated this method in the investigative surgical pathology. TMA cores as small as 0.6mm have been confirmed to be adequate for analyzing breast cancer specimens by IHC for the expression of estrogen and progesterone receptors and the tyrosine kinase receptor HER-2(11,12). Similarly, TMA immunohistochemical staining for p53, cyclin D1, bcl-2, bax, Cox-2, β-catenin, c-myc, PTEN and p-Akt1enabled high-throughput analysis of genetic alterations that might contribute to human colon cancer development and progression(13). TMA validation has also been conducted in endometrial cancer(14), esophageal squamous cell carcinoma(15), lung cancer(16), cervical adenocarcinoma(17), ovarian carcinoma(18) and in many other human neoplasias.

The use of TMA for molecular studies of SGTs is scarce **(Table 2)**(1, 19-26). Iwafuchi *et al.* (2004)(19) first used TMA for analyzing molecular features of these tumors. By evaluating the expression of a large panel of proteins in different salivary gland neoplasias, these authors concluded that SGTs may be well characterized using markers only toward myoepithelial, luminal and basal cells. Also by IHC and TMA, Mcm-2 has been proved to be a proliferative sensitive marker for SGTs and PLUNC proteins have been suggested to be useful diagnostic tools for mucoepidermoid carcinoma, whereas geminin has been strongly associated with reduced overall and relapse-free survival rates in patients affected by salivary gland carcinomas(1,20,21). Freier *et al.* (2005)(22) evaluated KIT expression in a large sample of histologically defined subgroups of adenoid cystic carcinoma, observing a stronger expression in cribriform and tubular subtypes when compared to the solid variant. Similarly, Freier *et al.* (2005)(23) analyzed the prevalence of chromosome 22q13 copy number gains in 70 ACC and found that it represents a decisive molecular event in early stages of ACC, irrespective of histologic differentiation. However, despite these interesting results previously described, there are no reports describing and evaluating the most important technical points to the construction of TMA blocks for studying SGTs.

TMAs present various relevant advantages for molecular studies of paraffin embedded tissues. It permits the concomitant use of a large number of cases and significantly reduces the

experimental handling time(3). Moreover, as the reactions are done in one single slide, the reagent concentrations, incubation times, temperature, wash conditions, and antigen retrieval are the same for all specimens. The necessary reagent volume is significantly reduced, making it a very cost-effective method(6,27,28). In our study, using ready-to-use recipient TMA blocks, it was possible to resume our whole 238 neoplastic samples to only 12 blocks, facilitating the evaluation of molecular markers in this large number of cases. In addition, the use of TMAs preserves precious and finite tissue resources and maximizes the number of experiments that can be performed with the material present in one paraffin block(6).

Whereas large tissue sections are used for histological diagnosis, TMA has been reserved for research purposes. However, tumor heterogeneity has traditionally been recognized as a potential problem for those using TMAs, and the most used 0.6mm cores have been perceived as too small and potentially not representative of the entire specimen. Taking multiple samples of each tumor seems to be the most direct way of combating the potential lack of representativeness in a certain tissue(7). Recent reports achieved 95% accuracy with only two cores, whereas most studies indicate that triplicate TMA cores have up to 98% concordance when compared with the results of full sections(3,17). Other alternative would be the use of larger punch needles; however, the increase in the number of cores and their diameters leads to a considerable damaged donor block and fewer samples arrayed(7). Evaluating the histological features present in the cores obtained in the present study, it could be noted that triplicate cores of 1.0, 2.0 and 3.0mm were all well representative of the original tissue, although 3.0mm cores evidently offered more neoplastic cells and structures to be evaluated than 1.0 and 2.0mm cores. However, only 9 cases could be inserted in a TMA recipient block when 3mm cylinders were used, whereas up to 39 could be arrayed when 1mm were taken; moreover, smaller diameters better preserved the donor blocks for future studies.

