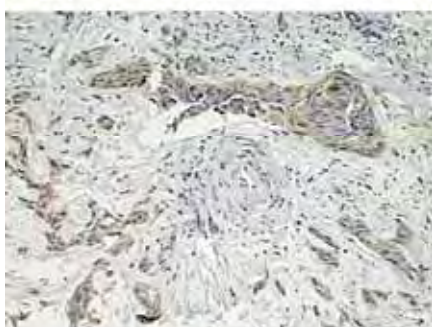
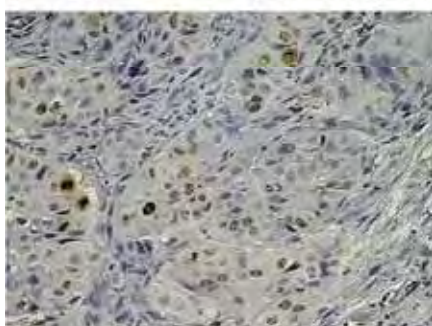


Ana Carolina Prado Ribeiro



**Estudo clínico, morfológico e
imunoistoquímico de carcinomas
espinocelulares em boca.
Análise comparativa entre pacientes
jovens e idosos.**



**ARAÇATUBA - SP
2008**

Ana Carolina Prado Ribeiro

**Estudo clínico, morfológico e imunoistoquímico
de carcinomas espinocelulares em boca. Análise
comparativa entre pacientes jovens e idosos.**

Dissertação apresentada à Faculdade de Odontologia do Câmpus de Araçatuba – Unesp, para a obtenção do Grau de “Mestre em Odontologia” – Área de Estomatologia.

Orientadora: Prof.^a Adj. Ana Maria Pires Soubhia

**ARAÇATUBA – SP
2008**

Catálogo-na-Publicação

Serviço Técnico de Biblioteca e Documentação – FOA / UNESP

R484e Ribeiro, Ana Carolina Prado
Estudo clínico, morfológico e imunoistoquímico de carcinoma
espinocelular em boca : análise comparativa entre pacientes jovens
idosos / Ana Carolina Prado Ribeiro. – Araçatuba : [s.n.], 2008
55 f. : il. ; tab. + 1 CD-ROM

Dissertação (Mestrado) – Universidade Estadual Paulista,
Faculdade de Odontologia, Araçatuba, 2008
Orientador: Profa. Dra. Ana Maria Pires Soubhia

1. Carcinoma de células escamosas 2. Imunoistoquímica
4. Grupos etários 5. Língua

Black D6
CDD 617.63

Dedicatória

Dedicatória

Aos meus pais, Antônio José Gracino Ribeiro e Renata Prado Ribeiro, pelo amor, compreensão e apoio incondicional em todos os momentos da minha vida. A eles o meu muito obrigado por sempre acreditarem em mim!

Aos meus irmãos, Juliana Prado Ribeiro e Marcelo Prado Ribeiro, pelos momentos de alegria, amizade e amor, que, apesar das diferenças, sempre nos tornaram cúmplices.

Ao Alan Roger dos Santos Silva, exemplo de profissionalismo e competência, incessante na busca de conhecimento. Com ele espero sempre dividir minha vida pessoal e profissional, com amor, companheirismo e em regime de mútuo incentivo.

Agradecimentos Especiais

Agradecimentos Especiais

À minha orientadora, Prof.^a Dr.^a Ana Maria Pires Soubhia, que desde a graduação me apóia e me incentiva. Nestes quatro anos de estreita convivência foram preciosos os seus ensinamentos, exemplos e atitudes, permitindo-me apropriar o real significado da pesquisa e da docência.

Agradecimentos

Agradecimentos

À Faculdade de Odontologia de Araçatuba - UNESP, por meio do de seu atual Diretor, Prof. Dr. Pedro Felício Estrada Bernabé, e de sua Vice-Diretora, Prof.^a Dr.^a Ana Maria Pires Soubhia.

Ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia de Araçatuba – UNESP, por meio de seu Coordenador, Prof. Dr. Idelmo Rangel Garcia Junior.

Aos docentes do Departamento de Patologia e Propedêutica Clínica da Faculdade de Odontologia de Araçatuba – UNESP, Prof.^a Dr.^a Ana Cláudia Okamoto, Prof. Dr. Antonio Augusto Ferreira de Carvalho, Prof. Dr. Éder Ricardo Biasoli, Prof. Dr. Élerson Gaetti Jardim Júnior, Prof. Dr. Gilberto Aparecido Coclete, Prof. Dr. Marcelo Macedo Crivelini, Prof. Dr. Norberto Perri Moraes e Prof.^a Dr.^a Renata Callestini Felipini, pela agradável convivência e conhecimentos transmitidos.

Aos funcionários do Departamento de Patologia e Propedêutica Clínica da Faculdade de Odontologia de Araçatuba – UNESP, Maria Aparecida Martins da Silva, Elaine Cristina Francischini Ferreira, José Marcelo Tamarin, João Batista Vieira, Katsuko Aparecida Anze Inoue, Marli Barbosa dos Santos, Mariana Bento Barbosa, Miriam Regina Mouro Ferraz Lima e Luzia Maria de Oliveira Francischini, pelo apoio, amizade, carinho e momentos de descontração que tornaram a realização deste trabalho muito mais agradável.

Às funcionárias do Centro de Oncologia Bucal (Unidade Auxiliar) da Faculdade de Odontologia do Câmpus de Araçatuba – UNESP, Jane Fátima Mendes Fernandes da Silva, Juliana Benevenuto Reis, Nair Ramos Macedo Cardoso e Shirleni Cantieri Cavazana, pela alegria, atenção e competência com que sempre me atenderem.

Aos funcionários da Seção de Pós-Graduação da Faculdade de Odontologia de Araçatuba – UNESP, Diogo Luís Reatto, Marina Midori Sakamoto Kawagoe e Valéria de Queiroz Marcondes Zagatto, pela atenção e paciência em todos os momentos.

Aos funcionários da Biblioteca da Faculdade de Odontologia do Câmpus de Araçatuba – UNESP, Ana Cláudia Martins Grieger Manzatti, Cláudio Hideo Matsumoto, Cláudio Maciel Júnior, Fernando Fukunish, Isabel Pereira de Matos, Ivone Rosa de Lima Munhoz, Izamar da Silva Freitas, Luzia Anderlini e Maria Cláudia de Castro Benez, pela atenção e carinho com que sempre atenderam às minhas solicitações.

Aos colegas pós-graduandos, Adriana Demathé, Ellen Cristina Gaetti Jardim, Evanice Menezes Marçal Vieira, Felipe Camargo Munhoz, Henrique José Baldo de Toledo, Iracy Costa, João Batista Vieira, Leandro Toyoji Kawata, Paulo de Tarso Coelho Jardim, Sâmira Âmbar Lins e Tereza Aparecida Delle Vedove

Semenoff, meu reconhecimento pelo convívio, pela amizade e pela troca de experiências.

À Prof.^a Dr.^a Maria Lucia Marçal Mazza Sundefeld, responsável pela análise estatística desse trabalho.

Ao Prof. Dr. Glauco Issamu Miyahara pelos ensinamentos e conselhos, fundamentais para a minha formação.

Ao Prof. Dr. Alvimar Lima de Castro, pelo aprendizado e oportunidade de convivência.

À Prof.^a Dr.^a Leda Maria Pescinini Salzedas, exemplo de dedicação e competência.

Ao Prof. Dr. Décio dos Santos Pinto e a Prof.^a Dr.^a Suzana C. Orsini Machado de Sousa, da disciplina de Patologia Bucal, da Faculdade de Odontologia da Universidade de São Paulo - USP, por terem cedido, gentilmente, o material biológico, os anticorpos e, ainda, por terem permitido a utilização do laboratório de imunoistoquímica viabilizando a execução desta dissertação.

