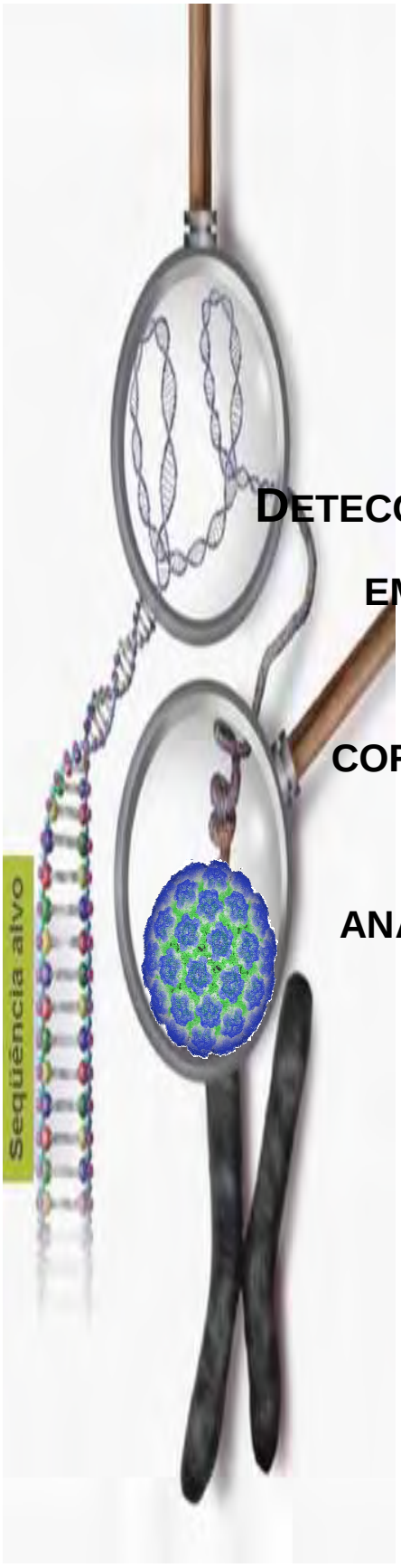
Adriana Demathé



DETECÇÃO DO PAPILOMAVÍRUS HUMANO (HPV) EM CARCINOMA EPIDERMÓIDE DE LÁBIO ATRAVÉS DA NESTED PCR E SUA CORRELAÇÃO COM OS FATORES DE RISCO, CARACTERÍSTICAS CLÍNICAS, ANATOMOPATOLÓGICAS E DE SOBREVIDA

ARAÇATUBA
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Detecção do papilomavírus humano (HPV) em carcinoma epidermóide de lábio através da nested PCR e sua correlação com os fatores de risco, características clínicas, anatomopatológicas e de sobrevida

Dissertação apresentada a Faculdade de Odontologia do Campus de Araçatuba – UNESP, para a obtenção do Grau de "Mestre em Odontologia" – Área de Estomatologia

Orientador: Prof. Dr. Glauco Issamu Miyahara
Co-orientador: Prof. Dr. Jose Fernando Garcia

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Detecção do papilomavírus humano (HPV) em carcinoma epidermóide de lábio através da nested PCR e sua correlação com fatores de risco, características clínicas, anatomopatológicas e de sobrevida. [dissertação]. Araçatuba: Faculdade de Odontologia da Universidade Estadual Paulista; 2007.

Resumo

O papilomavírus humano (HPV) está associado à um amplo espectro de lesões em humanos e tem sido associado à carcinogênese oral. Uma das metodologias mais sensíveis e especifica para sua detecção é a reação em cadeia de polimerase (PCR). O objetivo deste estudo foi investigar a presença do DNA do HPV em carcinoma epidermóide de lábio e correlacioná-la com fatores de risco, características clínicas, anatomopatológicas e sobrevida dos pacientes estudados. A presença do DNA do HPV foi investigada através da nested PCR em 30 amostras parafinadas de carcinoma epidermóide de lábio. O DNA viral foi detectado em 43,33% (13/30) das amostras. A presença do DNA do vírus foi relacionada com: idade, sexo, etnia, graduação histológica, etilismo, tabagismo, exposição freqüente à radiação solar, estadiamento clínico e sobrevida livre de doença e nenhuma correlação entre a presença do DNA viral e os parâmetros avaliados foi observada. Estes resultados sugerem que HPV não teve participação no processo de carcinogênese dos carcinomas epidermóides de lábio estudados.

Palavras-chave: Carcinoma epidermóide. Papillomaviridae. Reação em cadeia da polimerase.

Demathe A. Human papillomavirus (HPV) detection in lip squamous cell carcinoma by nested PCR and correlation with risk factors, clinical aspects, anatomophatologic observations and survival. [dissertation]. Araçatuba: UNESP – São Paulo State University; 2007.

Abstract

Human papillomavirus (HPV) is associated with a wide spectrum of lesions in humans and has been related to the oral carcinogenesis. The most sensitive and specific methodology for its detection is the polymerase chain reaction (PCR). The aim of this study was to investigate HPV DNA presence in lip squamous cell carcinoma and to correlate it with demographic, clinical-pathologic characteristics and survival data. HPV DNA was investigated by nested PCR in 30 paraffin-embedded tissues of lip squamous cell carcinomas. HPV DNA was detected in 43,33% (13/30) of samples. Presence of viral DNA was not associated with the following factors: age, sex, race, histologic grade, etilism, tabagism, history of solar radiation exposition, clinical staging and survival of the patients. This results suggest that HPV is not related to the lip squamous cell carcinomas carcinogenesis.

Keywords: Squamous cell carcinoma. Papillomaviridae. Polimerase chain reaction.

Epígrafe

“É melhor tentar e falhar,

que preocupar-se e ver a vida passar,

É melhor tentar, ainda que em vão,

que sentar-se fazendo nada até o final.

Eu prefiro na chuva caminhar,

que em dias tristes em casa me esconder.

Prefiro ser feliz, embora louco,

que em conformidade viver...”

Martin Luther King

Lista de Abreviaturas

HPV – do inglês Human Papillomavirus

DNA – do inglês Deoxyribonucleic Acid

PCR – do inglês Polymerase Chain Reaction

nPCR – do inglês nested Polymerase Chain Reaction

COB – Centro de Oncologia Bucal

FOA-UNESP – Faculdade de Odontologia do Campus de Araçatuba – UNESP

dNTP – do inglês Deoxyribonucleotide Triphosphate

pb - pares de base

CDC – do inglês Center of Disease Control

Sumário

1 INTRODUÇÃO

De acordo com a última estimativa mundial, o câncer é responsável por cerca de 7 milhões de mortes por ano (12% de todas as causas de óbitos), ficando atrás doenças cardiovasculares (30% das causas de óbito) e das doenças infecciosas e parasitárias (19% das causas de óbitos).[1]

Câncer de cabeça e pescoço é um termo coletivo que define tumores malignos localizados no trato aerodigestivo superior. Esta região anatômica inclui a cavidade oral, faringe e laringe. Cerca de 40% dos carcinomas de cabeça e pescoço ocorrem na cavidade oral (lábios, base da língua, língua, gengiva, assoalho bucal e palato), 15% na faringe e 25% na laringe, o restante afeta outras localizações como a tireóide e as glândulas salivares.[2]

O carcinoma oral é o sexto câncer mais freqüente entre os tumores malignos e representa a principal causa de morbidade e mortalidade entre os usuários de fumo e álcool em países em desenvolvimento.[1,3]

Para carcinomas de cavidade oral e orofaringe, a incidência mundial foi de mais de 460.000 com mortalidade de 250.900 indivíduos.[4] No Brasil, a estimativa de incidência de câncer da cavidade oral para 2008, incluindo lábio, é de 10.380 casos entre homens e 3.780 casos entre as mulheres variando nas regiões do Brasil.[5]

O carcinoma epidermóide corresponde a aproximadamente 90% de todas as neoplasias orais.[6] Sua etiologia é multifatorial e entre os principais fatores de risco estão o consumo de fumo e a ingestão de bebidas alcoólicas[7]. Por outro lado, cerca de 10% a 20% dos pacientes com câncer oral não fumam

e não bebem, sugerindo que outros fatores, incluindo certos vírus, possam ter implicação no processo de carcinogênese oral.[8,9]

A exposição solar é o principal agente etiológico nos casos de carcinoma de lábio, e ele se manifesta clinicamente como adelgaçamento do epitélio, formação de crostas ou ulceração.[10] Ocorre predominantemente em homens brancos.[11] Sua menor incidência entre mulheres parece ser devido ao uso freqüente de batom e protetor solar nos lábios, além da menor exposição ocupacional.[11] Dados epidemiológicos mostram que o carcinoma do vermelhão de lábio representa 0,47% e 0,09% de todos os carcinomas em homens e mulheres respectivamente.[11]

A maior prevalência ocorre entre a sexta e sétima décadas de vida. O lábio inferior é bem mais afetado que o superior e geralmente são diagnosticados no estádio I.[11] Mais de 90% dos tumores malignos de lábio são carcinomas epidermóides. Entre os locais com maior incidência de câncer de lábio está o sul da Austrália, com 13,49 casos/100,000 habitantes/ano em homens e 3,21 casos/100,000 habitantes/ano em mulheres. Na Europa, a Espanha apresenta uma das maiores incidências em homens (12,70 casos/100,000 habitantes/ano) e a Suíça a maior incidência em mulheres (0,83 casos/100,000 habitantes/ano).[12] Na França, a incidência de lábio, boca e faringe chega a atingir 38,5/100.000.[13] No Brasil, de acordo com a estimativa do INCA para 2008 a incidência de câncer oral no Brasil 14,45/100.000 em homens e 4,83/100.000 em mulheres. Na região Sudeste esta incidência é de 15,21/100.00 em homens e 4,64/100.000 em mulheres[5].

Entre os vírus que parecem estar relacionados com a etiologia das neoplasias orais está o papilomavírus humano (HPV)[9] (ANEXO A). Além

do seu papel bem definido na etiologia do carcinoma de cérvix, sugere-se sua participação na carcinogênese oral e também em outras localizações anatômicas como pele, esôfago, conjuntiva paranasal, brônquios, laringe e orofaringe[14].

Syrjänen e colaboradores descreveram pela primeira vez, em 1983[15], uma possível relação entre HPV e o câncer oral, devido à presença de lesões orais com alterações citopáticas tipicamente induzidas pelo HPV e idênticas aquelas previamente encontradas no câncer uterino.

As técnicas de detecção do DNA do HPV apresentam sensibilidade e especificidade amplamente variáveis[16]. Uma das técnicas com maior sensibilidade é a Reação em Cadeia de Polimerase (PCR)[17], devido a sua grande capacidade de detectar pequenos fragmentos de DNA do vírus[18].

Poucos estudos foram realizados para detectar o DNA do HPV em carcinomas epidermóides de lábio. Utilizando a PCR, Sugiyama et al.[19] estudaram 7 casos de carcinoma epidermóide de lábio e todos foram HPV negativos. Por outro lado, Shimizu et al.[20], em um total de 27 casos de carcinoma epidermóide de lábio, utilizando o mesmo método, encontraram 5 casos positivos (18,5%) para o DNA do HPV.