Improper selection of representative tumor areas on the H&E original slide by the pathologist, or incorrect punching of these representative areas can cause tissue cores that contain inadequate areas to be studied(6). In the current study, it was noted that in a small percentage of

cases, the neoplastic area inserted in the recipient block was not the exact area selected in the H&E slide, what may be attributable to differences in the tissue contraction in the original paraffin donor block and due to technical limitations. Moreover, it was seen that highly cystic tumors presented small areas of neoplastic cells to be studied, suggesting that TMA would be more indicated for solid tumors.

Finally, due to the small size of the cylinders and the high number of samples, TMA cores are much more prone to be lost during sectioning than full sections. The total number of lost cores due to technical reasons has been estimated to vary from 4 to 23%(15,17,29). In the building process of the TMA blocks in the present study, 3.7% of the cores were lost; however, this rate would probably increase if the samples were submitted to IHC procedures.

In conclusion, tissue microarray is a high-throughput, cost-effective and tissue-saving technique in molecular analysis of formalin-fixed, paraffin-embedded neoplasias, helping to overcome the ordinary time-consuming work. The present study showed the usefulness of this technique in the construction of SGTs TMA blocks, revealing that solid tumors is more indicated to be micro-arrayed than their cystic counterparts. These TMA blocks will now be used for immunohistochemical studies to better evaluate SGT's molecular features.

Acknowledgement

The authors state that they have no potential conflict of interest that could bias the results obtained in the current study and that this study has been supported by grants of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-Brasil) and from the São Paulo Research Foundation (FAPESP 2009/53839-2).

References

1. Yamazaki M, Fujii S, Murata Y, Hayashi R, Ochiai A. High expression level of geminin predicts a poor clinical outcome in salivary gland carcinomas. Histopathol 2010; 56: 883–892.

2. Vargas PA, Gerhard R, Araújo Filho VJF, Castro IV. Salivary gland tumors in a Brazilian Population: A retrospective study of 124 cases. Rev Hosp Clín Fac Med S Paulo 2002; 57(6): 271-276.
3. Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Köchli OR, et al. Tissue microarray for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001; 159(6): 2249-2256.
4. Simon R, Sauter G. Tissue microarrays for miniaturized high-throughput molecular profiling of tumors. Experiment Hematol 2002; 30: 1365–1372.
5. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4(7): 844-847.
6. Radhakrishnan R, Solomon M, Satyamoorthy K, Martin LE, Lingen MW. Tissue microarray – a high-throughput molecular analysis in head and neck cancer. J Oral Pathol Med 2008; 37: 166-176.
7. Nocito A, Kononen J, Kallioniemi O, Sauter AG. Tissue microarrays (TMAs) for high-throughput molecular pathology research. Int J Cancer 2001; 94: 1-5.
8. Voduc D, Kenney C, Nielsen TO. Tissue microarrays in clinical oncology. Semin Radiat Oncol 2008; 18: 89-97.
9. Barnes L, Eveson JW, Reichart PA, Sidranskiy D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. 1st Ed. Lyon, France: IARC Press; 2005.
10. Kallioniemi O, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high-throughput molecular profiling of cancer. Human Mol Gen 2001; 10(7): 657-662.
11. Henriksen KL, Rasmussen BB, Lykkesfeldt AE, Møller S, Ejlertsen B, Mouridsen HT. Semi-quantitative scoring of potentially predictive markers for endocrine treatment of breast cancer: a comparison between whole sections and tissue microarrays. J Clin Pathol 2007; 60: 397–404.