Aos funcionários da disciplina de Patologia Bucal, da Faculdade de Odontologia da Universidade de São Paulo - USP, Beatriz Costa, Elisa dos Santos, Nair Pereira e Neia Barbosa e Zilda Alves, pelo apoio e auxílio durante a realização deste trabalho.

Aos meus avós, Alice Silva, Heitor Prado, Drausio Ribeiro e Ladayr Ribeiro (*in memoriam*), que tornaram a minha infância cheia de sorrisos; não importa onde estejam, sei que olham por mim.

Aos meus tios, Carmem Ribeiro Mingoci e Roberto Mingoci, Ana Paula Prado Lorenção e Gustavo Lorenção, e aos meus primos, Rodrigo Ribeiro Mingoci, Guilherme Lorenção e Théo Lorenção, que me acolheram em suas casas com muito carinho.

Ao Prof. Eurípides Alves da Silva e Prof.^a Maria Batista dos Santos Silva que me acolheram em sua família com muito amor, sempre me incentivando e apoiando, assim como à Michelle dos Santos Silva e ao Cícero Luiz Reis Silva, à Vivian Lara dos Santos Silva Rossignolo e ao João Adriano Rossignolo e à pequena Catarina Silva Rossignolo, que tanto nos faz sorrir.

À Família Pires-Soubhia, da qual eu já me considero um membro, Dr. Silvio Soubhia, Silvinho, Mariana e Ana Silva, com o meu reconhecimento pelo carinho e amizade.

Aos meus amigos, Ana Cristina Murakawa, Carla de Sá, Daniel Galera Bernabé, Fátima Regina Nunes de Souza, Juliana Cristina Graça, Livia Bino

Marques, Melaine de Almeida Lawall, Rafael Akira Murawama, Thiago Macedo Marques e Valentim Adelino Ricardo Barão, por todo incentivo, carinho e amizade.

À minha amiga “dentista”, Valéria Cecília Albertone Durão, exemplo de responsabilidade, profissionalismo e competência em tudo que faz na vida.

À minha amiga Luciana Estevam Simonato, pessoa imprescindível em minha vida, com a qual divido meus momentos de alegrias e minhas tristezas; vibro com suas conquistas como se fossem minhas. Admiro sua perseverança e profissionalismo. Agradeço ainda por ter incorporado em nossas vidas o querido amigo Rodrigo Borges de Oliveira. Obrigada!

À FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo, pelo auxílio financeiro que viabilizou a realização desse trabalho de pesquisa.

Aos pacientes, que tornaram este trabalho realidade, a minha lembrança e gratidão.

A todos que colaboraram direta ou indiretamente para a concretização deste sonho, meus sinceros agradecimentos.

Ribeiro, ACP. Estudo clínico, morfológico e imunoistoquímico de carcinomas espinocelulares em boca. Análise comparativa entre pacientes jovens e idosos [dissertação]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2008.

Resumo

A incidência mundial de câncer em jovens tem aumentado e estudos recentes mostram que o câncer de boca também segue esta tendência. O objetivo deste trabalho foi avaliar e comparar as características clínicas, histopatológicas e imunoistoquímicas entre pacientes jovens, com idade igual ou inferior a 40 anos, e pacientes idosos, com idade igual ou superior a 65 anos, diagnosticados com carcinoma espinocelular em língua. Foram selecionados 19 casos de pacientes jovens e 19 casos de pacientes idosos e coletados dados clínicos dos prontuários. A gradação histológica foi realizada utilizando os critérios de classificação de Bryne et al (1992), na região do fronte tumoral. Também foi analisada a expressão imunoistoquímica das proteínas Bcl-2, Cerb-b2 e Ki-67. Neste estudo foi observado maior número de carcinomas espinocelulares moderadamente e pobremente diferenciados no grupo de pacientes jovens enquanto que no grupo de idosos houve maior prevalência de carcinomas bem diferenciados. Houve também no grupo de pacientes jovens um aumento do infiltrado linfoplasmocitário. A expressão imunoistoquímica das proteínas Bcl-2, Cerb-b2 e Ki-67 não mostrou diferenças significantes no fronte tumoral entre pacientes jovens e idosos. Na amostra estudada, foram detectadas diferenças morfológicas entre o grupo de pacientes jovens e idosos, no entanto, estas diferenças não foram expressas de forma significativa na análise imunoistoquímica.

Palavras-chave: Carcinoma de células escamosas. Imunoistoquímica. Grupos etários. Língua.

Ribeiro, ACP. Clinical, morphological and immunohistochemical study of oral squamous cell carcinoma. Comparative analysis between young and old patients[dissertation]. Araçatuba: UNESP – São Paulo State University; 2006.

Abstract

The worldwide incidence of cancer in young is increasing and recent studies show that the oral cancer also follows this trend. The objective of this study was to evaluate and to compare the clinical, histopathological and immunohistochemical features between young patients, with 40 years old or less, and elderly patients, with 65 years old or a superior age, diagnosed with tongue squamous cell carcinoma. Nineteen cases of young patients and 19 cases of elderly patients were selected and clinical data were collected from medical records. The histological grading was carried out using the criteria of classification of Bryne et al (1992) in the tumoral front region. The immunohistochemical expression of the proteins Bcl-2, Cerb-b2 and Ki-67 was also analyzed. In the present study, the group of young patients presented a higher number of moderately and poor differentiated squamous cell carcinomas whereas the elderly group had a greater prevalence of well differentiated carcinomas. The group of young patients also showed an increase in the lympho-plasmacytic infiltration. The immunohistochemical expression of the proteins Bcl-2, Cerb-b2 and Ki-67 did not show significant differences in the tumoral front region between young and elderly patients. In the studied sample, morphological differences between the group of young and elderly patients were detected, however, these differences were not expressed in in the immunohistochemical analysis.

Keywords: Squamous cell carcinoma. Immunohistochemistry. Age Groups. Tongue.

Lista de Figuras

- Figura 1** Fotomicografias mostrando as características morfológicas e marcações imunoistoquímicas na região do fronte tumoral em pacientes jovens e idosos. **A.** Aspectos morfológicos em HE (200X) – pacientes jovens. **B.** Aspectos morfológicos em HE (200X) – pacientes idosos. **C.** Bcl-2 (200X) – pacientes jovens. **D.** Bcl-2 (200X) – pacientes idosos. **E.** Cerb-b2 (200X) – pacientes jovens. **F.** Cerb-b2 (200X) – pacientes idosos. **G.** Ki-67 (400X) – pacientes jovens. **H.** Ki-67 (400X) – pacientes idosos. 32

Lista de Tabelas

Tabela 1	Anticorpos e protocolos utilizados no estudo.	26
Tabela 2	Distribuição de sexo e raça.	30
Tabela 3	Distribuição dos fatores histopatológicos.	31

Lista de Abreviaturas

μm - Micrômetro

Bcl-2 - B-cell lymphoma-2

Cerb-b2 - Epidermal Growth Factor Receptor

CEC - Carcinoma espinocelular

Ki-67 - Kiel 67.^a culture dishe, monoclonal antibody which recognizes a labile epitope on a nuclear antigen in cycling cells

IgG - Imunoglobulina

DAB – Diaminobenzidina

LSAB – Labeled Streptavidin Biotin

EDTA - Ethylenediamine tetraacetic acid

Sumário

1 Introdução	20
2 Proposição	22
3 Material e Método	24
4 Resultados	29
5 Discussão	34
6 Conclusões	39
Referências	41
Anexos	44

Introdução

1. Introdução*

O câncer de boca é a oitava neoplasia maligna mais freqüente em todo o mundo (1). No Brasil, estimam-se mais de 14.160 novos casos para o ano de 2008 (2).

O carcinoma espinocelular (CEC) bucal acomete principalmente homens entre a sexta e sétima décadas de vida, sendo raro em pacientes jovens, com idade inferior a 40 anos (3-7).

Apenas 4 a 6% de todos os casos de carcinomas espinocelulares bucais são relatados em pacientes jovens (8-10). No entanto, um aumento na incidência do câncer bucal em jovens vem sendo relatado em todo o mundo e tem sido sugerido que esta é uma entidade distinta, pelo seu aparente comportamento biológico mais agressivo (4, 11,12).