Em relação ao significado da presença do DNA do HPV no prognóstico dos pacientes com carcinomas epidermóides orais os estudos são controversos. Entretanto, alguns investigadores têm sugerido diferenças entre pacientes infectados com HPV e não infectados com relação ao sexo, idade, localização do tumor, grau de diferenciação dos tumores, metástase regional, taxa de recorrência e sobrevida.[21]

Paz et al.[22] estudaram 167 carcinomas epidermóides de cabeça e pescoço através da PCR. Com relação à idade, sexo, consumo de álcool e fumo, não houve diferença estatisticamente significante entre os casos com ou sem HPV. Os pacientes com tumores maiores e maior taxa de metástase regional apresentaram o DNA do HPV com maior freqüência, entretanto não houve diferença na taxa de sobrevida em três anos de controle entre os pacientes com ou sem DNA do HPV.

Em decorrência da grande variação nas taxas de detecção do HPV, os autores não são unânimes em indicar o HPV como um fator etiológico e fator prognóstico de sobrevida em casos de carcinoma epidermóide bucal. Alguns deles acreditam que a presença do HPV em lesões malignas pode ser puramente casualidade ou agir como co-fator junto com os fatores do meio ambiente, concluindo que somente a infecção do HPV não é suficiente para o desenvolvimento desta patologia.[23,24] No entanto, outros autores sugerem que a infecção do HPV pode ser um fator etiológico importante no desenvolvimento do carcinoma, já que verificaram a perda de um ou mais genes supressores de tumores no DNA das células envolvidas. [25,26]

Devido à grande controvérsia na literatura, novos estudos se fazem necessários para ajudar a elucidar muita das dúvidas ainda existentes sobre este tema.

2 PROPOSIÇÃO

Os objetivos deste trabalho foram:

- investigar a presença do HPV em pacientes portadores de carcinoma epidermóide de lábio por meio da reação em cadeia da polimerase (nPCR);

- avaliar a relação entre a presença do vírus com dados demográficos e características clínico-patológicas dos carcinomas epidermóides de lábio;

- determinar se a presença do vírus influencia na sobrevida dos pacientes.

3 MATERIAL E MÉTODO

Este trabalho foi analisado e aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Odontologia do Campus de Araçatuba – Universidade Estadual Paulista “Julio de Mesquita Filho” (FOA-UNESP), (Processo SISNEP Número: FOA 2006/01414) (Anexo B).

Utilizaram-se, retrospectivamente, 33 amostras parafinadas de carcinoma epidermóide de lábio (C00.0 e C00.1) [27] de pacientes atendidos no Centro de Oncologia Bucal da FOA – UNESP no período de 1991 a 2007. Os dados utilizados para avaliação das possíveis associações entre as variáveis foram obtidos dos prontuários e transcritos para uma ficha clínica individual (Anexo C).

Foram considerados tabagistas os indivíduos que fumaram regularmente durante alguma época da vida, mesmo aqueles que abandonaram o vicio. Os não-tabagistas foram aqueles que, em nenhum momento da vida, fizeram uso do fumo. O mesmo critério foi utilizado para classificação de etilistas e abstêmios.

A classificação TNM (Anexo D) que define a extensão da doença, analisando três parâmetros: extensão da neoplasia primaria (T), grau de comprometimento de linfonodos regionais (M) e metástase à distância (N), de acordo com a União Internacional Contra o Câncer foi utilizada para fazer o grupamento por estádios (Anexo E).[27] O diagnóstico histopatológico das peças cirúrgicas foi revisado por um único patologista, utilizando para a graduação histológica a classificação de Broders (bem diferenciado, moderadamente diferenciado e pouco diferenciado) [28].

Preparo do material genético

Foram coletados de seis a dez cortes histológicos com 08 micrômetros das peças parafinadas até obter 25 mg de material, procedendo-se a descontaminação do micrótomo e troca de lâminas entre cada exemplar. Após a coleta, os cortes foram identificados, acondicionados em tubos de polipropileno esterilizados de 1,5 ml e mantidos em temperatura ambiente até o momento da extração.

Para a realização da extração do DNA, foram seguidos três passos: desparafinização, digestão e purificação.

A desparafinização foi realizada pela dissolução da parafina em xileno e etanol, revertendo a embebição e desidratação dos tecidos processados. Aos tubos foram adicionados 1200 µl de xilol (Synth®) e agitados por 15 segundos em vortex (Thermoline® – EUA). Em seguida os tubos foram centrifugados (Centrifuge 5417C – Eppendorf®– Alemanha) a 14.000 rotações por minuto (rpm) durante cinco minutos. O sobrenadante foi descartado e sobre o sedimento foram adicionados 1200 µl de álcool etílico absoluto (Synth®) no tubo. Os tubos foram agitados em vórtex por 15 segundos e centrifugados a 14.000 rpm durante 5 minutos com posterior descarte do sobrenadante. Este ciclo (xilol-etanol) foi repetido novamente e ao final do processo as tampas foram abertas e os tubos colocados em um bloco térmico (Dri-block DB3 – Techne® - Inglaterra) a 37ºC por 15 minutos para evaporação do etanol remanescente.

A digestão dos tecidos foi feita adicionando-se ao tubo 20 µl de solução contendo proteinase K (20mg/ml) e 180 µl de ATL (tissue lysis buffer) do kit de extração de DNA (QIAamp DNA Mini Kit® – QIAGEN Ltd, Crawley,

UK), agitando-se no vortex por 1 minuto e mantendo-se o material em banho-maria (Modelo 146 – FANEM – São Paulo – Brasil) por 3 horas, a 56℃.

Para o isolamento dos ácidos nucléicos foi utilizado o sistema de extração de DNA QIAamp DNA Mini Kit®, segundo as instruções do fabricante (ANEXO F), e o DNA extraído (100 µl) foi transferido para tubos de polipropileno com tampa rosqueável. Após esta etapa, a quantidade e pureza do DNA genômico foram determinadas através da espectrofotometria (NanoDrop® ND-1000 UV-Vis).

Com a confirmação da presença e integridade do DNA, foram realizadas as PCRs com os oligonucleotídeos iniciadores para o gene controle da β-globina.

PCR para o gene da β-globina humana

Para a PCR do gene controle da β-globina, com 268 pares de base (pb), foram utilizados os oligonucleotídeos iniciadores GH20 (5'-GAAGAGCCAAGGACAG GTAC-3) e PC04 (5'-CAACTTCATCCACGTTCACC-3) descritos por Bell et al. [29]

A reação de amplificação foi realizada em um termociclador (PTC-100, MJ Research®, Inc., Watertown, MS, USA) e continha 20 µl de mistura com os seguintes componentes: 10,9 µl de água ultra-pura q.s.p. (Invitrogen Life Technologies®,Carlsbad, CA, EUA); 2,5 µl de tampão de PCR 10X (10 mM de Tris-HCl pH 8 e 50 mM de KCl) (Invitrogen Life Technologies®,Carlsbad, CA, EUA); 4mM de MgCl2 (Invitrogen Life Technologies®,Carlsbad, CA, EUA); 0,25 mM de dNTPs (deoxyribonucleoside 5'-triophosphates – dATP, dCTP, dGTP e dTTP – GEHealthcare®, Piscataway,

NJ, EUA); 15 pmol de cada oligonucleotídeo iniciador (Invitrogen Life Technologies®, Brasil) e 1U de Taq DNA polimerase (Invitrogen Life Technologies®, Brasil).

Após a mistura dos componentes, em capela de fluxo–laminar (Heto-Holter HV PCR, Dinamarca), foram adicionados 5 µl do DNA (150µg) de cada amostra totalizando um volume final de 25 µl. Como controle positivo para o gene da β-globina utilizou-se uma amostra de sangue humano e como controle negativo amostra contendo a mistura de reagentes e água ultra-pura sem o DNA.

Os fragmentos foram amplificados em termociclador (Peltier Effect Cycling modelo PTC – 100, MJ Research, EUA), sob as seguintes condições: desnaturação inicial a 95ºC por 5 minutos, 40 ciclos de desnaturação a 95ºC por 1 minuto, anelamento a 55ºC por 1 minuto e extensão a 72ºC por 2 minutos, com extensão final a 72ºC durante 8 minutos.

Para verificação da presença do DNA humano foi feita a análise através da eletroforese em gel de agarose a 2%, corada com brometo de etídeo, em tampão 1x TBE (Tris-borato 0,09 mM, EDTA 0,002M, pH 8,0) em fonte eletroforética (Amersham Pharmacia Biotech modelo EP3501, Suécia), durante 1 hora, a 100 volts. A visualização após coloração com brometo de etídeo foi feita em transiluminador, sob luz ultravioleta, e a documentação fotográfica com auxílio do sistema Kodak Digital Science 1D.

Após a confirmação da presença e integridade do DNA genômico através de PCR do gene da ß-globina, as amostras foram submetidas à

pesquisa do gene do HPV através de dois métodos: PCR e nPCR com oligonucleotídeos iniciadores para o DNA do HPV.

PCR para amplificação do DNA do HPV

Os oligonucleotídeos iniciadores utilizados neste método foram MY11 (5'-GCMCAGGGWCTATAAYAATGG-3') e MY09[30] (5'-CTCCMARRGGA WACTGATC-3') produzidos pela Invitrogen Life Technologies®, Brasil, para amplificar fragmentos de 450 pb da região tardia L1 do genoma viral (ANEXO G).

Os componentes da mistura de amplificação foram: 10,9 μl de água ultra-pura; 2,5 μl de tampão de PCR 10X (10 mM de Tris-HCl pH 8 e 50 mM de KCl – Invitrogen Life Technologies®, Carlsbad, CA, EUA); 4mM de $MgCl_2$ (Invitrogen Life Technologies®, Carlsbad, CA, EUA); 15 pmol de dNTPs; 1 U de Taq DNA polimerase e 0,02 mM de cada oligonucleotídeo iniciador. Em fluxo laminar, foram adicionados 5 μl de DNA genômico de cada amostra. Como controle positivo para infecção por HPV, empregou-se uma amostra de células HeLa, DNA de uma linhagem de células de carcinoma cervical uterino com ate 4 cópias de HPV-18 por célula. O controle negativo foi composto por mistura de amplificação e água ultra-pura sem a presença do DNA.

Os fragmentos foram amplificados em termociclador sob as seguintes condições: desnaturação inicial a 94ºC por 10 minutos, 40 ciclos de desnaturação a 94ºC por 1 minuto, anelamento a 55ºC por 1 minuto e extensão a 72ºC por 40 segundos, com extensão final a 72ºC por 4 minutos.

Os produtos da PCR foram submetidos à eletroforese em gel de poliacrilamida a 8%, durante 3 horas, sob voltagem constante de 100 volts. A

evidenciação das bandas foi realizada por coloração com solução de nitrato de prata e a documentação com auxílio do sistema Kodak Digital Science 1D.

nPCR para amplificação do DNA do HPV

Foram separados 2 μl do produto obtido na primeira PCR para utilização na nPCR. Foram utilizados os oligonucleotídeos iniciadores GP5+ (5'-TTTGTTACTGTGGTAGATAC YAC-3') e GP6+ (5'-GAAAAATAAACTTGTAAA TCATATTC-3') da Invitrogen Life Technologies®, Brasil, que geram fragmentos de 150 pares de bases.[31] A mistura de amplificação, os controles utilizados e as condições de temperatura foram semelhantes às da primeira etapa, com diferenças na quantidade de água ultra-pura q.s.p. (13,9 μl) e na temperatura de anelamento (43ºC).

Os produtos da nPCR foram submetidos a eletroforese em gel de poliacrilamida 8% sob as mesmas condições do método de PCR, sendo que após evidenciação foi realizada a documentação fotográfica.

As amostras foram divididas em dois grupos: grupo com e grupo sem a presença do DNA do HPV. Estes dois grupos foram comparados entre si, quanto às variáveis: sexo, faixa etária, cor, estadiamento clínico, gradação histológica (diferenciação celular), consumo de álcool, consumo de fumo e radiação solar, através do teste χ^2 e teste exato de Fisher.