12. Alkushi A. Validation of tissue microarray biomarker expression of breast carcinomas in Saudi women. Hematol Oncol Stem Cell Ther 2009; 2(3): 394-398.
13. Chen WC, Lin MS, Zhang BF, Fang J, Zhou Q, Hu Y, et al. Survey of molecular profiling during human colon cancer development and progression by immunohistochemical staining on tissue microarray. World J Gastroenterol 2007; 13(5): 699-708.
14. Fons G, Hasibuan SM, van der Velden J, ten Kate FJW. Validation of tissue microarray technology in endometrioid cancer of the endometrium. J Clin Pathol 2007; 60: 500–503.
15. Boone J, van Hillegersberg R, van Diest PJ, Johan GOA, Rinkes IHMB, Ten Kate FJW. Validation of tissue microarray technology in squamous cell carcinoma of the esophagus. Virchows Arch 2008; 452: 507–514.
16. Leversha MA, Fielding P, Watson S, Gosney JR, Field JK. Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. J Pathol 2003; 200: 610–619.
17. El-Mansi MT, Williams ARW. Validation of tissue microarray technology using cervical adenocarcinoma and its precursors as a model system. Int J Gynecol Cancer 2006; 16: 1225–1233.
18. Rosen DG, Huang X, Deavers MT, Malpica A, Silva EG, Liu J. Validation of tissue microarray technology in ovarian carcinoma. Mod Pathol 2004; 17: 790-797.
19. Iwafuchi H, Mori N, Takahashi T, Yatabe Y. Phenotypic composition of salivary gland tumors: an application of principle component analysis to tissue microarray data. Mod Pathol 2004; 17: 803–810.
20. Vargas PA, Cheng Y, Barrett AW, Craig GT, Speight PM. Expression of Mcm-2, Ki-67 and geminin in benign and malignant salivary gland tumours. J Oral Pathol Med 2008; 37: 309-318.
21. Vargas PA, Speight PM, Bingle CD, Barrett AW, Bingle L. Expression of PLUNC family members in benign and malignant salivary gland tumours. Oral Dis 2008; 14: 613-619.

22. Freier K, Flechtenmacher C, Walch A, Devens F, Mühling J, Lichter P, et al. Differential KIT expression in histological subtypes of adenoid cystic carcinoma (ACC) of the salivary gland. Oral Oncol 2005; 41: 934-939.

23. Freier K, Flechtenmacher C, Walchd A, Ohl S, Devens F, Burke B, et al. Copy number gains on 22q13 in adenoid cystic carcinoma of the salivary gland revealed by comparative genomic hybridization and tissue microarray analysis. Cancer Gen Cytogen 2005; 159: 89–95.

24. Clauditz TS, Reiff M, Gravert L, Gnoss A, Tsourlakis MC, Munscher A, et al. Human epidermal growth factor receptor 2 (HER2) in salivary gland carcinomas. Pathol 2011; 43(5): 459-464.

25. Heiduschka G, Erovic BM, Pammer J, Kotowski U, Kaider A, Ch Grasl M, et al. Mcl-1 expression is up-regulated in malignancies of the parotid gland. Dis Markers 2011; 30(5): 229-233.

26. Williams MD, Roberts DB, Kies MS, Mao L, Weber RS, El-Naggar AK. Genetic and expression analysis of her-2 and egfr genes in salivary duct carcinoma: Empirical and therapeutic significance. Clin Cancer Res 2010; 16: 2266-2274.

27. Goldstine J, Seligson DB, Beizai P, Miyata H, Vinters HV. Tissue microarrays in the study of non-neoplastic disease of the nervous system. J Neuropathol Experiment Neurol 2002; 61(8): 653-662.

28. Parsons M, Grabsch H. How to make tissue microarrays. Diagn Histopathol 2009; 15(3): 142-150.

29. Hager M, Kolbitsch C, Tiefenthaler W, Haufe H, Kemmerling R, Moser PL. Tissue microarrays from renal cell tumors: exclusion criteria and rate of exclusion. Scand J Urol Nephrol 2007; 41: 485–9.

Figures Legends

Figure 1. Example of full tissue sections previously and after the acquisition of TMA cores of **A)** 1.0, **B)** 2.0 and **C)** 3.0 mm. It can be seen that sections that provided larger cylinders became significantly more damaged than those that provided smaller cylinders. Microscopic images acquired using the Aperio ScanScope SC scanner.