A gradação histopatológica de CECs bucais vem sendo usada na tentativa de avaliar o comportamento biológico do tumor. O CEC possui populações celulares distintas e acredita-se que as células do fronte tumoral tenham características próprias quando comparadas a diversas áreas do mesmo tumor e que poderiam expressar, de forma mais precisa, o comportamento clínico do tumor (13,14). No entanto, são poucos os estudos na literatura que comparam pacientes jovens e idosos acometidos por CEC bucal no que diz respeito às diferenças histopatológicas e imunoistoquímicas no fronte tumoral.

*Normalização segundo as Normas para publicação da Revista Journal Of Oral Pathology & Medicine (Anexo B)

Proposição

2. Proposição

O objetivo deste trabalho foi avaliar e comparar as características clínicas, histopatológicas e imunoistoquímicas entre pacientes jovens, com idade igual ou inferior a 40 anos, e pacientes idosos, com idade igual ou superior a 65 anos diagnosticados com carcinoma espinocelular em língua.

Material e Métodos

3. Material e Métodos

Amostras estudadas

Foi realizada uma análise retrospectiva nos arquivos do Serviço de Histopatologia Bucal da Faculdade de Odontologia de Araçatuba – UNESP e da Faculdade de Odontologia da Universidade de São Paulo – FOUASP. Foram selecionados 19 casos de pacientes jovens, com idade igual ou inferior a 40 anos, e 19 casos de pacientes idosos, com idade igual ou superior a 65 anos, com o diagnóstico de carcinoma espinocelular em língua. Dos prontuários de cada paciente selecionado foram coletados os seguintes dados: sexo, raça e idade.

Avaliação Histopatológica

Após a seleção dos casos, foram realizados novos cortes de 5 µm de espessura do material arquivado. As lâminas obtidas foram coradas pelo método de Hematoxilina e Eosina e submetidas à reavaliação por estudo morfológico em microscopia de luz por dois observadores distintos, que aplicaram os critérios da classificação de Bryne et al (1992)¹³ na região do fronte tumoral. Cinco parâmetros foram avaliados individualmente: ceratinização, pleomorfismo nuclear, número de mitoses (campo de 400X), padrão de invasão e infiltrado linfoplasmocitário.

Análise Imunoistoquímica

A fim de realizar as reações imunoistoquímicas para os anticorpos Bcl-2, Cerb-b2 e Ki-67, foi utilizada a técnica da estreptavidina-biotina. Foram obtidos

cortes de 3µm de espessura e os fragmentos colocados sobre lâminas previamente tratadas com 3-aminopropil-trietoxi-silano (Sigma Chemical Company, EUA). Os cortes foram desparafinizados, reidratados em soluções com concentrações decrescentes de etanol, e realizada a remoção de pigmentos formólicos por meio da incubação em hidróxido de amônia a 10% em solução alcoólica (etanol 95%) por 10 minutos. Realizou-se recuperação antigênica e, ao final, os cortes foram imediatamente lavados em água corrente por 10 minutos. Em seguida foram levados para a etapa de desbloqueio da peroxidase endógena tecidual, realizada pela incubação em dois banhos de 15 minutos cada em solução de 1:1 de peróxido de hidrogênio a 6% e etanol.

Repetida a lavagem em água, os cortes foram imersos em solução de Tris pH 7,6, acrescidos de albumina bovina a 1%, e os anticorpos primários foram incubados sobre os cortes por 30 minutos. As reações foram reveladas pela diaminobenzidina (DAKO, Liquid DAB+) através da incubação por 10 minutos. Na seqüência os cortes foram contra-corados com hematoxilina de Mayer.

Os anticorpos primários, o clone, a diluição, o complexo terciário utilizado para incubação do anticorpo de ligação, o tratamento realizado, a marca comercial e a origem dos anticorpos utilizados no estudo podem ser observados na tabela 1.

Todos os passos, desde a utilização dos anticorpos primários até a contra-coloração, foram realizados automaticamente com o auxílio do equipamento Autostainer DAKO (DAKO, Carpenteria, CA, USA).

Tabela 1. Anticorpos e protocolos utilizados no estudo.

Anticorpo primário	Clone	Diluição	Complexo	Tratamento	Marca
Bcl-2	100	1:100	LSAB	Ácido cítrico 95°C, 30 min.	DakoCytomation
Cerb-2 (Neu)	Policlonal	1:100	LSAB	Ácido cítrico, 95°C, 30 min.	Santa Cruz Biotechnology
Ki-67	MIB-1	1:75	LSAB	EDTA, 95°C, 30 min.	DakoCytomation

Análise dos Resultados

Após a realização das reações imunoistoquímicas os resultados foram analisados em microscopia de luz. No caso de marcação citoplasmática do Bcl-2 e Cerb-b2, as marcações imunoistoquímicas foram analisadas por dois observadores distintos na região do fronte tumoral mediante um aumento de 200 X e agrupadas nas seguintes categorias: reação negativa e reação positiva.

A quantificação das células com positividade nuclear para Ki-67 foi realizada utilizando-se o software LEICA Qwin (Leica Imaging Systems Ltda, Cambridge, England). O índice de positividade foi obtido por meio da leitura dos resultados na região do fronte tumoral (aumento de 400x). Um mínimo de quinhentas células, considerando as células positivas e as células negativas, de cada caso, foram contadas manualmente e os resultados foram expressos em porcentagem de positividade.

Análise estatística

Para avaliar o grau de concordância entre os observadores durante a análise da gradação histológica e a intensidade de marcação imunoistoquímica no fronte tumoral, foi aplicado o teste Kappa com o auxílio do programa SAS v.8.2. Para analisar a correlação entre os grupos de pacientes jovens e idosos utilizaram-se os testes Qui-quadrado ou Exato de Fisher. Para análise do

Índice Ki-67 foi utilizado um teste de proporção entre os grupos estudados. O nível de significância considerado foi de 5%.

Resultados

4. Resultados

No grupo de pacientes jovens encontramos 13 (68,42%) homens e 6 (34,58%) mulheres, enquanto que no grupo de pacientes idosos encontramos 12 (63,18%) homens e 7 (36,84%) mulheres. A média de idade no grupo jovem foi de 34,6 anos, com idade mínima de 20 anos. Com relação à raça, tanto no grupo de jovens quanto no de idosos, predominou a raça branca como mostrado na tabela 2.

A análise histopatológica do fronte tumoral utilizando os critérios de classificação de Bryne et al (1992)¹³ mostrou que no grupo de pacientes jovens 10,53% dos carcinomas foram considerados bem diferenciados, 57,89% moderadamente diferenciados e 31,58% pobremente diferenciados, enquanto que no grupo de idosos 52,63% foram bem diferenciados, 21,05% moderadamente diferenciados e 26,32% pobremente diferenciados. Esta diferença foi estatisticamente significativa ($p < 0,05$). A análise individual dos fatores em cada grupo revelou aumento do infiltrado linfoplasmocitário em pacientes jovens ($p < 0,05$), como é mostrado na tabela 3.

O padrão de marcação imunoistoquímica para a proteína Bcl-2 revelou negatividade em 18 entre 19 pacientes jovens e em 15 entre 19 pacientes idosos; quando comparados estatisticamente, estes resultados não se mostraram significantes ($p > 0,05$). Com relação à proteína Cerb-b2, foi observado positividade em 15 entre 19 amostras de pacientes com idade igual ou inferior a 40 anos e em 9 entre 19 amostras de pacientes com idade igual ou superior a 65 anos; estes resultados não foram estatisticamente significantes ($p > 0,05$). O índice de marcação para o Ki-67 mostrou proliferação celular discretamente mais acentuada no grupo de pacientes idosos. No entanto o

teste de proporção aplicado mostrou não haver diferenças estatisticamente significantes ($p > 0,05$) (Figura 1).