A taxa de sobrevida foi calculada em anos, da data do diagnóstico até o óbito ou até a data do último contato com o paciente vivo. Pacientes que morreram de outras causas que não câncer de lábio foram considerados censurados para as observações específicas de análise de sobrevida livre de doença.

4 RESULTADOS

Das 33 amostras analisadas, as amostras 7, 25 e 32 não amplificaram o gene controle da β-globina humana (Fig. 1 - ANEXO H). Desta forma, a análise da presença do HPV foi efetuada em 30 amostras que foram submetidas aos métodos de PCR e nPCR.

Ao analisarmos as amostras através da PCR com os oligonucleotídeos MY09/MY11 não evidenciamos o DNA do HPV em nenhuma das amostras estudadas (Fig. 2 - ANEXO I).

Utilizando a nPCR com os oligonucleotídeos GP5+/GP6+, o DNA do HPV foi detectado nas amostras 1, 2, 5, 6, 10, 12, 16, 24, 28, 29, 30, 31 e 33 correspondendo a 43,33% (13 de 30 amostras), como podemos observar na Figura 3.

Todos os pacientes foram caucasianos.

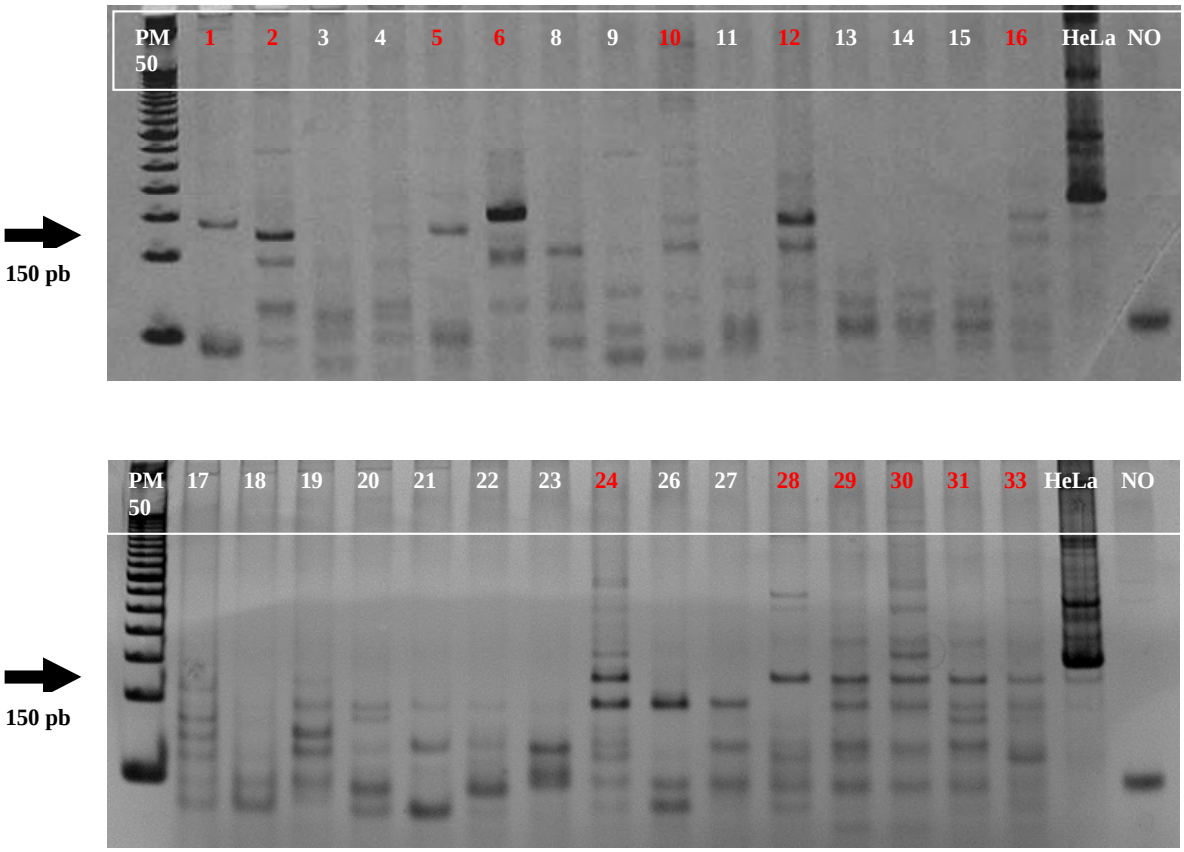

Figura 3 – Eletroforese em gel de poliacrilamida 8% mostrando resultado da amplificação do HPV (150 pb) por nPCR nas 30 amostras de carcinoma epidermóide de lábio. PM 50 = peso molecular de 50 pb; HeLa (controle positivo para DNA do HPV); NO (controle negativo, mistura de amplificação e água ultra-pura sem DNA).

A taxa de sobrevida de 5 anos, livre da doença, foi de 89,6% (Fig. 4) e não houve diferença estatística entre pacientes HPV positivos e negativos. Dos 21 pacientes vivos sem doença, a média de seguimento foi de 9,3 anos com um máximo de 13,9 anos taxa de sobrevida livre da doença. Ocorreram 7 óbitos por outras causas, um caso de óbito por câncer iniciado em lábio, lesão de 4 cm de diâmetro, que posteriormente teve metástase cervical e recidiva. A sobrevida deste paciente a partir do diagnóstico foi de 14 meses. Desta maneira com apenas 1 óbito não havia razão para realizar o teste Log-

Rank, que compara as curvas de probabilidade acumulada de sobrevida. Foi perdido o seguimento de um paciente.

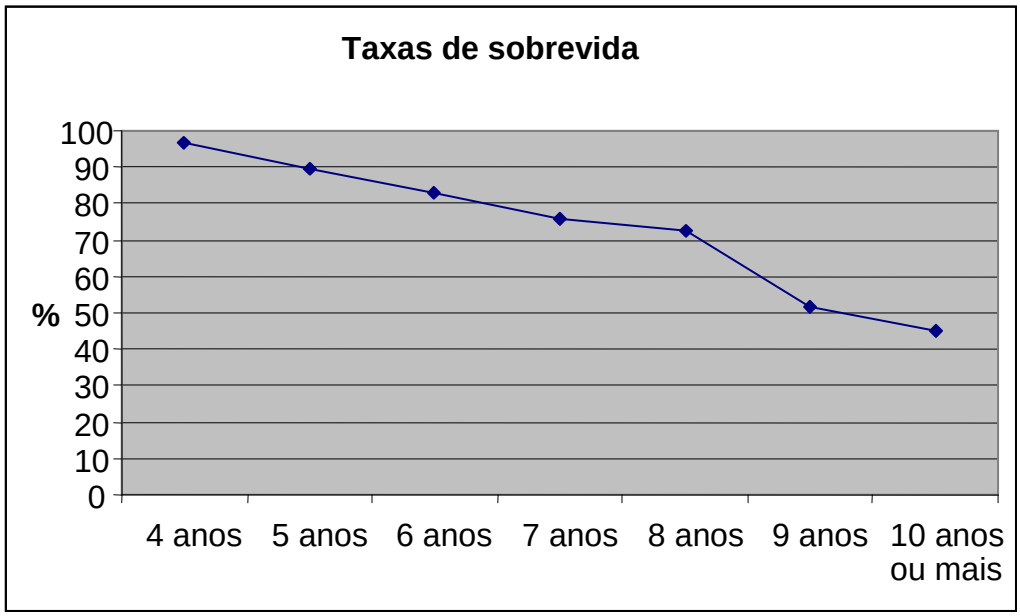

Figura 4 – Taxas de sobrevida livre de doença de pacientes com carcinoma epidermóide de lábio

A relação entre a presença do HPV e as variáveis estudadas (sexo, idade, estadiamento clinico, gradação histológica, tabaco e álcool) é mostrada na Tabela 1. Não foi observada nenhuma significância estatística entre essas variáveis.

Tabela 1 - Relação entre a presença do HPV com as variáveis clínico patológicas e os fatores de risco dos pacientes com carcinoma epidermóide de lábio

Variáveis	Pacientes (%) (n=30)	HPV + (%) (n=13)	HPV - (%) (n=17)	P*
Sexo				
Masculino	26(86,7)	10(76,9)	16(94,1)	
Feminino	04(13,3)	03(23,1)	01 (5,9)	0,20
Idade				
<60	14(46,7)	06(46,2)	08(47,1)	
≥60	16(53,3)	07(53,8)	09(52,9)	0,96
Estadiamento Clínico				
I	18(60,0)	07(53,8)	11(64,7)	
II	05(16,7)	04(30,8)	01 (5,9)	0,27
III/IV	07(23,3)	02(15,4)	05(29,4)	
Gradação histológica				
Bem diferenciado	09(30,0)	05(38,5)	04(23,5)	
Moderadamente	20(66,7)	08(61,5)	12(70,6)	0,49
Pobremente	01(3,3)	--	01 (5,9)	
Radiação solar				
Sim	23(76,7)	08(61,5)	15(88,2)	0,10
Não	07(23,3)	05(38,5)	02(11,8)	
Tabaco				
Tabagista	19(63,3)	07(61,5)	12(64,7)	
Não tabagista	11(36,7)	04(38,5)	07(35,3)	0,86
Álcool				
Etilista	15(50,0)	09(69,2)	06(35,3)	
Abstêmio	15(50,0)	04(30,8)	11(64,7)	0,06

Teste x^2 ou teste exato de Fisher

5 DISCUSSÃO

Os trabalhos encontrados na literatura apresentam taxas de detecção variáveis. Estas diferenças na detecção do DNA do HPV sugerem uma diferença potencial na habilidade para amplificar fragmentos de diferentes tamanhos e tipos específicos de HPV, de acordo com os métodos de detecção de DNA utilizados. Também podem ser resultado dos tipos de material estudados (esfregaços, material congelado, material parafinado), localização anatômica, questões populacionais, desenho dos oligonucleotídeos e o número de amostras estudadas.

Um dos métodos utilizados mais sensíveis é a PCR e os oligonucleotídeos iniciadores mais empregados em pesquisas de DNA viral para o HPV são: MY09/MY11,[30] GP5/GP6[17] ou GP5+/GP6+.[31] A PCR com os oligonucleotídeos M09/MY11 tem se mostrado menos sensível quando comparada à PCR em duas etapas (nPCR),[32-34] o que também ocorreu no presente estudo.

Quando se utiliza a nPCR mesmo com tecidos parafinados a detecção é bastante eficaz,[35-37] o que foi verificado neste experimento quando não ocorreu nenhuma amplificação na PCR e com o uso da nPCR houve detecção do DNA do HPV.

As diferenças populacionais podem ser constatadas na análise de uma revisão sistemática que avaliou estudos de detecção do DNA do HPV em vários continentes. [38] Para países europeus e asiáticos esta taxa ficou entre 16% e 33%[38], porém quando considerada a população latinoamericana esta taxa variou entre 50% e 60% para carcinomas epidermóides da cavidade

oral[39,40.] Corroboramos com Cañadas[41] que relata que a contaminação com HPV está diretamente ligada a experiências sexuais precoces, número de parceiros sexuais e contato sexual com parceiros promíscuos e estes fatos podem aumentar as taxas de detecção de HPV.