Figure 2. Highly representative areas of pleomorphic adenoma in tissue cores of 1.0, 2.0 and 3.0mm.

Figure 3. In cases rich in cystic spaces as low-grade mucoepidermoid carcinoma and Warthin tumor, only few neoplastic tissue are found on the TMA cores.

Table 1. Histopathological distribution of the 238 salivary gland tumors used for construction of 12 TMA blocks.

Tumors	*N*	%
Benign		
Pleomorphic adenoma	173	72.7
Warthin Tumor	24	10.0
Canalicular adenoma	3	1.3
Malignant		
Adenoid cystic carcnoma	10	4.2
Mucoepidermoid Carcinoma	10	4.2
Adenocarcinoma, Not Otherwise Specified	4	1.7
Carcinoma Ex-Pleomorphic Adenoma	4	1.7
Polimorphous Low-grade Adenocarcinoma	4	1.7
Acinic Cell Carcinoma	3	1.3
Epithelial-Myoepithelial Carcinoma	2	0.8
Myoepithelial Carcinoma	1	0.4
Total	238	100.0

Table 2. Studies previously published in the English literature using tissue microarray for molecular analysis of salivary gland tumors.

Authors	Country	Sample	Molecular markers	TMA Cores		Methods	Results
				Number	Size		
Heiduschka *et al.,* 2011(25)	Austria	108	Mcl-1	NS	NS	IHC	Parotid gland malignancies produce high levels of Mcl-1 protein.
Clauditz *et al.,* 2011(24)	Germany	1109	HER-2	NS	NS	FISH, IHQ	HER-2 overexpression was observed in about 20% of patients with salivary duct cancers.
Williams *et al.,* 2010(26)	USA	66	HER-2, EGFR, Chromosomes 7 and 17	NS	1.0mm	FISH, IHQ	HER-2 gene amplification and protein high expression, may be selected for targeted therapy.
Yamazaki *et al.,* 2010(1)	Japan	170	Geminin and Ki-67	02	2.0mm	IHQ	Geminin expression is a useful marker for predicting salivary gland carcinoma aggressiveness.
Vargas *et al.,* 2008(21)	UK	64	SPLUNC1, SPLUNC2 and LPLUNC1	03	1.0mm	IHC	Presence of an intense expression of two PLUNC proteins in mucous cells and mucin plugs of mucoepidermoid carcinoma and papillary cystadenocarcinoma.
Vargas *et al.,* 2008(20)	UK	62	Mcm-2, Ki-67 and Geminin	03	1.0mm	IHC	Mcm-2 may be a sensitive proliferation marker in SGTs and may be useful for differential diagnosis.
Freier *et al.,* 2005(22)	Germany	70	Chromosome 22q13	02	0.6mm	FISH	Copy number gain of 22q13 as a frequent finding in ACC irrespective of histological variant.
Freier *et al.,* 2005(23)	Germany	55 for IHC 49 for FISH	KIT	NS	0.6mm	FISH, IHC	Stronger expression in cribriform and tubular subtypes if compared to solid variant.
Iwafuchi *et al.,* 2004(19)	Japan	88	Caldesmon, αSMA, CD10, calponin, CD44v6, Ck7, Ck19, Ck8, p63, Ck14, 14-3-3σ and Maspin	04	0.6mm	IHC	SGT may be well characterized by using makers toward only three components, myoepithelial, luminal and basal cells.

Figure 1.