Tabela 2. Distribuição de sexo e raça.

	Número (%) de cada grupo	
	Pacientes \leq 40 anos	Pacientes \geq 65 anos
Sexo		
Masculino	13 (68.42)	12 (63.18)
Feminino	6 (34.58)	7 (36.84)
Raça		
Branca	12 (63.18)	16 (84.21)
Outras	7 (36.84)	3 (15.79)

Tabela 3. Distribuição dos fatores histopatológicos*

Fatores Histopatológicos	Número (%) de cada grupo		Valor-P
	Pacientes ≤ 40 anos	Pacientes ≥ 65 anos	
Diferenciação			
Bem	2 (10,53)	10 (52,63)	0,0130
Moderadamente	11 (57,89)	4 (21,05)	
Pouco	6 (31,58)	5 (26,32)	
Ceratinização			
	5 (26,32)	4 (21,05)	ns [†]
Grau 1	4 (21,05)	5 (26,32)	
Grau 2	4 (21,05)	5 (26,32)	
Grau 3	6 (31,58)	5 (26,32)	
Grau 4			
Pleomorfismo Nuclear			
Grau 1	0 (0)	3 (15,79)	ns
Grau 2	9 (47,37)	9 (47,37)	
Grau 3	9 (47,37)	7 (36,84)	
Grau 4	1 (5,26)	0 (0)	
Mitose			
Grau 1	13 (68,42)	14 (73,68)	ns
Grau 2	5 (26,32)	3 (15,79)	
Grau 3	1 (5,26)	2 (10,53)	
Grau 4	0 (0)	0 (0)	
Padrão de Invasão			
Grau 1	0 (0)	3 (15,79)	ns
Grau 2	11 (57,89)	7 (36,84)	
Grau 3	7 (36,84)	6 (31,58)	
Grau 4	1 (5,26)	3 (15,79)	
Infiltrado Linfoplasmocitário			
Grau 1	3 (15,79)	8 (42,11)	0,0184
Grau 2	8 (42,11)	2 (10,53)	
Grau 3	5 (26,32)	9 (47,37)	
Grau 4	3 (15,79)	0 (0)	

* Bryne et al (1992)

†ns: não significativo (Testes Qui-quadrado ou Fischer)

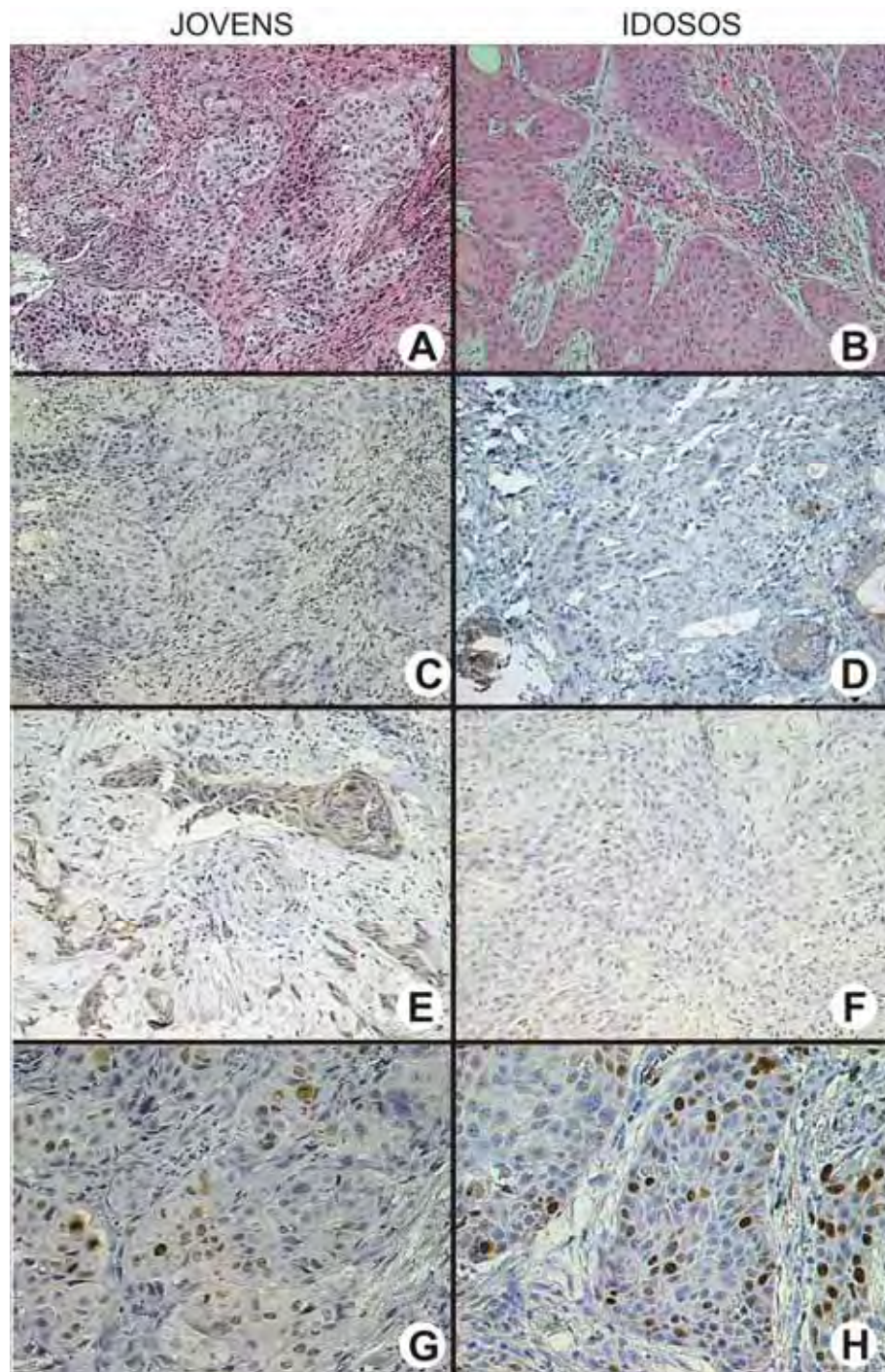


Figura 1. Fotomicrografias mostrando as características morfológicas e marcações imunoistoquímicas na região do fronte tumoral em pacientes jovens e idosos. **A.** Aspectos morfológicos em HE (200X) – pacientes jovens. **B.** Aspectos morfológicos em HE (200X) – pacientes idosos. **C.** Bcl-2 (200X) – pacientes jovens. **D.** Bcl-2 (200X) – pacientes idosos. **E.** Cerb-b2 (200X) – pacientes jovens. **F.** Cerb-b2 (200X) – pacientes idosos. **G.** Ki-67 (400X) – pacientes jovens. **H.** Ki-67 (400X) – pacientes idosos.

Discussão

5. Discussão

Um aumento na incidência de câncer bucal está sendo relatado em todo o mundo. Sugere-se que tanto a etiologia quanto a patogênese do CEC bucal diferem entre pacientes jovens e idosos, principalmente devido ao curto período de exposição a agentes carcinogênicos, à provável ausência de lesões potencialmente malignas preexistentes e ao seu aparente comportamento biológico mais agressivo (3-5,11,15).

O CEC bucal ocorre mais freqüentemente em homens do que em mulheres (12,16). Entretanto, quando analisamos os dados da literatura relacionados à distribuição de sexos em pacientes jovens com câncer de boca, encontramos divergências. Kuriakose et al (1992)¹¹ encontraram uma maior prevalência em mulheres, com uma proporção de 1 para 1,2 em um grupo de pacientes com idade igual ou inferior a 35 anos. No presente estudo, 68,42% dos casos de CEC em pacientes jovens foram observados em homens e 34,58% diagnosticados em mulheres, achados semelhantes aos de Sasaki et al (2005)¹² que observaram uma maior incidência no sexo masculino em amostras de pacientes com até 40 anos.