Poucos trabalhos avaliados estudaram exclusivamente carcinoma epidermóide em lábio. Alguns estudos incluíram esta localização entre os carcinomas epidermóides da cavidade oral. A maioria das amostras variou de 1 a 7 casos e as taxas de detecção de 0% a 33,34%.[22,26,35,42] A maior amostra que avaliou detecção do DNA do HPV em carcinoma epidermóide de lábio foi um estudo multicêntrico realizado na Suécia, Noruega e Finlândia que incluía 57 casos e encontrou uma positividade de 4%, porém em uma população bem diferente da latinoamericana.[42]

Taxa de detecção do DNA do HPV semelhantes a este trabalho foram encontradas por Riethdorf[44] que avaliou 18 casos de carcinoma epidermóide de lábio através da PCR utilizando tecidos congelados e encontrou 50% de positividade.

Em relação à taxa de sobrevida para 5 anos em lábio, estudo de Ogura[45] apresentou uma taxa de sobrevida de 82,5%, apesar do fato que seu estudo tinha 75% das lesões com estadiamento clínico I e cerca de 43% eram bem diferenciadas. No presente estudo 60% das lesões se encontravam no estádio I e 30% eram bem diferenciadas, apesar disto a taxa de sobrevida encontrada foi maior (89,6%).

Quanto a outras variáveis avaliadas (sexo, raça, idade, estadiamento clinico, gradação histológica, tabaco e álcool) não houve diferença estatisticamente significante para a associação de nenhuma destas

variáveis e a presença do HPV. Esta ausência de associação entre a infecção pelo HPV e estas variáveis também foi constatada em outros estudos.[44,46] Cremos que o lábio possa ser somente um reservatório para o papilomavírus humano.

A associação mais próxima de ser estatisticamente significante foi entre a presença do HPV e os pacientes etilistas (p = 0.06544). É possível que com uma casuística maior se pudesse encontrar uma correlação significante.

Um achado interessante no presente estudo foi o fato que dois dos casos HPV positivos não tiveram exposição a nenhum dos fatores etiológicos avaliados (radiação solar, uso de fumo e consumo de álcool), sendo um do sexo feminino e um do sexo masculino e lesões classificadas como moderadamente diferenciadas, merecendo estudos mais aprofundados para elucidar o papel do HPV nestas lesões.

6 CONCLUSÃO

Os resultados obtidos com a metodologia descrita permitiram concluir que:

- a presença do DNA do HPV foi detectada em 43,33% das amostras de carcinoma epidermóide de lábio estudadas;

- a presença de partículas virais do DNA do HPV não teve correlação com idade, sexo, consumo de fumo e álcool, radiação solar, estadiamento clínico e gradação histológica;

- a presença de partículas virais do DNA do HPV não teve correlação com a sobrevida dos pacientes acometidos pelo carcinoma epidermóide de lábio.

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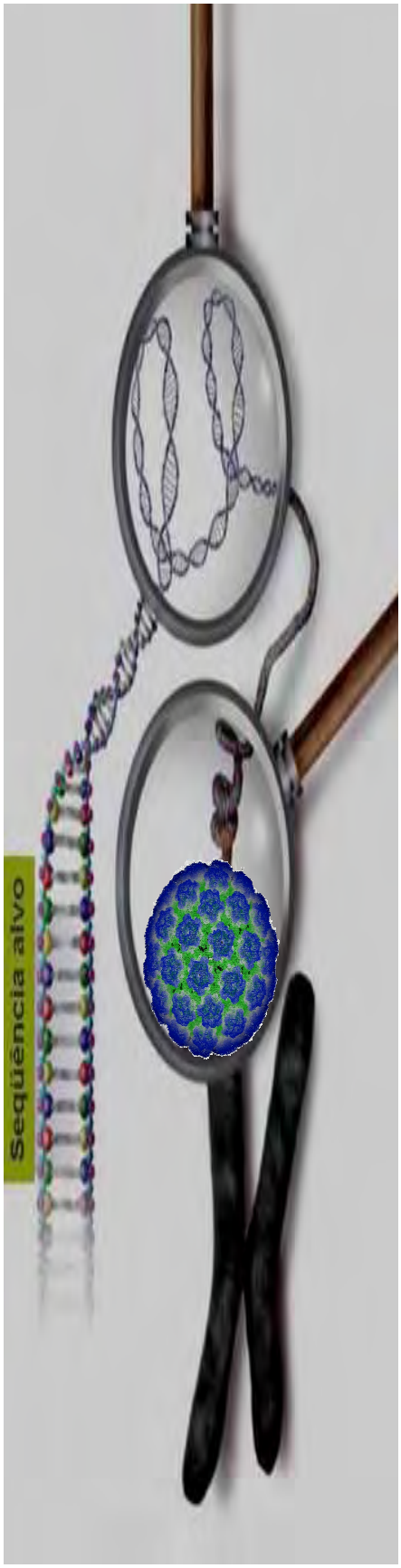
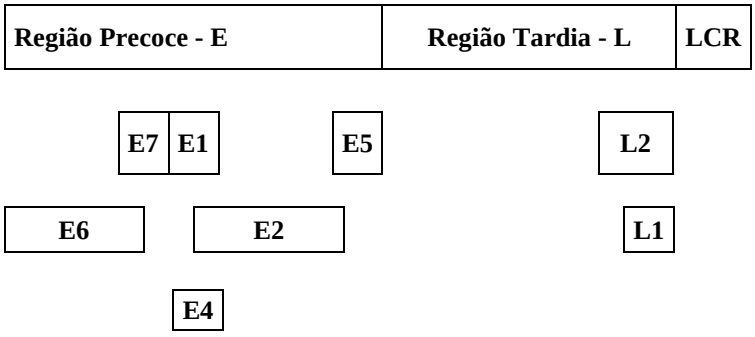


Anexos

ANEXO A – PAPILOMAVÍRUS HUMANO – HPV

O papilomavírus humano é um pequeno vírus de fita simples de DNA circular é epiteliotrópico e pode infectar o epitélio cutâneo e mucoso.[4,10,18] É formado por 8000 pares de bases (8 kilo bases), as quais são ligadas por histonas e contém uma cobertura protéica.[15,30]

O genoma do HPV (Fig. 1) pode ser dividido em 3 três regiões: uma região longa de controle (LCR) que regula a expressão do gene, compreendendo cerca de 10% do genoma e as regiões precoce (E) e tardia (L). Geralmente, as regiões E são expressas logo após a infecção e codificam as proteínas envolvidas na indução e regulação. Já as regiões L são expressas em estágios posteriores da infecção e codificam as proteínas do capsídeo viral. As regiões E são designadas E1 a E7, e a região L é dividida em regiões L1 e L2. Do ponto de vista da transformação celular, as regiões E5, E6 e E7 são as mais importantes.[15,18]



Proteína Viral	Função
E1	Síntese do DNA viral
E2	Proteína reguladora da transcrição
E4	Romper citoqueratinas
E5	Interagir com fatores de crescimento
E6	Transformar proteínas, ligante e iniciadora da degradação de p53; ativador transcricional, ativação telomerase
E7	Transformar proteínas, ligante da família de genes retinoblastoma (Rb1, p107, p103), ciclina A
L1	Proteína capsídeo maior
L2	Proteína capsídeo menor

Figura 1 – Mapa genético do papilomavírus humano.

Este vírus pode induzir lesões papilomatosas e lesões verrucosas de células escamosas no epitélio escamoso estratificado da pele e mucosa, incluindo a mucosa oral.[22]

Recentemente mais de 100 tipos de HPV têm sido identificados em várias lesões.[13,18] São classificados em vírus de alto e baixo risco de acordo com sua capacidade de malignização.[4,30]

Os modos de transmissão do HPV para as regiões de cabeça e pescoço não estão completamente elucidados; entretanto, teorias tem proposto a transmissão sexual pelo contato oral-genital, transmissão perinatal e autoinfecção pelo contato genital-oral com as mãos. Também foi sugerido que a cavidade oral pode ser uma reserva para infecção pelo HPV.[19,21,23,27]

Uma forte associação entre o câncer cervical e o oral com papilomavírus humano (HPV) de alto risco 16 e 18 ressalta a importância do

vírus na patogênese dos carcinomas epidermóides e contribuição deles na carcinogênese e progressão tumoral destes tipos de carcinomas. Esta contribuição ocorre predominantemente através da ação de dois oncogenes virais, E6 e E7. Os genes E6 e E7 têm sido estudados em várias populações diferentes e mais de 40 variantes já foram descritas e relacionadas à diferença na progressão de lesões intraepiteliais escamosas.[17]

Na cavidade oral, 24 tipos foram identificados, destes os mais importantes são os HPVs 6 e 11 associados com lesões benignas e os HPVs 16 e 18 relacionados com lesões malignas.[18]

Em carcinomas epidermóides tem sido detectados os tipos de HPVs 2, 3, 6, 11, 13, 16, 18, 31, 32, 33, 35, 52 e 57. Destes os HPVs 16 e 18 representam um importante papel na transformação maligna em carcinomas epidermóides[13]. O potencial carcinogênico dos HPVs 16 e 18 está associado principalmente às oncoproteínas E6 e E7 produzidas pelos mesmos. A E6 liga-se, seqüestra e degrada a p53, importante proteína supressora de tumor. A E7 seqüestra outra proteína supressora de tumor, a pRb e facilita a liberação de um dos fatores de transcrição, o E2F.[18]

Cerca de 60% dos carcinomas epidermóides de cabeça e pescoço podem ser HPV positivos. Em uma revisão sistemática de sobre detecção de HPV, de 5046 casos de carcinomas epidermóides, incluindo 2.642 casos da cavidade oral, 969 casos de orofaringe e 1.435 casos de laringe a maior prevalência de HPV positivo foi em carcinomas epidermóides de orofaringe (35.6%) , seguida pela laringe (24%) e orais (23.5%). O HPV 16 foi o tipo mais comum detectado.[14]

Em relação ao significado do prognóstico destes tumores os estudos são controversos. Entretanto, alguns investigadores tem sugerido diferenças entre pacientes infectados com HPV e não infectados com relação ao sexo, idade, localização do tumor, grau de diferenciação, linfadenopatia local ou regional, taxa de recorrência e sobrevida.[1]

Para o estudo do HPV, uma grande variedade de técnicas de biologia molecular tem sido empregada para a detecção do DNA do HPV, tais como: captura híbrida[9], Southern blot[8,16], Northern blot, dot blot [5] e hibridização in situ[8] além da reação em cadeia de polimerase – PCR.[20,26,29]

Um dos primeiros trabalhos utilizando PCR com oligonucleotídeos GP5/GP6 para detectar a prevalência de HPV em carcinomas epidermóides de cabeça e pescoço foi realizado por Snidjers em 1996[24]. Analisando 63 amostras de tecidos congelados de diferentes localizações anatômicas (cavidade oral, hipofaringe, laringe e orofaringe) encontrou positividade para o HPV em 20,6% dos casos.

A infecção pelo HPV em boca tem um baixo número de cópias de DNA viral[12] necessitando de um sistema de detecção altamente sensível de forma que se obtenham informações mais seguras sobre a presença do vírus nas amostras de carcinoma epidermóide bucal. Para aumentar a especificidade e a eficiência da amplificação do DNA alvo foi desenvolvida a nested PCR (nPCR), uma das variações da PCR[11], que consiste na realização da PCR em duas etapas. Neste método o produto da amplificação da primeira PCR é utilizado na segunda etapa. No final das duas etapas, obtém-se um produto menor que o da primeira amplificação, porém o DNA-molde da segunda etapa está em concentrações altíssimas e os *oligonucleotídeos* da segunda etapa

têm menos chances de anelamento em seqüências inespecíficas, dada a redução do tamanho do molde.