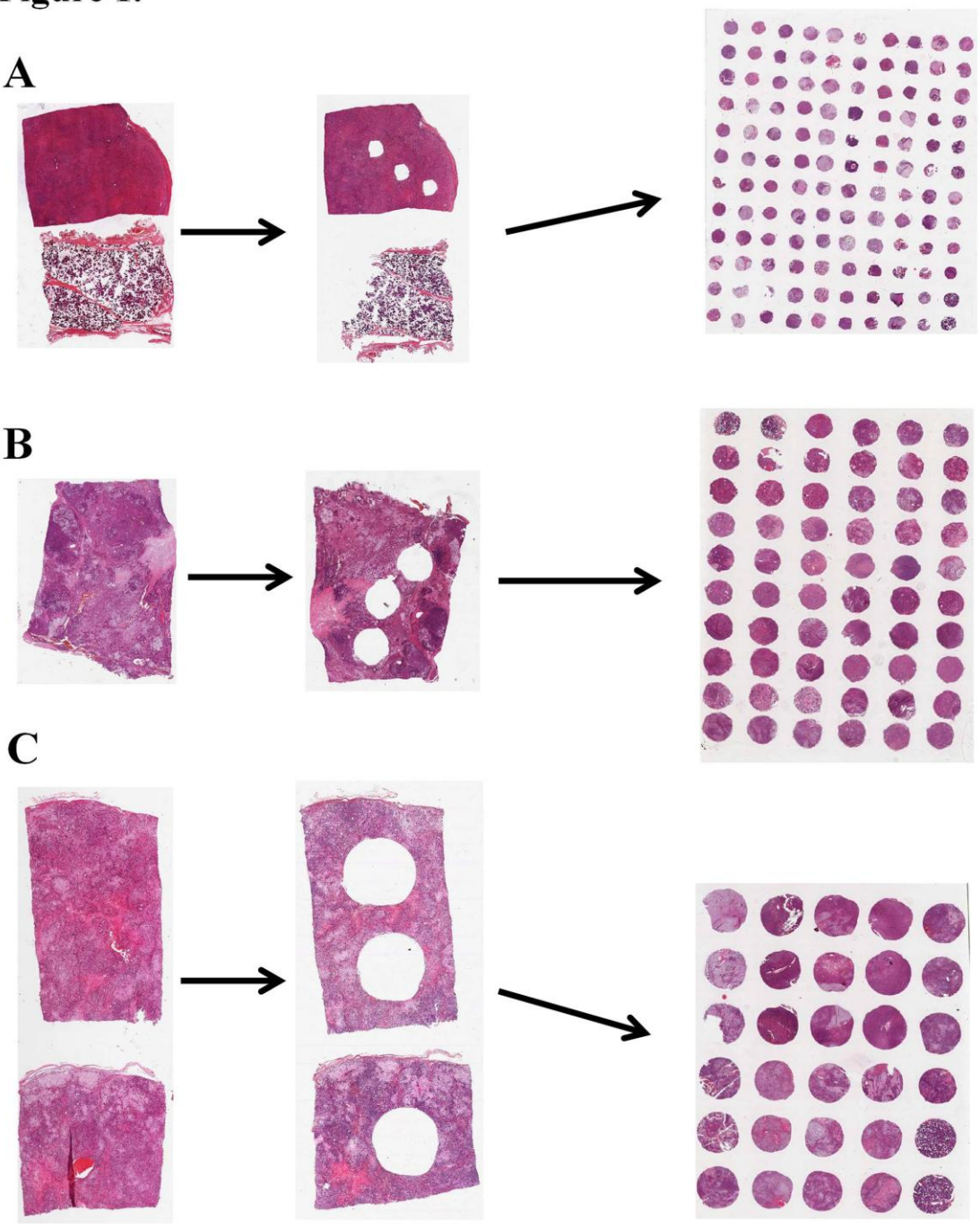


Figure 2

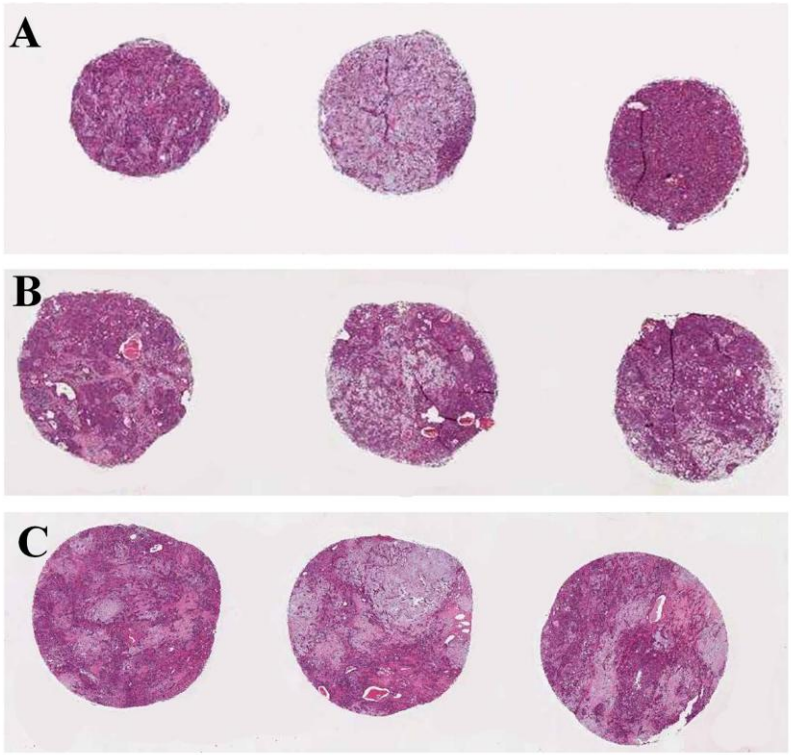