Kuriakose et al (1992)¹¹ sugerem que o CEC bucal em pacientes jovens tende a ser menos diferenciado histologicamente quando comparado ao CEC bucal, que acomete pacientes idosos. No presente estudo, ao analisar o fronte tumoral, observou-se que no grupo de pacientes jovens o maior número das amostras foi classificado como moderadamente diferenciado (57,89%) e pobremente diferenciado (31,58%). No entanto, no grupo de pacientes idosos 52,63% foram classificadas como bem diferenciados, 21,05% moderadamente diferenciados e 26,32% pobremente diferenciados ($p < 0,05$). Regezi et al

(1999)¹⁵, ao analisarem os padrões morfológicos de uma amostra de pacientes com idade inferior a 35 anos e compará-los aos padrões morfológicos de pacientes com idade superior a 75 anos, não obtiveram diferenças estatisticamente significantes; o mesmo ocorreu no estudo de Sasaki et al (2006)¹² ao analisarem uma amostra de pacientes jovens com idade inferior a 40 anos e compará-la com idosos. Esses achados reforçam a importância de se desenvolver novos estudos que busquem compreender melhor a patogênese do CEC bucal em jovens, a fim de se obter resultados mais confiáveis em relação às possíveis diferenças de classificação morfológica entre os dois grupos. A análise individual dos critérios estabelecidos por Bryne et al (1992)¹³ na região do fronte tumoral, em cada um dos grupos constatou um aumento do infiltrado linfoplasmocitário no grupo de pacientes jovens ($p < 0,05$). Este achado pode sugerir uma menor diferenciação celular neste grupo, pois recentes estudos de Suwa et al (2006)¹⁷ e Keberle et al (2003)¹⁸ sugerem que pacientes acometidos por CECs bucais com intenso infiltrado linfoplasmocitário apresentem maior incidência de metástase regional para linfonodos cervicais.

A carcinogênese pode estar relacionada não apenas ao aumento da proliferação celular, mas, também, à diminuição da morte celular (apoptose) (Loro et al 1999¹⁹). Uma das proteínas que tem sido relacionada à carcinogênese por prevenir a morte celular e aumentar a taxa de divisão celular é a Bcl-2 (20,21). Popovic et al (2007)²¹ e Loro et al (1999)¹⁹, ao analisarem casos de câncer bucal, encontraram uma associação entre a baixa expressão da Bcl-2 e um melhor prognóstico. Entretanto, Lo Muzio et al (2003)²² observaram que a baixa expressividade da Bcl-2 foi diretamente relacionada a

um pior prognóstico do paciente. No presente estudo, observou-se 18 pacientes jovens e 15 pacientes idosos com ausência da expressão da proteína Bcl-2. Embora estes resultados não tenham sido estatisticamente significantes, houve uma menor expressão de Bcl-2 no grupo de pacientes jovens, o que poderia estar relacionado com o maior número de carcinomas pobremente diferenciados neste grupo.

O Cerb-b2 é um dos membros da família de crescimento epidermal (ErbB) de receptores transmembrânicos com atividade tirosino quinase. Todos os membros desta família estão envolvidos na regulação do crescimento, diferenciação ou migração celular. A expressão aumentada do Cerb-b2 está sendo associada a um pior comportamento clínico em vários cânceres humanos, incluindo o carcinoma espinocelular bucal (23-27). Ulanovski et al (2004)²⁵, ao analisarem 27 amostras de CECs de língua encontraram correlação entre a positividade do Cerb-b2 e carcinomas pobremente diferenciados. Observou-se, neste estudo, positividade para o Cerb-b2 em 15 pacientes jovens e em 9 pacientes idosos, embora estes achados não tenham sido estatisticamente significantes. Assim como na imunomarcagem para o Bcl-2, estes resultados poderiam relacionar-se ao maior número de carcinomas pobremente diferenciados no grupo de jovens.

O antígeno Ki-67 é uma proteína nuclear expressa, principalmente, durante as fases S e G2 do ciclo celular. Somente as células em proliferação expressam Ki-67, ao contrário das células quiescentes (fase G0 do ciclo celular) que não produzem esta proteína (14,27-29). Elevados índices de Ki-67 são observados em CECs e correlacionados com a progressão da doença e com um pior prognóstico (14). Surpreendentemente, neste estudo observou-se

uma marcação discretamente mais acentuada no grupo de pacientes idosos. Este fato contrasta com os achados anteriores, que relacionavam o grupo de pacientes jovens a uma menor diferenciação celular e sugeriam maior proliferação.

Derka et al (2006)²⁸, ao realizarem um estudo experimental em carcinogênese bucal em hamsters, constataram um aumento do índice de Ki-67 nos estágios iniciais da oncogênese e uma diminuição em carcinomas menos diferenciados. Estes achados são contrastantes com outros vários estudos da literatura (14, 27, 29), que relataram um aumento da expressão do Ki-67 acompanhando, a progressão da displasia epitelial para o CEC bucal. Esta discrepância pode ser atribuída, em parte, ao pequeno número de casos das amostras estudadas.

Conclusões

6. Conclusões

1. No grupo de pacientes jovens com carcinoma espinocelular de língua estudado houve maior prevalência em homens da raça branca, não diferindo do grupo de pacientes idosos.
2. Embora o grupo de pacientes jovens com carcinoma espinocelular bucal tenha apresentado menor diferenciação celular, o grupo de pacientes idosos apresentou uma tendência maior para proliferação celular;
3. Não foram detectadas diferenças significantes entre a expressividade das proteínas Bcl-2, Cerb-b2 e Ki-67 no fronte tumoral de pacientes jovens e idosos;
4. Diferenças morfológicas entre o grupo de pacientes jovens e idosos foram detectadas, no entanto não foram expressas de forma significativa na análise imunoistoquímica.

Referências

Referências*

01. Massano J, Regateiro FS, Januário G, Ferreira A. Oral squamous cell carcinoma: review of prognostic and predictive factors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006; 102: 67-76.
02. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Instituto Nacional de Câncer. Coordenação de Prevenção e Vigilância. Estimativa 2008: Incidência de Câncer no Brasil. Rio de Janeiro: INCA; 2007.
03. Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for squamous cell carcinoma of the oral cavity in young people--a comprehensive literature review. *Oral Oncol.* 2001; 37: 401-18.
04. Llewellyn CD, Johnson NW, Warnakulasuriya KAAS. Risk factors for oral cancer in newly diagnosed patients aged 45 years and younger: a case-control study in Southern England. *J Oral Pathol Med,* 2004; 33: 525-32.
05. Chitapanarux I, Lorvidhaya V, Sittitrai P et al. Oral cavity cancers at a young age: analysis of patient, tumor and treatment characteristics in Chiang Mai University Hospital. *Oral Oncol.* 2006; 42: 83-8.
06. Warnakulasuriya S, Mak V, Möller H. Oral cancer survival in young people in South East England. *Oral Oncol.* 2007; 43: 982-6.
07. Garavello W, Spreafico R, Gaini RM. Oral tongue cancer in young patients: A matched analysis. *Oral Oncol.* 2007; 43: 894-7.
08. Iamaroon A, Pattanaporn K, Pongsiriwet S et al. Analysis of 587 cases of oral squamous cell carcinoma in northern Thailand with a focus on young people. *Int J Oral Maxillofac Surg.* 2004; 33: 84-8.
09. Siriwardena BS, Tilakaratne A, Amaratunga EA, Tilakaratne WM. Demographic, aetiological and survival differences of oral squamous cell carcinoma in the young and the old in Sri Lanka. *Oral Oncol.* 2006; 42: 831-6.
10. Siriwardena BS, Tilakaratne A, Amaratunga EA et al. Analysis of histopathological and immunohistochemical differences of oral squamous cell carcinoma in young and old patients in Sri Lanka. *J Oral Pathol Med.* 2007; 36: 357-62.
11. Kuriakose M, Sankaranarayanan M, Nair MK et al. Comparison of oral squamous cell carcinoma in younger and older patients in India. *Eur J Cancer B Oral Oncol.* 1992; 28B: 113-20.
12. Sasaki T, Moles DR, Imai Y, Speight PM. Clinico-pathological features of squamous cell carcinoma of the oral cavity in patients <40 years of age. *J Oral Pathol Med.* 2005; 34: 129-33.
13. Bryne M, Koppang HS, Lilleng R, Kjaerheim A. Malignancy grading of the deep invasive margins of oral squamous cell carcinomas has high prognostic value. *J Pathol.* 1992; 166: 375-81.
14. Kurokawa H, Zhang M, Matsumoto S et al. The relationship of the histologic grade at the deep invasive front and the expression of Ki-67 antigen and p53 protein in oral squamous cell carcinoma. *J Oral Pathol Med.* 2005; 34: 602-7.
15. Regezi JA, Dekker NP, McMillan A, Ramirez-Amador V, Meneses-Garcia A, Ruiz-Godoy Rivera LM, et al. p53, p21, Rb, and MDM2