Os oligonucleotídeos utilizados são direcionados à região longa (LCR) do HPV e os oligonucleotídeos de consenso, ou gerais utilizados em pesquisas de DNA viral (HPV) são MY09/MY11 e GP5/GP6.[28]

O papilomavírus humano também tem sido detectado em mucosa normal de adultos e crianças. Em crianças encontrou-se uma positividade acima de 40% para a presença do DNA do HPV em mucosa normal, sugerindo que a cavidade oral é um reservatório de HPV em crianças.[13,25]

Mais de 25% dos carcinomas epidermóides de cabeça e pescoço estão associados a HPVs de alto risco.[14,23]

A taxa de detecção do HPV é muito variável indo de 0 a 100%, dependendo do método empregado, localização anatômica, tipo de tecido e oligonucleotídeos iniciadores empregados.

A tabela abaixo mostra alguns destes estudos realizados através da reação em cadeia de polimerase - PCR e seus resultados:

Estudo	**Metodologia Utilizada**	**Amostras positivas**	**%**
Balaram et al., 1995[2]	Consensus PCR	67/91	73,6
Cruz et al., 1996[6]	Consensus PCR	19/35	54,3
Elamin et al., 1998[7]	Nested L1 PCR	14/28	50
Bouda et al., 2000[3]	Nested consensus PCR	17/19	89,5

Uobe et al., 2001[28]	L1 in situ PCR	20/20	100

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ANEXO B - APROVAÇÃO COMITÊ DE ÉTICA EM PESQUISA

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

COMITÊ DE ÉTICA EM PESQUISA CEP-

Fls. 52
Proc. 2006-01414
Rub.

OF. 134/2006
CEP
SFCD/bri

Araçatuba, 18 de setembro de 2006.

Referência Processo FOA 2006-01414

O Coordenador do Comitê de Ética em Pesquisa desta Unidade, tendo em vista o parecer favorável do relator que analisou o projeto **"DETECÇÃO DO PAPILOMA VÍRUS HUMANO (HPV) ATRAVÉS DE PCR EM CARCINOMA EPIDERMÓIDE DE LÁBIO E SUA INFLUÊNCIA COM O PROGNÓSTICO"** 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 14/09/2007 e o relatório final em 14/09/2008.**

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

Ciente.De acordo.
06/10/06
Dr.Glauco Issamu Miyahara

Ilmo. Senhor
Dr.GLAUCO ISSAMU MIYAHARA
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ANEXO C - FICHA COLETA DADOS

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"

Faculdade de Odontologia – Câmpus de Araçatuba
Departamento de Patologia e Propedêutica Clínica
Disciplina de Estomatologia

Pesquisa: **Detecção do papilomavírus humano (HPV) em carcinoma epidermóide de lábio através de PCR e influência com o prognóstico e sobrevida.**

Aluna: Adriana Demathé

Nome do paciente:.....

N° do caso:.....N° do prontuário: .....

1 - Sexo: (1) masculino (2) feminino

2 - Raça: (1) branca (2) não branca

3 – Idade:.....

4 - Tabagista: (1) sim (2) não

5 - Etilista: (1) sim (2) não

6 – Radiação Solar: (1) sim (2) não

7a - Categoria T: (1)To (2) Tis (3)T1 (4)T2 (5)T3 (6)T4 (7)Tx

7b - Categoria N: (1)No (2)N1 (3)N2a (4)N2b (5)N2c (6)N3 (7)Nx

7c - Categoria M: (1)Mo (2)M1 (3)Mx

8 - Anatomopatológico:

(1) bem diferenciado (2) moderadamente diferenciado (3) pouco diferenciado

9 – Estádio Clínico:

(1) Estádio I (2) Estádio II (3) Estádio III (4) Estádio IV

10 - Status:

(1) vivo sem doença (2) vivo com doença (3) morte por câncer oral

(4) morte sem outras especificações (5) perda do segmento

ANEXO D – CLASSIFICAÇÃO TNM LÁBIO E CAVIDADE ORAL

T - Tumor Primário	
TX	O tumor primário não pode ser avaliado
T0	Não há evidência de tumor primário
Tis	Carcinoma *in situ*
T1	Tumor com 2 cm ou menos em sua maior dimensão
T2	Tumor com mais de 2 cm e até 4 cm em sua maior dimensão
T3	Tumor com mais de 4 cm em sua maior dimensão
T4a	*(Lábio)* Tumor que invade estruturas adjacentes: cortical óssea, nervo alveolar inferior, assoalho da boca, ou pele da face (queixo ou nariz)
N - Linfonodos Regionais	
NX	Os linfonodos regionais não podem ser avaliados
N0	Ausência de metástase em linfonodos regionais
N1	Homolateral, único, < 3 cm
N2	(a) Homolateral, único, > 3 até 6 cm (b) Homolateral, múltiplo, < 6 cm (c) Bilateral, contralateral, < 6 cm
N3	> 6 cm
	M - Metástase à Distância
MX	A presença de metástase à distância não pode ser avaliada
M0	Ausência de metástase à distância
M1	Metástase à distância

ANEXO E - GRUPAMENTO POR ESTÁDIOS PARA CARCINOMA DE LÁBIO

ESTÁDIO	T	N	M
Estádio 0	Tis	N0	M0
Estádio I	T1	N0	M0
Estádio II	T2	N0	M0
Estádio III	T1, T2 T3	N1 N0, N1	M0 M0
Estádio IVA	T1, T2, T3 T4a	N2 N0, N1, N2	M0 M0
Estádio IVB	Qualquer T T4b	N3 Qualquer N	M0 M0
Estádio IVC	Qualquer T	Qualquer N	M1

ANEXO F – PROTOCOLO DE EXTRAÇÃO DE DNA QIAamp DNA minikit®

1. Após incubar o tubo em banho-maria centrifugar o tubo por 10 segundos a 20800 rcf e adicionar 200 µL de AL e agitar o tubo por 15 segundos no vórtex e, em seguida, colocar na secadora a 70ºC por 10 minutos, para que ocorra a inativação na proteinase K residual;

2. Acrescentar 100 µL de etanol absoluto, agitar por 15 segundos no vórtex e centrifugar por 10 segundos a 20800 rcf;

3. Transferir a mistura para a coluna sobreposta ao tubo e centrifugar durante 1 minuto a 6000 rcf, apos a centrifugação, desprezar o filtrado;

4. Adicionar 500 µL de AW1 (Wash Buffer 1) e centrifugar por 1 minuto a 6000 rcf, descartando o filtrado e o tubo;

5. Adicionar 500 µL de AW2 (Wash Buffer 2) e centrifugar por 3 minutos a 20800 rcf, descartando o filtrado e o tubo;

6. Acrescentar 50 µL de AE (Tris-HCl 10 mM, 0,5 mM EDTA, pH9.0) para eluir o DNA, aguardar 1 minuto e centrifugar por 1 minuto a 6000 rcf;

7. Acrescentar 50 µL de AE a coluna; repousar por 5 minutos e centrifugar durante 1 minuto a 6000 rcf;

8. Após a eluição do DNA, transferir a amostra para um tubo com rosca e armazenar a temperatura de -20ºC.

ANEXO G – GENOMA VIRAL DO HPV-16 e OLIGONUCLEOTÍDEOS MY/GP

ACTACAATAATTCATGTATAAAACTAAGGGCGTAACCGAAATCGGTTGAACCGAAACCGGTTAGTA
TAAAAGCAGACATTTTATGCACCAAAAGAGAACTGCAATGTTTCAGGACCCACAGGAGCGACCCAG
AAAGTTACCACAGTTATGCACAGAGCTGCAAACAACTATACATGATATAATATTAGAATGTGTGTA
CTGCAAGCAACAGTTACTGCGACGTGAGGTATATGACTTTGCTTTTCGGGATTTATGCATAGTATA
TAGAGATGGGAATCCATATGCTGTATGTGATAAATGTTTAAAGTTTTATTCTAAAATTAGTGAGTA
TAGACATTATTGTTATAGTTTGTATGGAACAACATTAGAACAGCAATACAACAAACCGTTGTGTGA
TTTGTTAATTAGGTGTATTAACTGTCAAAAGCCACTGTGTCCTGAAGAAAAGCAAAGACATCTGGA
CAAAAAGCAAAGATTCCATAATATAAGGGGTCGGTGGACCGGTCGATGTATGTCTTGTTGCAGATC
ATCAAGAACACGTAGAGAAACCCAGCTGTAATCATGCATGGAGATACACCTACATTGCATGAATAT
ATGTTAGATTTGCAACCAGAGACAACTGATCTCTACTGTTATGAGCAATTAAATGACAGCTCAGAG
GAGGAGGATGAAATAGATGGTCCAGCTGGACAAGCAGAACCGGACAGAGCCCATTACAATATTGTA
ACCTTTTGTTGCAAGTGTGACTCTACGCTTCGGTTGTGCGTACAAAGCACACACGTAGACATTCGT
ACTTTGGAAGACCTGTTAATGGGCACACTAGGAATTGTGTGCCCCATCTGTTCTCAGAAACCATAA
TCTACCATGGCTGATCCTGCAGGTACCAATGGGGAAGAGGGTACGGGATGTAATGGATGGTTTTAT
GTAGAGGCTGTAGTGGAAAAAAAAACAGGGGATGCTATATCAGATGACGAGAACGAAAATGACAGT
GATACAGGTGAAGATTTGGTAGATTTTATAGTAAATGATAATGATTATTTAACACAGGCAGAAACA
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GTGGGGGAGAGGGTGTTAGTGAAAGACACACTATATGCCAAACACCACTTACAAATATTTTAAATG
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GTAATTCACAGGCAAAAATTGTAAAGGATTGTGCAACAATGTGTAGACATTATAAACGAGCAGAAA
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TACTACCACTTCACATGCAGCCTCACCTACTTCTATTAATAATGGATTATATGATATTTATGCAGA
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TATACCCATTAATATAACTGACCAAGCTCCTTCATTAATTCCTATAGTTCCAGGGTCTCCACAATA
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TAAACGTTTACCATATTTTTTTTCAGATGTCTCTTTGGCTGCCTAGTGAGGCCACTGTCTACTTGC
CTCCTGTCCCAGTATCTAAGGTTGTAAGCACGGATGAATATGTTGCACGCACAAACATATATTATC
ATGCAGGAACATCCAGACTACTTGCAGTTGGACATCCCTATTTTCCTATTAAAAAACCTAACAATA
ACAAAATATTAGTTCCTAAAGTATCAGGATTACAATACAGGGTATTTAGAATACATTTACCTGACC
CCAATAAGTTTGGTTTTCCTGACACCTCATTTTATAATCCAGATACACAGCGGCTGGTTTGGGCCT
GTGTAGGTGTTGAGGTAGGTCGTGGTCAGCCATTAGGTGTGGGCATTAGTGGCCATCCTTTATTAA
ATAAATTGGATGACACAGAAAATGCTAGTGCTTATGCAGCAAATGCAGGTGTGGATAATAGAGAAT
GTATATCTATGGATTACAAACAAACACAATTGTGTTTAATTGGTTGCAAACCACCTATAGGGGAAC
ACTGGGGCAAAGGATCCCCATGTACCAATGTTGCAGTAAATCCAGGTGATTGTCCACCATTAGAGT
TAATAAACACAGTTATTCAGGATGGTGATATGGTTCATACTGGCTTTGGTGCTATGGACTTTACTA
CATTACAGGCTAACAAAAGTGAAGTTCCACTGGATATTTGTACATCTATTTGCAAATATCCAGATT
ATATTAAAATGGTGTCAGAACCATATGGCGACAGCTTATTTTTTTATTTACGAAGGGAACAAATGT
TTGTTAGACATTTATTTAATAGGGCTGGTACTGTTGGTGAAAATGTACCAGACGATTTATACATTA
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TTACCTCTGATGCCCAAATATTCAATAAACCTTATTGGTTACAACGAGCACAGGGCCACAATAATG
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TATGTGCTGCCATATCTACTTCAGAAACTACATATAAAAATACTAACTTTAAGGAGTACCTACGAC
ATGGGGAGGAATATGATTTACAGTTTATTTTTCAACTGTGCAAAATAACCTTAACTGCAGACGTTA
TGACATACATACATTCTATGAATTCCACTATTTTGGAGGACTGGAATTTTGGTCTACAACCTCCCC
CAGGAGGCACACTAGAAGATACTTATAGGTTTGTAACCCAGGCAATTGCTTGTCAAAAACATACAC
CTCCAGCACCTAAAGAAGATGATCCCCTTAAAAAATACACTTTTTGGGAAGTAAATTTAAAGGAAA
AGTTTTCTGCAGACCTAGATCAGTTTCCTTTAGGACGTAAATTTTTACTACAAGCAGGATTGAAGG
CCAAACCAAAATTTACATTAGGAAAACGAAAAGCTACACCCACCACCTCATCTACCTCTACAACTG
CTAAACGCAAAAAACGTAAGCTGTAAGTATTGTATGTATGTTGAATTAGTGTTGTTTGTTGTGTAT
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ATAAACACGTGTGTATGTGTTTTTAAATGCTTGTGTAACTATTGTGTCATGCAACATAA
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CTGTTTTTGTTTTATATATACTATATTTTGTAGCGCCAGGCCCATTTTGTAGCTTCAAC
GGTTGCATGCTTTTTGGCACAAAATGTGTTTTTTTAAATAGTTCTATGTCAGCAACTAT
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CCATTCCATTGTTTTTTACACTGCACTATGTGCAACTACTGAATCACTATGTACATTGT
AAAATAAATCACTATGCGCCAACGCCTTACATACCGCTGTTAGGCACATATTTTTGGCT
ACTAACCTAATTGCATATTTGGCATAAGGTTTAAACTTCTAAGGCCAACTAAATGTCAC
CATACATGAACTGTGTAAAGGTTAGTCATACATTGTTCATTTGTAAAACTGCACATGGG
AAACCGATTTTGGGTTACACATTTACAAGCAACTTATATAATAATACTAA