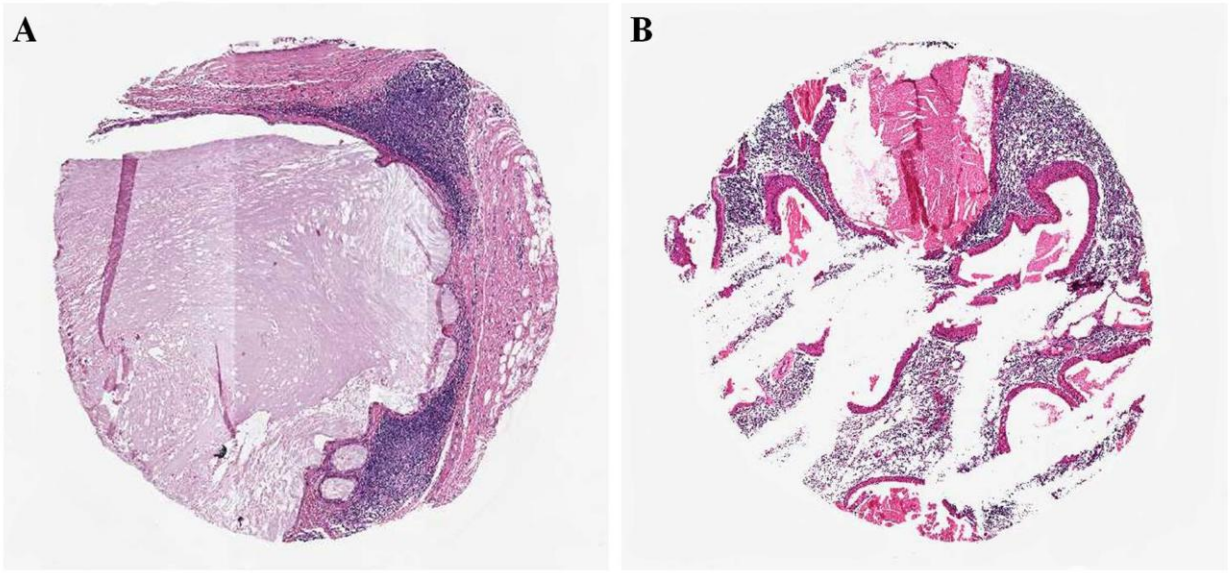


Figure 3.