- proteins in tongue carcinoma from patients < 35 versus > 75 years. *Oral Oncol.* 1999; 35: 379-83.
16. Bsoul SA, Huber MA, Terezhalmay GT. Squamous Cell Carcinoma of the Oral Tissues: A Comprehensive Review for Oral Healthcare Providers. *J Contemp Dent Pract.* 2005;15: 1-16.
 17. Suwa T, Saio M, Umemura N et al. Preoperative radiotherapy contributes to induction of proliferative activity of CD8+ tumor-infiltrating T-cells in oral squamous cell carcinoma. *Oncol Rep.* 2006; 15: 757-63.
 18. Keberle M, Ströbel P, Marx A, Hahn D, Hoppe F. CT determination of lymphocytic infiltration around head and neck squamous cell carcinomas may be a predictor of lymph node metastases. *Eur Arch Otorhinolaryngol.* 2003; 260: 558-64.
 19. Loro LL, Vintermyr OK, Liavaag PG, Jonsson R, Johannessen AC. Oral squamous cell carcinoma is associated with decreased bcl-2/bax expression ratio and increased apoptosis. *Hum Pathol.* 1999; 30: 1097-105.
 20. Loro LL, Johannessen AC, Vintermyr OK. Decreased expression of bcl-2 in moderate and severe oral epithelia dysplasias. *Oral Oncol.* 2002; 38: 691-8.
 21. Popović B, Jekić B, Novaković I et al. Bcl-2 expression in oral squamous cell carcinoma. *Ann N Y Acad Sci.* 2007; 1095: 19-25.
 22. Lo Muzio L, Mignogna MD, Pannone G et al. Expression of bcl-2 in oral squamous cell carcinoma: an immunohistochemical study of 90 cases with clinico-pathological correlations. *Oncol Rep.* 2003; 10: 285-91.
 23. Angiero F, Del Sordo R, Dessy E et al. Comparative analysis of c-erbB-2 (HER-2/neu) in squamous cell carcinoma of the tongue: does over-expression exist? And what is its correlation with traditional diagnostic parameters? *J Oral Pathol Med.* 2008 (impress).
 24. Kazkayasi M, Hücümenoğlu S, Siriner GI, Hücümenoğlu M. Over-expression of p53 and c-erbB-2 oncoproteins in laryngeal carcinoma. *Eur Arch Otorhinolaryngol.* 2001; 258: 329-35.
 25. Ulanovski D, Stern Y, Roizman P, Shpitzer T, Popovtzer A, Feinmesser R. Expression of EGFR and Cerb-B2 as prognostic factors in cancer of the tongue. *Oral Oncol.* 2004; 40: 532-7.
 26. Shiga H, Rasmussen AA, Johnston PG et al. Prognostic value of c-erbB2 and other markers in patients treated with chemotherapy for recurrent head and neck cancer. *Head Neck.* 2000; 22: 599-608.
 27. Silva SD, Agostini M, Nishimoto IN et al. Expression of fatty acid synthase, ErbB2 and Ki-67 in head and neck squamous cell carcinoma. A clinicopathological study. *Oral Oncol.* 2004; 40: 688-96.
 28. Derka S, Vairaktaris E, Papakosta V et al. Cell proliferation and apoptosis culminate in early stages of oral oncogenesis. *Oral Oncol.* 2006; 42: 540-50.
 29. Myoung H, Kim M-J, Lee J-H, Ok Y-J, PaengJ-Y, Yun P-Y. Correlation of proliferative markers (Ki-67 and PCNA) with survival and lymph node metastasis in oral squamous cell carcinoma: a clinical and histopathological analysis of 113 patients. *Int J Oral Maxillofac Surg.* 2006; 35: 1005–1010.

Anexos

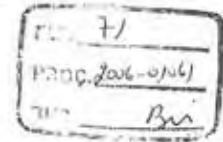
ANEXO A - Certificado do Comitê de Ética em Pesquisa



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba



COMITÊ DE ÉTICA EM PESQUISA – CEP-



OF. 067/2006
CEP
SFCD/br1

Araçatuba, 29 de maio de 2006.

Referência Processo FOA 2006-01061

O Coordenador do Comitê de Ética em Pesquisa desta Unidade, tendo em vista o parecer favorável do relator que analisou o projeto "ESTUDO CLÍNICO, MORFOLÓGICO E IMUNOISTOQUÍMICO DE CARCINOMA ESPINOCELULAR EM BOCA. ANÁLISE COMPARATIVA ENTRE PACIENTES JOVENS E IDOSOS" expede o seguinte parecer:

Aprovado:

Informamos a Vossa Senhoria que de acordo com as normas contidas na resolução CNS 215, deverá ser enviado relatório parcial em 04/05/2007 e o relatório final em 04/05/2008.

Prof. Dr. Stefan Fiúza de Carvalho Dekon
Coordenador do CEP

Ilma. Senhora
Dr^a. ANA MARIA PIRES SOUBHIA
Araçatuba-SP-

Ciente.De acordo.

27/10/06

Dr^a. Ana Maria Pires Soubhia

ANEXO B – Normas para publicação da Revista Journal of Oral Pathology & Medicine.



Journal of Oral Pathology & Medicine

Edited by:

Erik Dabelsteen

Print ISSN: 0904-2512

Online ISSN: 1600-0714

Frequency: Ten times a year

ISI Journal Citation Reports® Ranking: 2006: 20/49 (Dentistry, Oral Surgery & Medicine); 36/65 (Pathology)

Impact Factor: 1.530

Guide for Authors

1. General

Journal of Oral Pathology & Medicine publishes manuscripts of high scientific quality representing original clinical, diagnostic or experimental work in oral pathology and oral medicine. Papers advancing the science or practice of these disciplines will be welcomed, especially those which bring new knowledge and observations from the application of techniques within the spheres of light and electron microscopy, tissue and organ culture, immunology, histochemistry, immunocytochemistry and molecular biology. Review papers on topical and relevant subjects will receive a high priority and articles requiring rapid publication because of their significance and timeliness will be included as brief reports not exceeding three printed pages. **Please note that as of 1st September 2007, Journal of Oral Pathology & Medicine no longer accepts submission of case reports.** All submitted manuscripts falling within the overall scope of the Journal will be assessed by suitably qualified reviewers, but manuscripts in an incorrect format will be returned to the author without review.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Journal of Oral Pathology & Medicine*. Authors are encouraged to visit Blackwell Publishing Author Services for further information on the preparation and submission of articles and figures.

2. Ethical Guidelines

Journal of Oral Pathology & Medicine adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the work has not been published before, is not being considered for publication elsewhere and has been read and approved by all authors.

Journal of Oral Pathology & Medicine adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE authorship criteria should be based on substantial contributions to conception and design of, or acquisition of data or analysis and interpretation of data, and drafting the article or revising it critically for important intellectual content.

It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002 www.wma.net/e/policy/b3.htm) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

2.3. Clinical Trials

Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A [CONSORT checklist](#) should also be included in the submission material.