MY11 - GCMCAGGGWCTATAAYAATGG
GCACAGGGCCAC AATAATGG

452 pb

MY09 - CGTCCMARRGGAWACTGATC
GCAGGMTRRCCTWTGACTAG (COMPLEMENTAR)
GATCAGTWTCCRRTMGGACG (INVERSO)
GATCAGTTTCCTTTAGGACG

GP5+ - TTTGTTACTGTGGTAGATACYAC
TTTGTTACTGTTGTTGATACTAC

142 pb

GP6+ - GAAAAATAAACTGTAAATCATATTC
CTTTTTATTTGACATTTAGTATAAG (COMPLEMENTAR)
GAATATGATTTACAGTTTATTTTTC (INVERSO)

ANEXO H - PCR PARA AMPLIFICAÇÃO DO GENE CONTROLE ß-GLOBINA

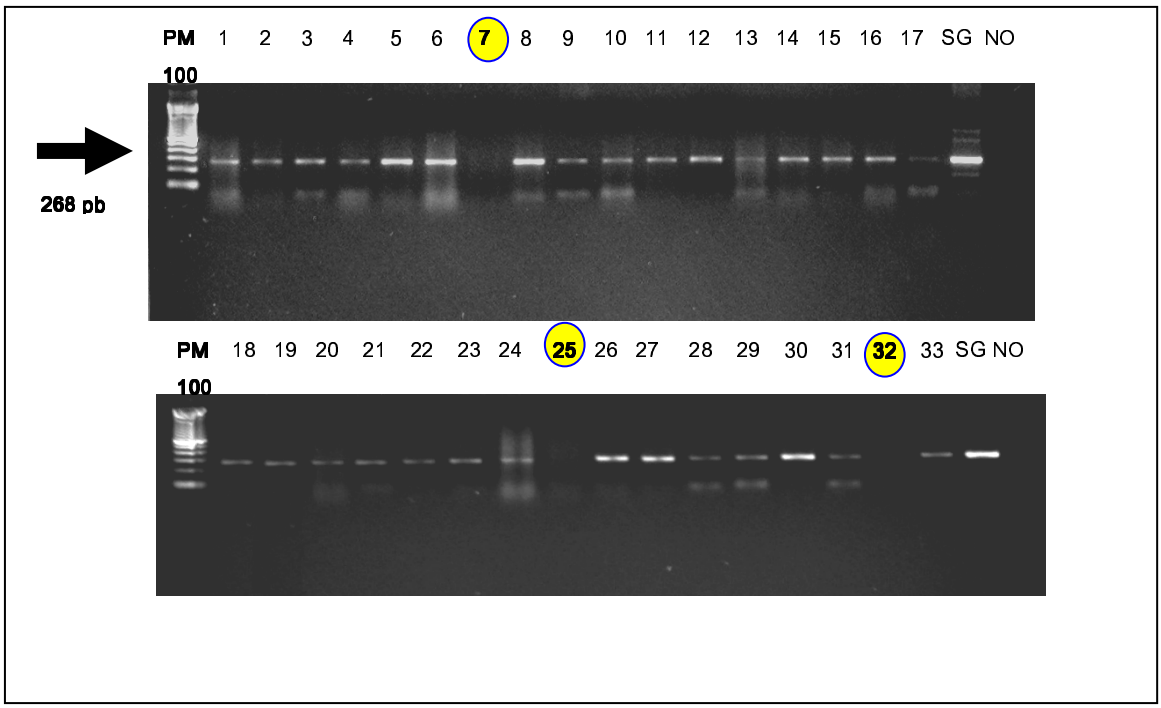

Figura 1 Eletroforese em gel de agarose 2% mostrando resultado da amplificação da β-globina (268 pb) das 33 amostras de carcinoma epidermóide de lábio. PM = peso molecular de 100 pb; CO = controle positivo (DNA extraído de sangue humano); NO = controle negativo (mistura de amplificação e água ultra-pura sem DNA)

ANEXO I - PCR PARA DETECÇÃO DO DNA DO HPV COM OLIGONUCLEOTÍDEOS MY09/MY11

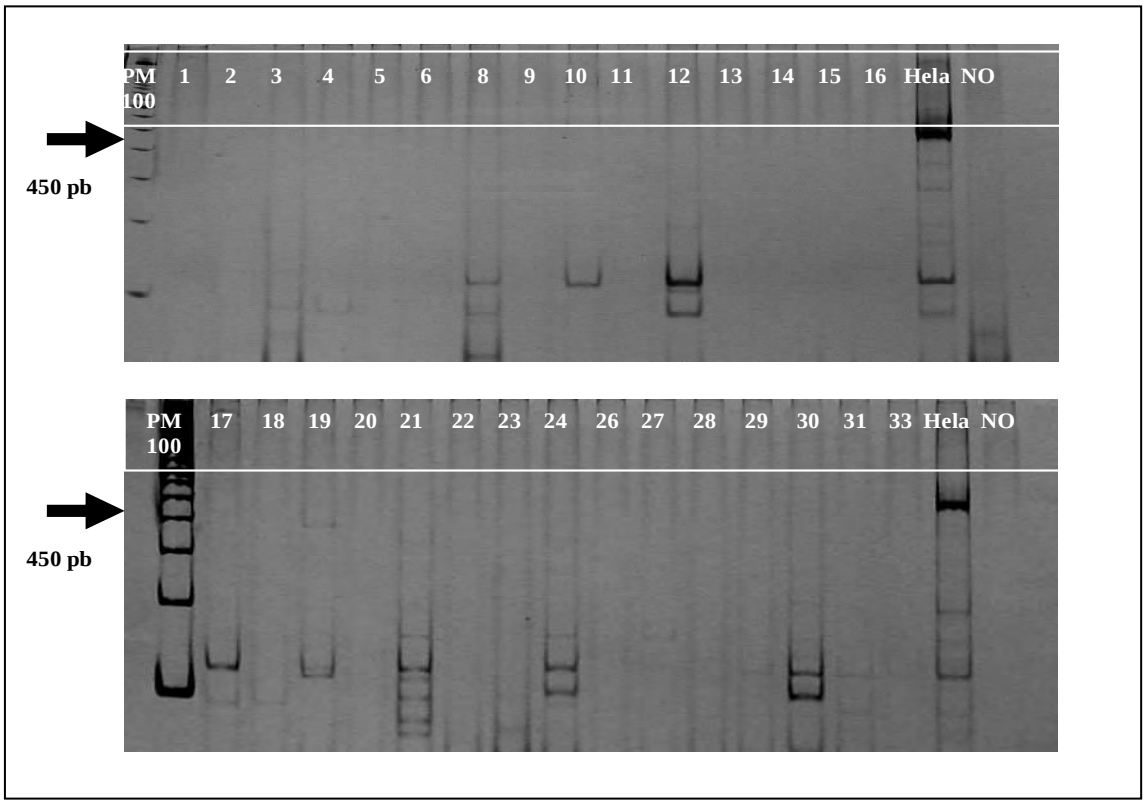

Figura 2 – Eletroforese em gel de poliacrilamida 8% mostrando resultado da amplificação do HPV (450 pb) por PCR nas 30 amostras de carcinoma epidermóide de lábio. PM 100 = peso molecular de 100 pb; HeLa (controle positivo para DNA do HPV); NO (controle negativo = mistura de amplificação e água ultra-pura sem DNA)

ANEXO J - NORMAS DO PERIÓDICO - Cancer Epidemiology, Biomarkers & Prevention

Information for Authors

Important Notice Regarding New Electronic Manuscript Submission System. *Cancer Epidemiology, Biomarkers & Prevention* has changed to a new manuscript submission and peer review system.

- Effective August 22, 2007, authors submitting new manuscripts to *Cancer Epidemiology, Biomarkers & Prevention* are required to submit manuscripts online via **AACR SmartSubmit** (http://cebp.msubmit.net). New submissions will be given a manuscript number higher than CEBP-07-1000.
- If you are submitting a REVISED version of a manuscript originally submitted via *Cancer Epidemiology, Biomarkers & Prevention's* previous system, Rapid Review, you must submit your revision via Rapid Review (http://www.rapidreview.com/AACR2/CALogon.jsp). Revised manuscripts will have a manuscript number lower than CEBP-07-1000.

Submit a Manuscript to *Clinical Cancer Epidemiology, Biomarkers & Prevention*:

1. Categories of Publication	8. Submission of Manuscript Files
2. Publication Policies	9. Correcting Proofs
3. Page Charges	10. Publication Fees and Reprints
4. Submission Procedures	11. Copyright and Permissions
5. Format and Style	12. Advertisements
6. Abbreviations	13. Subscriptions and Business Inquiries
7. Terminology	

Cancer Epidemiology, Biomarkers & Prevention (CEBP) publishes original research on cancer causation, mechanisms of carcinogenesis, prevention, and survivorship. The following topics are of special interest: descriptive, analytical, and molecular epidemiology; the use of biomarkers to study the neoplastic and preneoplastic processes; chemoprevention and other types of prevention trials; and the role of behavioral factors in cancer etiology and prevention.

Particular attention will be given to the identification of factors associated with various aspects of the carcinogenic process, including genetic susceptibility, host factors, infectious agents, chemical and physical carcinogens, environmental contaminants, dietary components, and behavioral factors such as tobacco use and sun exposure.