4 CONCLUSÕES

1. A incidência e as características clínico-patológicas dos tumores de glândulas salivares da amostra estudada são compatíveis com os resultados previamente descritos na literatura, em especial com os estudos já desenvolvidos em outras populações brasileiras.
2. A técnica de *tissue microarray* representa uma metodologia de alto rendimento e de baixo custo, que apesar de diminuir o tempo necessário para a realização de reações imunoistoquímicas, exige grande dispensa de tempo nas suas primeiras etapas, devendo a construção dos blocos de TMA ser considerada um projeto por si só.
3. Utilizando-se 3 cilindros de 1,0, 2,0 ou 3,0mm de diâmetro é possível obter boa representatividade dos tumores de glândulas salivares.
4. Neoplasias sólidas são mais indicadas para serem arranjadas em blocos de TMA do que neoplasias císticas, haja vista a limitação de células neoplásicas disponíveis nestas últimas.

5 REFERÊNCIAS*

1. Ananthanarayanan V, Deaton RJ, Amatya A, Macias V, Luther E, Kajdacsy-Balla A, et al. Subcellular localization of p27 and prostate cancer recurrence: automated digital microscopy analysis of tissue microarrays. Human Pathol 2011; 42: 873-881.
2. Andrade VP, Cunha IW, Silva EM, Ayala F, Sato Y, Ferreira SS, et al. O arranjo em matriz de amostras teciduais (*tissue microarray*): larga escala e baixo custo ao alcance do patologista. J Bras Patol Med Lab 2007; 43(1): 55-60.
3. Barnes L, Eveson JW, Reichart PA, Sidranskiy D. World Health Organization Classification of Tumours. Pathology and Genetics of Head and Neck Tumours. 1st Ed. Lyon, France: IARC Press; 2005.
4. Bjørndal K, Krogdahl A, Therkildsen MH, Overgaard J, Johansen J, Kristensen CA, et al. Salivary gland carcinoma in Denmark 1990–2005: Outcome and prognostic factors. Results of the Danish Head and Neck Cancer Group (DAHANCA). Oral Oncol 2011 (in press).
5. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Breast Cancer Res 2001; 3: 68443.
6. Freier K, Flechtenmacher C, Walch A, Devens F, Mühling J, Lichter P, et al. Differential KIT expression in histological subtypes of adenoid cystic carcinoma (ACC) of the salivary gland. Oral Oncol 2005a; 41: 934-939.
7. Guzzo M, Locati LD, Prott FJ, Gatta G, McGurkc M, Licitra L. Major and minor salivary gland tumors. Crit Rev Oncol/Hematol 2010; 74(2): 134-148.
8. Ito FA, Ito K, Vargas PA, Almeida OP, Lopes MA. Salivary gland tumors in a Brazilian population: a retrospective study of 496 cases. Int J Oral Maxillofac Surg 2005; 34: 533–536.
9. Jones AV, Craig GT, Speight PM, Franklin CD. The range and demographics of salivary gland tumours diagnosed in a UK population. Oral Oncol 2008; 44: 407– 417.
10. Kallioniemi O, Wagner U, Kononen J, Sauter G. Tissue microarray technology for high-throughput molecular profiling of cancer. Human Mol Gen 2001; 10(7): 657-662.

* De acordo com a norma da UNICAMP/FOP, baseada na norma do International Committee of Medical Journal Editors – Grupo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

11. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4(7): 844-847.
12. Koul R, Dubey A, Butler J, Cooke AL, Abdoh A, Nason R. Prognostic factores depicting disease-specific survival in parotid-gland tumors. Int J Radiation Oncol Biol Phys 2007; 68(3): 714–718.
13. Lloyd BAS, Yu JB, Ross DA, Wilson LD, Decker RH. A prognostic index for predicting lymph node metastasis in minor salivary gland cancer. Int J Radiation Oncology Biol Phys 2010; 76(1): 169–175.
14. Loh KS, Barker E, Bruch G, O'Sullivan B, Brown DH, Goldstein DP, et al. Prognostic factors in malignancy of the minor salivary glands. Head Neck 2009; 31: 58–63.
15. Luukkaa H, Laitakari J, Vahlberg T, Klemi P, Stenbäck F, Grénman R. Morphometric analysis of CD34-positive vessels in salivary gland adenoid cystic and mucoepidermoid carcinomas. J Oral Pathol Med 2009; 38: 695-700.
16. Mahmood U, Koshy M, Goloubeva O, Suntharalingam M. Adjuvant Radiation Therapy for High-Grade and/or Locally Advanced Major Salivary Gland Tumors. Arch Otolaryngol Head Neck Surg 2011; 137(10): 1025-1030.
17. Marek S, Brad S, James DP. Tissue Microarrays: A Powerful Tool for High-Throughput Analysis of Clinical Specimens: A Review of the Method With Validation Data. Appl Immunohistochem Mol Morphol 2002; 10(1): 1-6.
18. Mariano FV, da Silva SD, Chulan TC, de Almeida OP, Kowalski LP. Clinicopathological factors are predictors of distant metastasis from major salivary gland carcinomas. Int J Oral Maxillofac Surg 2011; 40: 504–509.
19. Mücke T, Robitzky LK, Kesting MR, Wagenpfeil S, Holhweg-Majert B, Wolff KD, et al. Advanced malignant minor salivary glands tumors of the oral cavity. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108: 81-89.
20. Neville BW, Damm DD, Allen CM, Bouquot JE. Patologia oral e maxillofacial. 2ªed. Rio de Janeiro: Ed. Guanabra 2004.
21. Nocito A, Kononen J, Kallioniemi O, Sauter AG. Tissue microarrays (TMAs) for high-throughput molecular pathology research. Int J Cancer 2001; 94: 1-5.
22. Radhakrishnan R, Solomon M, Satyamoorthy K, Martin LE, Lingen MW. Tissue microarray – a high-throughput molecular analysis in head and neck cancer. J Oral Pathol Med 2008; 37: 166-176.

23. Rapidis AD, Givalos N, Gakiopoulou H, Stavrianos SD, Faratzis G, Lagogiannis GA, et al. Mucoepidermoid carcinoma of the salivary glands. Review of the literature and clinicopathological analysis of 18 patients. Oral Oncol 2007; 43: 130– 136.
24. Rimm DL, Camp RL, Charette LA, Olsen DA, Provost E. Amplification of Tissue by Construction of Tissue Microarrays. Experiment Molecular Pathol 2001; 70(3): 255-264.
25. Seethala RR. Histologic grading and prognostic biomarkers in salivary gland carcinomas. Adv Anat Pathol 2011; 18: 29–45.
26. Speight PM, Barrett AW. Prognostic factor in malignant tumours of the salivary glands. Br J Oral Maxillofac Surg 2009; 47: 587-593.
27. Tian Z, Li L, Wang L, Hu Y, Li J. Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6982 cases in an eastern Chinese population. Int J Oral Maxillofac Surg 2010; 39: 235–242.
28. Vargas PA, Speight PM, Bingle CD, Barrett AW, Bingle L. Expression of PLUNC family members in benign and malignant salivary gland tumours. Oral Dis 2008b; 14: 613-619.

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Análise clinicopatológica de 493 casos de tumores de glândulas salivares utilizando a técnica de tissue microarray"**, protocolo nº 141/2011, dos pesquisadores Felipe Paiva Fonseca e Pablo Agustin Vargas, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 23/12/2011.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Clinicopathologic analysis of 493 cases of salivary gland tumors using the tissue microarray approach"**, register number 141/2011, of Felipe Paiva Fonseca and Pablo Agustin Vargas, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 12/23/2011.

Profa. Dra. Lívia Maria Andaló Tenuta
Secretária
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.