Journal of Oral Pathology & Medicine encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, <http://clinicaltrials-dev.ifpma.org/>, <http://isrctn.org/>. The clinical trial registration number and name of the trial register will then be published with the paper.

2.4. Conflict of Interest

All sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential grant holders should be listed. Please see [Conflicts of Interest](#) for generally accepted definitions on conflict of interest? Please enclose this information under the heading "Conflict of Interest Statement".

2.5. Appeal of Decision

Authors who wish to appeal the decision on their submitted paper may do so by emailing the editor with a detailed explanation for why they find reasons to appeal the decision.

2.6. Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

2.7. Copyright Assignment

Authors submitting a paper do so on the understanding that the work and its essential substance have not been published before and is not being considered for publication elsewhere. The submission of the manuscript by the authors means that the authors automatically agree to assign exclusive copyright to Blackwell Publishing if and when the manuscript is accepted for publication. The work shall not be published elsewhere in any language without the written consent of the publisher. The articles published in this journal are protected by copyright, which covers translation rights and the exclusive right to reproduce and distribute all of the articles printed in the journal. No material published in the journal may be stored on microfilm or videocassettes or in electronic database and the like or reproduced photographically without the prior written permission of the publisher.

Upon acceptance of a paper, authors are required to assign the exclusive licence to publish their paper to Blackwell Publishing. Assignment of the exclusive licence is a condition of publication and papers will not be passed to the publisher for production unless licence has been assigned. (Papers subject to government or Crown copyright are exempt from this requirement; however, the form still has to be signed). A completed [Exclusive Licence Form](#) must be sent to the address specified on the Exclusive Licence Form, before any manuscript can be published. Authors must send the completed original Exclusive Licence Form by regular mail upon receiving notice of manuscript acceptance, i.e., do not send the Exclusive Licence form at submission. Faxing or e-mailing the Exclusive Licence Form does not meet requirements.

For questions concerning copyright, please visit [Blackwell Publishing's Copyright FAQ](#).

3. Manuscript Submission Procedure

Manuscripts should be submitted electronically via the online submission site <http://mc.manuscriptcentral.com/jopm>. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below. For further instructions, please contact Editorial Assistant Anne-Marie Engel at ame@dadlnet.dk.

3.1. Getting Started

Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: <http://mc.manuscriptcentral.com/jopm>.

- Log-in or, if you are a new user, click on "register here".
- If you are registering as new user.
 - After clicking on "register here", enter your name and e-mail information and click "Next". Your e-mail information is very important.
 - Enter your institution and address information as appropriate, and then click "Next."
 - Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click "Finish".
- If you are registered as user, but have forgotten your log in details, enter your e-mail address under "Password Help". The system will send you an automatic user ID and a new temporary password.
- Log-in and select "Author Centre".

3.2. Submitting Your Manuscript

- After you have logged into your "Author Centre", submit your manuscript by clicking the submission link under "Author Resources".
- Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.
- Click the "Next" button on each screen to save your work and advance to the next screen.
- You are required to upload your files.
 - Click on the "Browse" button and locate the file on your computer.
 - Select the designation of each file in the drop down next to the Browse button.
 - When you have selected all files you wish to upload, click the "Upload Files" button.
- Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the "Submit" button when you are finished reviewing.

3.3. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rtf) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the entire manuscript including title page, abstract, text, references, acknowledgements and conflict of interest statement, tables, and figure legends, but *no* embedded figures. In the text, please reference figures as for instance "Figure 1", "Figure 2" etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below. Please note that any manuscripts uploaded as Word 2007 (.docx) will be automatically rejected. Please save any .docx file as .doc before uploading.

3.4. Blinded Review

All manuscripts submitted to *Journal of Oral Pathology & Medicine* will be reviewed by two experts in the field. *Journal of Oral Pathology & Medicine* uses single blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper.

3.5. Suggest a Reviewer

Journal of Oral Pathology & Medicine attempts to keep the review process as short as possible to enable rapid publication of new scientific data. In order to facilitate this process, the name and current email address of a potential international reviewer whom you consider capable of reviewing your manuscript is requested. Additionally, you may mention non-preferred reviewers as well.

3.6. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the "Submit" button and save it to submit later. The manuscript can then be located under "Unsubmitted Manuscripts" and you can click on "Continue Submission" to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status

You can access Manuscript Central any time to check your "Author Centre" for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts

To submit a revised manuscripts please locate your manuscript under "Manuscripts with Decisions" and click on "Submit a Revision". Please remember to delete any old files uploaded when you upload your revised manuscript.

4. Manuscript Types Accepted

Original Research Articles: of high scientific quality representing original clinical, diagnostic or experimental work in oral pathology and oral medicine. Papers advancing the science or practice of these disciplines will be welcomed, especially those which bring new knowledge and observations from the application of techniques within the spheres of light and electron microscopy, tissue and organ culture, immunology, histochemistry, immunocytochemistry and molecular biology.

Review Papers: *Journal of Oral Pathology & Medicine* commissions review papers and also welcomes uninvited reviews. Reviews should be submitted via the online submission site: <http://mc.manuscriptcentral.com/jopm> and are subject to peer-review.

Case Reports: Please note that as of 1st September 2007, *Journal of Oral Pathology & Medicine* no longer accepts submission of case reports.

Brief Reports: Original research material requiring rapid publication because of their significance and timeliness will be included as Brief Reports. They should not exceed three pages.

Letters to the Editor: Letters, if of broad interest, are encouraged. Letters should not be confused with Brief Reports. Letters may deal with material in papers published in *Journal of Oral Pathology & Medicine* or they may raise new issues, but should have important implications.

5. Manuscript Format and Structure

5.1. Page Charge

Articles exceeding 6 published pages (excluding figures and tables) are subject to a charge of USD163.00 per additional page. One published page amounts approximately to 5,500 characters (excluding figures and tables).

5.2. Format

Language: The language of publication is English. Authors for whom English is a second language may choose to have their manuscript professionally edited before submission to improve the English. A list of independent suppliers of editing services can be found at www.blackwellpublishing.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Abbreviations, Symbols and Nomenclature: Use only standard abbreviations (Vancouver System). All units will be metric. Use no roman numerals in the text. In decimals, a decimal point, and not a comma, will be used. Avoid abbreviations in the title. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement. Useful is Baren DN, ed. *Units, symbols, and abbreviations. A guide for biological and medical editors and authors.* 4. ed. London: Royal Society of Medicine.

Font: When preparing your file, please use only standard fonts such as Times, Times New Roman or Arial for text, and Symbol font for Greek letters, to avoid inadvertent character substitutions. In particular, please do not use Japanese or other Asian fonts. Do not use automated or manual hyphenation.

5.3. Structure

All papers submitted to *Journal of Oral Pathology & Medicine* should include: title page, abstract, main text, references and tables, figures, figure legends and conflict of interest statement where appropriate. Manuscripts must conform to the journal style. Manuscripts not complying with the journal format will be returned to the author(s).

Title Page: Should be part of the manuscript document uploaded for review and include: The title of the article, a running title of no more than 50 letters and spaces, 2-5 keywords, complete names and institution for each author, corresponding author's name, address, email address and fax number.

Abstract: is limited to 250 words in length and should contain no abbreviations. The abstract should be included in the manuscript document uploaded for review as well as inserted separately where specified in the submission process. The abstract should convey the essential purpose and message of the paper in an abbreviated form. For original articles the abstract should be structured with the following headings in accordance with Index Medicus (Medical Subject Headings): background, methods, results and conclusions. For other article types, please choose headings appropriate for the article.

Main Text of Original Articles: should be divided into introduction, material and methods, results and discussion.

Introduction: should clearly state the purpose of the article. Give only strictly pertinent references. Exhaustive literature reviews are inappropriate.

Materials and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced. As a condition of publication, authors are required to make materials and methods used freely available to academic researchers for their own use. This may for example include antibodies etc. Other supporting data sets must be made available on the publication date from the authors directly.