Besides welcoming manuscripts that address individual subjects in any of the relevant disciplines, the Editors encourage the submission of manuscripts with an interdisciplinary approach.

1. CATEGORIES OF PUBLICATION

Descriptions of the types of articles considered for publication (and page limits, if any) are as follows:

Original Research Papers

(1) **Research Articles.** Manuscripts that report original studies in epidemiology, biomarkers, prevention, or behavioral sciences relating to cancer which are well-documented, novel, and of high scientific merit. Research articles should not exceed 5,000 words, have a total of 6 or fewer tables and figures, an abstract of 250 words or less, and about 50 references. Meta-analyses and similar articles may require more references.

(2) **Short Communications.** Original research articles of scientific merit that are limited in scope. Short communications should be no more than 2,000 words, with 3 figures or tables and fewer than 25 references.

(3) **Null Results in Brief.** Original reports of null results of important *a priori* hypotheses tested with sufficient statistical power. These brief reports should be no more than 1,000 words and must follow the specified format exactly. Please indicate in your covering letter that your submission is for the Null Results in Brief category. Note that authors of submissions to this category will be permitted to make only minor revisions. In addition, reviewers' comments may not be provided to authors of rejected manuscripts. Relatively rigid criteria are applied during the evaluation. The submitted manuscript should fulfill the following criteria:

- The manuscript clearly should add to the current knowledge of cancer in humans (not animals) and be useful to future investigators making decisions regarding replication and/or inclusion in meta-analysis.
- The manuscript should provide a sufficient description of the study so that readers can evaluate the results.
- The methodology needs to have been described elsewhere, given limitations to manuscript length. These publications should be submitted as supplemental information.
- A greater weight for acceptance will be for studies where some prior publication(s) on the topic will have reported an association.

- The authors should exhibit a clear biological rationale, such as an established effect of the polymorphism on function or expression of the protein.
- The biomarker should be well validated and biologically meaningful.
- The statistical power should be equal to power reported in prior empirical publications.
- The manuscript should contain no more than 2 tables or figures and an Introduction of no more than 6 sentences.

Special Sections

(4) **Letters to the Editor.** Readers are welcome to submit comments on articles recently published in the journal. Letters should be no more than 400 words, with 5 or fewer references and no tables or figures, unless agreed to by the Editors. Please supply a title of 5 to 8 words that includes the main topic of the letter.

(5) **Reviews and Minireviews.** Reviews and minireviews on timely subjects of interest and importance to the readers of CEBP are welcome. Unsolicited reviews will be considered for publication, and others will be invited by the Editor. Reviews should be no more than 6,000 words in length. Minireviews are more focused articles and should be no more than 3,500 words in length.

(6) **Meeting Reports.** Reports on relevant symposia and conferences should include a statement of purpose, a summary of the findings presented, and recommendations for future research. Meeting reports should be no more than 1,500 words in length.

(7) **Hypotheses/Commentaries.** Creative new insights or commentaries that present original ideas relating to cancer causes and prevention. Hypotheses and Commentaries should be no more than 3,500 words in length.

(8) **Global Perspectives.** Summaries of current national research programs which increase international awareness within the scientific community are welcome. These summaries should be no more than 2,000 words in length.

(9) **Looking Farther Afield.** Summaries of recent articles published in other journals and commentary about their relevance to *CEBP* readers.

Invited Articles

(10) **Editorials.** Brief papers commenting on articles in the journal.

(11) **Point-Counterpoint.** Articles featuring two or more experts in a particular field who present counter-balancing views on a topic of interest to the readership.

2. PUBLICATION POLICIES

Submission of a manuscript to *CEBP* implies that the author(s) of the paper understand and fully accept the policies of the journal as detailed in the &ldquot;Information for Authors.&rdquot;

No Prior or Subsequent Publication. When a manuscript is submitted for consideration, the authors should confirm in writing that neither the submitted

paper nor any similar paper, in whole or in part, other than an abstract or preliminary communication, has been or will be submitted to or published in any other scientific journal. Permission to reproduce all or parts of articles published in AACR journals must be sought from the AACR Publications Office [phone: (215) 440-9300; fax: (215) 440-9354; e-mail: permissions@aacr.org]

Embargo Policy. Once submitted, contributions cannot be discussed with the media (including other scientific journals) until one week before the publication date. The information in accepted articles is embargoed from reporting by all media until 12:01 AM (EST) on the mail date of the issue (contact the Publications Office at cebp@aacr.org for exact mail dates). Authors who discuss their work with the media during the week before publication must ensure that the media representatives know the embargo policy and the embargo date. Authors arranging their own publicity on their articles are advised to notify the AACR Communications Department in advance [phone: (215) 440-9300; fax: (215) 440-9410; e-mail: communications@aacr.org].

Authorship. Who should be listed as an author is determined by the authors or by policies at their institutions, or both. As a general guideline, persons listed as authors should have contributed substantially to: 1) the conception and design of the study, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it for important content; and 3) final approval of the version to be published. The corresponding author is responsible for

ensuring that all authors have agreed to be authors and have agreed to the manuscript's content and its submission to the journal. If any changes are proposed to authorship after the manuscript is submitted, including the order of author listing, the corresponding author must provide the AACR Publications Department with signed documentation that the authors involved agree to the changes. *CEBP* accepts no responsibility for deciding matters of authorship.

Image Acquisition and Analysis

It is the authors' responsibility to exercise discretion during data acquisition, where misrepresentation must be avoided. Acquisition of images for comparative purposes must be standardized. Specimen areas should be selected which objectively represent the critical features being presented. Images should be captured in a non-compressing format such as .tif, or .bmp. Authors should retain their unprocessed images and metadata files, as editors may request them to aid in manuscript evaluation. If unprocessed data is unavailable, manuscript evaluation may be delayed until the issue is resolved. Files which have been adjusted in any way should be saved separately from the originals, also in a non-compressed format. Compressing formats, such as .jpg, should only be used for presentation of final figures, where requested, to keep files sizes small for electronic transmission.

8 bit monochrome, or 24 bit RGB acquisition is acceptable for visual documentation, but capture at higher bit depths is generally required for fine analysis of intensity data. Only non-adjusted original files should be used for analysis. If data is presented which includes mathematical representations of pixel intensities and locations, the original unprocessed files must be provided for review. A description of the analysis preparation and techniques should be included in the supplementary data.

Image Manipulation

The *American Association for Cancer Research* allows that minimal image adjustment is acceptable for publication in its journals; however, the final image must remain representative of the original data. Adjustments of brightness, contrast, or color balance are acceptable only if they are applied to the whole image and as long as they do not obscure or eliminate any information present in the original, including backgrounds. Non-linear manipulation, such as 'gamma' should only be used to adjust the overall presentation of the image, to make sure details are visible in the printed form. Alteration to specific features within the image is generally not acceptable. Sub-forms of an image may not be enhanced, obscured, moved or removed in relation to the larger image.

Non-linear algorithms to enhance overall presentation such as background subtraction, shading correction, sharpening, despeckling and flattening may be acceptable, but disclosure of adjustment must be included in the legend and the

specific techniques must be described in the supplemental data. Descriptions must include the original, unprocessed files for comparison.

Image Composites

The grouping of images from different originals must be made explicit, both by the arrangement of the figure (i.e., adding dividing lines) and in the text of the figure legend. This also applies to multiple fields taken from the same image (such as individual lanes combined from a single electrophoresis gel), and separate images acquired with different conditions. If dividing lines are not included, they will be added by our production department, and may result in publication delays.

Figures presenting merged color images from fluorescence originals must include the original single channel images used to make the merged file. Original images captured as color files are acceptable, but grayscale images are preferred, laid out in sequence as part of the figure.

Multiple images may be combined into a single photomontage when the area of interest cannot be captured in a single image. In such a case, all images which make up the montage must be captured using a standardized method. Each smaller image must overlap its neighboring image by ¼ of the shared field in each direction. The outer boundary of the combined image must be clearly

delineated with a line. Any post-processing must be done to the total, combined montage. All original images must also be submitted as supplementary data.

Electrophoretic gels and blots

Include positive and negative controls, as well as molecular size markers, on each gel and blot. Provide a citation for previously characterized antibodies. For antibodies less well characterized in the system under study, we require a detailed characterization that demonstrates not only the specificity of the antibody, but also the range of reactivity of the reagent in the assay, which will be published as supplementary data. Clearly separate vertically sliced gels that juxtapose lanes that were not contiguous in the experiment or include a line delineating the boundary between the gels.

The display of cropped gels and blots in the main paper is encouraged if it improves the clarity and conciseness of the presentation. In such cases, the cropping must be mentioned in the figure legend and the supplementary information should include full-length gels and blots wherever possible. These uncropped images should be labeled as in the main text and placed in a single supplementary figure. The manuscript's figure legends should state that "full-length blots/gels are presented in Supplemental Figure X."

- Cropped gels in the paper must retain important bands.

- Cropped blots in the body of the paper should retain at least six band widths above and below the band.
- High-contrast gels and blots are discouraged, as overexposure may mask additional bands. Authors should strive for exposures with gray backgrounds. Multiple exposures should be presented in supplementary information if high contrast is unavoidable. High-contrast immunoblots should be surrounded by a black line to indicate the borders of the blot.
- Describe all image acquisition tools and image processing software.
- Document key image-gathering settings and processing manipulations in the Supplementary Data.

Microscopy

The most important images should be made available to referees in images that are at least 300 dpi at the size which they will be published. Adjustments should be applied to the entire image. Threshold manipulation, expansion or contraction of signal ranges and the altering of high signals should be avoided. ’Pseudo-coloring’ and nonlinear adjustment (for example ’gamma changes’) are only allowed if unavoidable and must be disclosed. Include the following with the final revised version of the manuscript for publication:

- Include a magnification scale bar for each image.

- In the Methods section, specify the type of equipment (microscopes/objective lenses, cameras, detectors) used. Acquisition software should also be specified, as well as a description of specialized techniques requiring large amounts of processing, such as confocal, deconvolution, 3D reconstructions, or surface and volume rendering.
- In Supplementary Data, provide additional acquisition information for each image, including time and space resolution data (xyzt and pixel dimensions), image bit depth, experimental conditions such as temperature and imaging medium, and fluorochromes.

Review Process. All submitted manuscripts are assessed by a Senior Editor, who makes the final recommendation on acceptance or rejection. The Senior Editor will select peer reviewers and makes a recommendation based on their comments; directly select peer reviewers for the manuscript; or determine that the manuscript is not suitable for the journal and return it, without peer review. All reviewers and editors are required to adhere to ethical guidelines that mandate strict confidentiality concerning all aspects of the manuscript and its content. Manuscripts submitted for consideration for publication are privileged communications, and the status of the manuscript and details regarding it are available only to AACR editorial staff, authors, and the editors and peer reviewers involved.

Submission of a manuscript implies acceptance by all authors of the strict policy of the AACR that under no circumstances will the identities, or information leading to the identities, of the Senior Editors and reviewers be revealed.

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Depositing Data in Public Databases. The AACR requires that authors submitting manuscripts describing microarray data be prepared to supply peer reviewers with the data in a format that conforms to the Minimum Information About a Microarray Gene Experiment (MIAME) guidelines of the Microarray Gene Expression Data society (MGED). These guidelines include a checklist of information to be included with each new microarray submission; the checklist is available online

(http://www.mged.org/Workgroups/MIAME/miame_checklist.html). Authors will also be required to deposit the data with either of two public repositories: GEO (www.ncbi.nlm.nih.gov/geo/) or Array Express (www.ebi.ac.uk/arrayexpress) and to have the accession numbers available to be published in the article.