- (i) **Clinical trials:** Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A [CONSORT checklist](#) should also be included in the submission material. *Journal of Oral Pathology & Medicine* encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, <http://clinicaltrials-dev.ifpma.org/>, <http://isrctn.org/>. The clinical trial registration number and name of the trial register will then be published with the paper.
- (ii) **Experimental subjects:** Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version, 2002 www.wma.net/e/policy/b3.htm) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental

procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

- (iii) **Suppliers:** Suppliers of materials should be named and their location (town, state/county, country) included.

Results: Present your results in logical sequence in the text, tables, and illustrations. Do not repeat in the text all the data in the tables, illustrations, or both: emphasize or summarize only important observations.

Discussion: Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat in detail data given in the Results section. Include in the Discussion the implications of the findings and their limitations and relate the observations to other relevant studies.

Main Text of Review Articles comprise an introduction and a running text structured in a suitable way according to the subject treated. A final section with conclusions may be added.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name because readers may infer their endorsement of the data and conclusions. See also above under Ethical Guidelines.

Conflict of Interest Statement: All sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential grant holders should be listed. Please see [Conflicts of Interest](#) for generally accepted definitions on conflict of interest? See also above under Ethical Guidelines.

5.4. References

References should be kept to the pertinent minimum and numbered consecutively in the order in which they appear in the text. Identify references in text, tables, and legends by Arabic numerals (in parentheses). References cited only in the tables or figure legends should be numbered in accordance with a sequence established by the first identification of that figure or table in the text. Use the style of the examples below, which are based on the formats used in Index Medicus. Try to avoid using abstracts as references. Include manuscripts accepted, but not published; designate the abbreviated title of the journal followed by (in press). Information from manuscripts not yet accepted, should be cited in the text as personal communication. The references must be verified by the author(s) against the original documents. Titles should be abbreviated in accordance with the style used in Index Medicus and the Vancouver System.

We recommend the use of a tool such as [EndNote](#) or [Reference Manager](#) for reference management and formatting. EndNote reference styles can be searched for here: www.endnote.com/support/enstyles.asp . Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

Examples of the Journal's reference style:

(1) Standard journal article

(List all authors when 6 or less; when 7 or more, list only the first 3 and add et al.)
BUCHNER A, SCIUBBA JJ. Peripheral epithelial odontogenic tumors: a review. Oral Surg Oral Med Oral Pathol 1987; 63: 688-97.

HEINIC GS, GREENSPAN D, MACPHAIL LA, et al. Oral Histoplasma capsulatum infection in association with HIV infection: a case report. J Oral Pathol Med 1992; 21: 85-9.

(2) Corporate author

European Collaborative Study. Risk factors for mother-to-child transmission of HIV-1. *Lancet* 1992; 339: 1007-12.

(3) No author given

Anonymous. 'The importance of being early' [leader]. *Br Dent J* 1991; 170: 167.

(4) Journal supplement

MØLLER-PETERSEN J. Evaluation of diagnostic tests. Design and phases. *Scand J Clin Lab Invest* 1992; 52: suppl. (208): 35-50.

CROSS SS, SCHOLFIELD JH, KENNEDY A, COTTON DWK. Measuring the fractal dimension of tumour borders. *J Pathol* 1992; 168: 117A (abstr).

(5) Journal paginated by issue

HILLAM C. Dentistry in Europe in the 1790's. *Dent Historian* 1992; 22: (May): 31-4.

(6) Book

PINDBORG JJ. Atlas of diseases of the oral mucosa. Copenhagen: Munksgaard, 1992: 50-66.

(7) Chapter in a book

VAN DER WAAL I. Salivary gland neoplasms. In: PRABHU SR, WILSON DF, DAFTARY DK, JOHNSON NW, eds. *Oral diseases in the tropics*. Oxford: Oxford Medical, 1992; 478-86.

(8) Published proceedings paper

DRINNAN AJ. Review of the literature: educational aspects of oral medicine. In: MILLARD HD, MASON DK, eds. *World workshop on oral medicine*. Chicago: Year Book Medical, 1989; 5-11.

(9) Agency publication

MUIR C, WATERHOUSE J, MACK T, POWELL J, WHELAN S. Cancer incidence in five continents: Vol. 5. Lyon: International Agency for Research on Cancer, 1987; IARC Scientific Publications No. 88.

(10) Dissertation or thesis

CHUNGPANICH S. The diagnostic and prognostic potential of nucleolar organizer regions in oral epithelial dysplasia. MMedSci Thesis, University of Sheffield, 1989.

a. Tables, Figures and Figure Legends

Tables: should be numbered consecutively with Arabic numerals. Type each table on a separate sheet, with titles making them self-explanatory. Due regard should be given to the proportions of the printed page.

Figures: All figures should clarify the text and their number be kept to a minimum. Text on figures should be in CAPITALS. Line drawings should be professionally drawn; half-tones should exhibit high contrast.

All figures and artwork must be provided in electronic format. Please save vector graphics (e.g. line artwork) in Encapsulated Postscript Format (EPS) and bitmap files (e.g. half-tones) or clinical or in vitro pictures in Tagged Image Format (TIFF). Detailed information on our digital illustration standards can be found at www.blackwellpublishing.com/bauthor/illustration.asp

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected.

Figures divided into parts should be labeled with a lower-case, boldface, roman letter, a, b, and so on, in the same type size as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and unit, and follow SI nomenclature common to a particular field. Unusual units and abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc).

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible).

For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Blackwell Publishing's guidelines for figures: www.blackwellpublishing.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: www.blackwellpublishing.com/bauthor/eachecklist.asp.

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

Figure Legends: should be a separate section of the manuscript, and should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used: they should not contain any details of methods.

b. Supplementary Material

Supplementary Material, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted.

It should be clearly stated at the time of submission that the Supplementary Material is intended to be made available through the online edition. If the size or format of the Supplementary Material is such that it cannot be accommodated on the journal's Web site,

the author agrees to make the Supplementary Material available free of charge on a permanent Web site, to which links will be set up from the journal's Web site. The author must advise Blackwell Publishing if the URL of the Web site where the Supplementary Material is located changes. The content of the Supplementary Material must not be altered after the paper has been accepted for publication.

The availability of Supplementary Material should be indicated in the main manuscript by a paragraph, to appear after the References, headed "Supplementary Material" and providing titles of figures, tables, etc. In order to protect reviewer anonymity, material posted on the authors Web site cannot be reviewed. The Supplementary Material is an integral part of the article and will be reviewed accordingly.

6. After Acceptance

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1 Proof Corrections

Proofs will be sent via e-mail as an Acrobat PDF (portable document format) file. The e-mail server must be able to accept attachments up to 4 MB in size. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html.

This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs.

Proofs of the paper should be corrected, signed and returned without delay. Please ensure that you can be contacted during this time. As changes to proofs are costly, we ask you to only correct typesetting errors. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

6.2 Early Online Publication Prior to Print

Journal of Oral Pathology & Medicine is covered by Blackwell Publishing's OnlineEarly service. OnlineEarly articles are complete full-text articles published online in advance of their publication in a printed issue. OnlineEarly articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of OnlineEarly articles means that they do not yet have volume, issue or page numbers, so OnlineEarly articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

6.3 Online Production Tracking

Online production tracking is available for your article through Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit www.blackwellpublishing.com/bauthor for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

6.4 Author Material Archive Policy

Please note that unless specifically requested, Blackwell Publishing will dispose of all hardcopy or electronic material submitted two months after publication. If you require the return of any material submitted, please inform the editorial office or production editor as soon as possible.

6.5 Offprints

A PDF offprint of the online published article will be provided free of charge to the corresponding author, and may be distributed subject to the Publisher's terms and conditions. Additional paper offprints may be ordered online. Please click on the following link, fill in the necessary details and ensure that you type information in all of the required fields: [Offprint Cosprinters](#) If you have queries about offprints please email offprint@cosprinters.com

6.6 Author Services

For more substantial information on the services provided for authors, please see [Blackwell Publishing Author Services](#).