Authors of manuscripts with new nucleotide or amino acids sequences are asked to deposit the sequence information with GenBank (National Center for Biotechnology Information, Building 38A, Rm. 8N-803, 8600 Rockville Pike, Bethesda, MD 20894; phone: (301) 496-2475; fax: (301) 480-9241; e-mail for information: info@ncbi.nlm.nih.gov ; e-mail for submission: gb-sub@ncbi.nlm.nih.gov). Authors outside of the United States may elect to deposit sequence information in the European Molecular Biology Laboratory (EMBL) database (e-mail: datasubs@ebi.ac.uk or the DNA Databank of Japan (e-mail: datasub@ddbj.nig.ac.jp). The accession numbers for deposited sequences will be published with the article.

3. PAGE CHARGES

A per-page payment of $60 for the first 6 published pages and $75 for each additional published page will be required for all manuscripts accepted for publication. It is understood at the time of submission that the author(s) agree to pay this charge in the event of publication. Please refer to the section titled

Publication Fees and Reprints for details. Under exceptional circumstances, when no grant or other source of support exists, the author(s) may apply to the Publisher at the time of submission for a waiver of the page charges. All such applications must be countersigned by an appropriate institutional official stating that no funds are available for the payment of page charges.

4. SUBMISSION PROCEDURES

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Cancer Epidemiology, Biomarkers & Prevention requires that submissions be made electronically through the **SmartSubmit** system (cebp.msubmit.net). Complete details on how to submit or resubmit a manuscript can be found when you log on to **SmartSubmit** to create an author account or on the AACR Website (click here for Author Instructions).

Registration

When you register online, you will be asked to provide or select the following:

- Title of the manuscript.
- Running title that does not exceed 50 characters in length to appear at the top of each printed page.
- Full name and affiliations of all authors, complete with first and middle names or initials, but not academic degrees, and contact information for each.
- A journal section that you believe to be the best match for your manuscript. Please note, however, that the final section assignments are made at the discretion of the editors.
- The type of manuscript and an indication of whether or not the paper was invited.
- At least otwo keyword from the pull-down list of terms to classify your manuscript. In addition to the keyword selection, you may also provide your own keywords in the text boxes provided.
- Upload PDFs or other files for Supplemental Data (if necessary).
- Cover letter typed into the box provided. Include a description of the novel and salient findings of the work, as well as any information not covered elsewhere in the submission form.
- Authors are required to recommend at least one Editorial Board member or other referee with appropriate expertise to review the manuscript. A current list of Associate Editors can be found at cebp.aacrjournals.org/misc/edboard.shtml. Final assignment of the

manuscript to peer-review, however, is made at the discretion of the Editors.

- Abstract (not to exceed 250 words) for most types of articles (see **Categories of Publication**) typed into the box provided. Abstracts are often copied directly by the secondary services, so they should recapitulate in abbreviated form the purpose of the study and the experimental technique, results, and interpretations of the data. Include a synopsis of all pertinent data, but do not include references. Avoid abbreviations and acronyms.
- Answers to questions about the manuscript, such as statement of authorship, notification of color reproduction costs, and disclosure of conflicts of interest.

When you have completed the submission form, you will be able to submit your cover letter, manuscript and graphics files, and supplemental data (if necessary). The following are acceptable formats for manuscript files: PDF (for original submissions only; not for revisions), Word, WordPerfect, EPS, text, Postscript, or RTF. The following are acceptable formats for graphics files: TIFF, GIF, JPG, Postscript, or EPS format. Figures/images should NOT be embedded in the manuscript file. PDF files for figures/images are not acceptable.

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Revisions

If you have been asked to revised your paper and you are ready to submit it, log on to the AACR SmartSubmit system (cebp.msubmit.net) and, on your author homepage, click the Revised Manuscript link of the manuscript you wish to resubmit. You will be asked to review the information you originally submitted to confirm its accuracy. In your cover letter, please be sure to provide a point by point reply to the reviewers' comments as well as a listing of the changes made and page numbers where the changes appear.

You should use the rebuttal box to provide a listing of the changes made and page numbers

When you have successfully resubmit your manuscript, you will receive acknowledgement via e-mail.

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The revised version of your manuscript may undergo another review if the original submission required extensive changes or if the authors' responses to

the criticisms entail rebuttal rather than revision. Authors of regular research articles are asked to submit revised versions within 3–4 weeks from the notification of the decision on a manuscript; authors of Advances in Brief are asked to resubmit within 2 weeks. The Editors acknowledge that a longer period might be needed to make the revisions in some cases; however, authors must request an extension by contacting the Publications Office or the resubmission will be considered a new manuscript and it will be subject to all of the conditions of an original submission, including a new manuscript number and a new date of receipt.

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Manuscripts that have been declined for publication will be reconsidered only at the Editor-in-Chief's or Senior Editor's discretion. Authors who wish to request reconsideration of a previously rejected manuscript must do so in writing by sending correspondence that includes the manuscript ID number to the Editorial Office either via e-mail to cebp@aacr.org or via fax to (215) 440-9323. Requests for reconsideration sent to a location other than the Editorial Office will not receive a reply. In the correspondence, please explain in detail the reasons why you feel the paper should be reconsidered. If the Editor-in-Chief or Senior Editor determines that the paper should be reconsidered, you will be asked to submit it electronically, and it will be assigned a new manuscript

identification number and date of receipt, and the paper will undergo review as a new submission.

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Papers are to be written in clear, grammatical English and must be typed double-spaced. Papers that are not in *CEBP* style or that are not in good idiomatic English may be returned to the author without review. Laboratory jargon as well as terminology and abbreviations not consistent with internationally accepted guidelines should be avoided.

The AACR journals generally conform to usage guidelines in the following publications: *Stedman's Medical Dictionary* (Twenty-seventh Edition, 2000, Lippincott Williams & Wilkins, Baltimore, MD); *CBE Style Manual* (Sixth Edition, 1994, published by the Council of Biology Editors, Inc., Northbrook, IL). For general guidance on manuscript preparation, consult the documents issued by the International Committee of Medical Journal Editors (www.icmje.org).

Large data sets of peripheral significance to the main thesis of the investigation will not be published in *CEBP* but may be published in the Data Supplements section of *CEBP* online. Contact the AACR Publications Department for more information. Supplementary data should be submitted for review with the manuscript.

Manuscripts should be arranged in the following order: title, author(s) and complete name(s) and location(s) of institution(s) or laboratory(ies), running title, key words, footnotes, text and references, tables, legends for all illustrations, illustrations, and other material. Numbered and lettered sections in the text should be avoided. Each table and illustration must be cited in order in the text. Simple chemical formulas or mathematical equations should be presented in a form that allows their reproduction in single horizontal lines of type; more complicated mathematical formulas or chemical structures difficult to set in type should be provided for reproduction in the form of line drawings, glossy photographs, or digital files (see **Illustrations** for more details).

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- Genetic Epidemiology
- Molecular Epidemiology
- Biomarkers in Prevention
- Nutrition in Prevention
- Behavior in Prevention
- Chemoprevention
- Screening and Early Detection

- Survivorship and Supportive Care
- Statistical Methods and Models

Title. Write a brief, informative title limited, if possible, to about 100 characters. It is important for literature retrieval to include in the title the key words that identify the nature of the subject matter, including, if applicable, the species on which the work is done. Avoid expressions such as “Studies on . . .” or “Observations of . . .”, and avoid chemical formulas or abbreviations. Also, do not use Roman or Arabic numerals to designate that the paper is part of a series (see section below on **Footnotes**).

Authors and Affiliations. Authors are urged to include their full names, complete with first and middle names or initials. Academic degrees should not be included. The names and locations of institutions and the laboratories or names and locations of companies should be given. If several institutions are listed on a paper, it should be clearly indicated with which department and institution each author is affiliated by using superscript numbers that correspond to each author’s affiliation.

Include the following footnotes to the title page (if applicable) in this order:

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- Full name, mailing address, and e-mail address (optional) of the person to whom reprint requests should be sent.
- Other notes about the paper as a whole (whether part of a series, conflict of interest statements, etc.).

Running Title. A brief running title of about 50 characters should be provided.

Key Words. On the title page, provide five key words identifying the subject of your article.

Footnotes. In most instances information should be presented in the text, not in footnotes. If footnotes are necessary, use superscript Arabic numerals consecutively throughout the text.

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References Examples

Giovannucci E, Pollak M, Liu Y, et al. Nutritional predictors of Insulin-like Growth Factor I and Their Relationships to Cancer in Men. Cancer Epidemiol Biomarkers Prev 2003;12:84–9.

Yuspa SH, Hennings H, Roop D, Strickland J, Greenhalgh DA. Genes and mechanisms involved in malignant conversion. In: Harris CC, Liotta LA, editors. Genetic mechanisms in carcinogenesis and tumor progression. New York: Wiley-Liss; 1990. p.115–26.

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Standard Abbreviations. Authors may use, without definition, abbreviations of units of measure when they are used with units (1.5 cm). The following are examples of standard abbreviations that may be used in the text, without explanation:

ABVD*	Adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine
ADP, dADP	
AMP, dAMP	
AATP, dATP	
bp	base pair(s)
CDP, cCDP	
CMP, cCMP	
CNBr	cyanogen bromide
cDNA	complementary DNA
CoA	coenzyme A
CTL	cytotoxic T-lymphocyte
CTP, dCTP	

cyclic ADP	adenosine 3[prime],5[prime]-diphosphate
cyclic AMP	adenosine 3[prime],5[prime]-monophosphate
DEAE	diethylaminoethyl
DNA, cDNA	
EDTA	ethylenediaminetetraacetic acid
EGTA	ethyleneglycotetraacetic acid
EtOH	etomidate
GDP, dGDP	
GMP, dGMP	
GTP, dGTP	
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid or
	N-2-hydroxyethylpiperazine-*N*[prime]-2-ethanesulfonic acid
IDP, dIDP	
IMP, dIMP	
IR	infrared
ITP, dITP	
MOPP	mechlorethamine, oncovin (vincristine), procarbazine, and prednisone
NAD^+	NADH
$NADP^+$	nicotinamide adenine dinucleotide phosphate (oxidized form)

NADPH	nicotinamide adenine dinucleotide phosphate (reduced form)
PBS	phosphate-buffered saline
PIPES	1,4-piperazinediethanesulfonic acid
POPOP	1,4-bis[lsqb]2-(5-phenyloxazolyl)[rsqb]benzene
PPO	2,5 diphenyloxazole
Pi, Ppi	orthophosphate, pyrophosphate
R_f	retardation factor
RFLP	restriction fragment length polymorphism
RIA	radioimmunoassay
RKO cells	
RNA, aRNA, mRNA, nRNA, tRNA	
Rnase, DNase	
RPMI	
Roswell Park Memorial Institute	
rRNA	spell out as "Ribosomal RNA" in title only
SD	standard deviation
SDS	sodium dodecyl sulfate

SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	standard error of the mean
SSC	standard saline-citrate
SV40	Simian Virus 40
TBS	Tris-buffered saline
TDP, dTDP	
TEAE	triethylaminoethyl
TMP, dTMP	
TTP, dTTP	
Tris	tris(hydroxymethyl)aminomethane and tris(hydroxymethyl)methylamine
UDP, dUDP	
UMP, dump	
UTP, dUTP	
USP units	
USPHS	United States Public Health Service
UTP	uridine 5c-triphosphate
UV	ultraviolet
w/v	weight per volume
w/w	weight for weight

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