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Avaliação da eficácia do sal de cozinha na redução da permeabilidade dentinária: estudo ultraestrutural

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RESUMO | Numerosos agentes dessensibilizantes têm sido utilizados para alívio da dor decorrente da hiperestesia dentinária. Este trabalho é resultado de um estudo *in vivo* que comprovou que o sal de cozinha pode ser utilizado como agente dessensibilizante da dentina. Entretanto, não se conhece os possíveis mecanismos que justificariam sua eficiência como agente anti-hiperestésico. Assim, com objetivo de investigar o possível efeito da solução à base de NaCl (sal de cozinha) na redução do diâmetro dos túbulos dentinários, realizou-se um estudo *in vitro* no qual se mensurou o diâmetro dos túbulos dentinários de discos de dentina submetidos a uma solução à base de NaCl. Foram selecionados cinco terceiros molares humanos hígidos, não irrompidos, extraídos de pacientes jovens, com idade entre 19 e 25 anos, provenientes de consultórios particulares do município de Belo Horizonte (MG). Discos de dentina foram obtidos pela secção transversal da coroa dentária em duas regiões, de forma a constituírem discos de aproximadamente 1 milímetro de espessura. O grupo controle foi tratado com ácido fosfórico a 37% por 15 segundos e, em seguida, lavado com água destilada durante 30 segundos. Os grupos experimentais receberam o mesmo tratamento com ácido e, em seguida, um foi imerso por 2 e o outro por 4 minutos, ambos em solução à base de NaCl. Os espécimes foram então analisados por microscópio eletrônico de varredura (MEV). O grupo tratado com NaCl por 2 minutos não apresentou obliteração dos túbulos dentinários, já o tratado por 4 minutos apresentou redução significativa do diâmetro na maioria dos túbulos dentinários, sendo que em alguns, inclusive, a obliteração foi total.

DESCRITORES | Dentina; Cloreto de Sódio; Microscopia Eletrônica de Varredura.

ABSTRACT | **Evaluation of the effectiveness of table salt in reducing dentin permeability: an ultra-structural study** • Many desensibilizing agents have been used to decrease pain due to hypersensitive dentine. Studies *in vivo* show that NaCl may be used as a dentine desensibilizing agent – in addition to its effect on the reduction of pain, it would be a simple, low cost and very easy procedure. However, the mechanisms of NaCl as a desensibilizing agent are yet unknown. To investigate the potential effect of NaCl in the reduction of the dentin tubules diameter, an *in vitro* study was performed, in which the dentin tubules diameter submitted to a NaCl solution were measured. Five non erupted third molars were selected. They were extracted from young patients (age range from 19 to 25 years old) in private dental offices in Belo Horizonte, Brazil. Dentine discs were obtained by transversal section of the crown in two parts, in order to obtain discs 1 millimeter thick. The dental discs in the control group were treated with a 37% phosphoric acid solution for 15 seconds and then washed with distilled water for 30 seconds. The dental discs in the experimental groups received the same treatment with acid and, afterwards, one group was emerged in a NaCl solution for 2 minutes, and the other one was emerged for 4 minutes in a similar solution. The material was analyzed by a scanning electron microscope (SEM). The group treated with NaCl for 2 minutes did not show a decrease in the dentin tubules diameter, but the group treated with NaCl for 4 minutes showed an expressive reduction of the dentin tubules diameter, with a few tubules being totally obliterated.

DESCRIPTORS | Dentine; Sodium Chloride; Electronic Scanning Microscopy.

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INTRODUÇÃO

A hiperestesia dentinária é um dos eventos clínicos odontológicos mais desconfortáveis para o paciente e de mais difícil solução¹. Segundo Krüger² (2001), somente na América do Norte, 40 milhões de adultos apresentam hiperestesia dentinária de modo esporádico e 10 milhões de modo crônico. As dificuldades parecem iniciar-se com a própria terminologia empregada, sendo citados na literatura vários termos para caracterizar essa mesma manifestação sensorial da dentina³.

A hiperestesia dentinária está associada à exposição da dentina à cavidade bucal, e é caracterizada por uma dor aguda, bem marcada e transitória, causada por estímulos táteis, térmicos, evaporativos e osmóticos^{3,4}.

Entre as várias teorias que explicam o mecanismo de transmissão da dor pela dentina, a mais aceita é a teoria hidrodinâmica de Brännström⁵ (1966). Assim, a grande variedade de estímulos induz a movimentação do fluido ao longo da dentina e, conseqüentemente, excita os mecanorreceptores na periferia da polpa, causando dor⁶. Com base nessa teoria, o bloqueio dos túbulos dentinários reduz a permeabilidade, o que provoca redução da hiperestesia dentinária⁷.

De acordo com Matias *et al.*⁸ (2010), um agente anti-hiperestésico dentinário deve preencher todos os critérios preconizados por Grossman⁹ (1935), como promover efeito imediato e duradouro, ser de fácil aplicação, não ser irritante à polpa, ser bem tolerado pelo paciente, além de ser de baixo custo e não manchar o dente.

Fonseca *et al.*¹⁰ (2001) avaliaram clinicamente o efeito do sal de cozinha no tratamento da hiperestesia dentinária e concluíram que o uso de solução à base de NaCl, na escovação dentinária diária, diminui a hiperestesia. Apesar de detectada essa diminuição, não foram avaliados os mecanismos pelos quais ocorreria a redução da dor; sendo assim, foi necessário avaliar os parâmetros *in vitro*

que justifiquem a redução da hiperestesia, para que posteriormente essa técnica possa ser aplicada com segurança na clínica odontológica. Ademais, como exposto por Martinelli¹¹ (2001), na maioria das pesquisas científicas, o uso de um modelo de laboratório para ilustrar a operação de um mecanismo postulado e testar sua validade é essencial para o entendimento da natureza das observações clínicas.

Anteriormente, Cuenin *et al.*¹² (1991) já haviam demonstrado, após análise por MEV, que a solução de NaCl de baixo pH pode ser um forte adjunto no tratamento da hiperestesia dentinária, cuja redução é mais efetiva do que com oxalato de potássio. Porém, a solução de NaCl utilizada na preparação do sal de cozinha apresenta ainda, em pequena concentração, iodeto de potássio, ferrocianeto de sódio e alumínio silicato de sódio¹³.

Com base nesses requisitos, este estudo tem como objetivo avaliar qualitativa e quantitativamente o padrão de obliteração dos túbulos dentinários de discos de dentina submetidos a uma solução à base de NaCl.

MATERIAIS E MÉTODOS

Desenho do estudo

Foi realizado um estudo experimental cego, comparando três grupos compostos por segmentos de discos de dentina que posteriormente foram analisados por MEV.

Amostra do estudo

Foram avaliados cinco discos de dentina, cada um dividido em três segmentos, originando quinze amostras. Esses discos foram obtidos de cinco terceiros molares humanos hígidos não irrompidos, extraídos – depois de assinado o Termo de Consentimento Livre e Esclarecido (TCLE) – de cinco pacientes com idade entre 19 e 25 anos, provenientes de consultórios particulares de Belo Horizonte. Os dentes foram

armazenados em água destilada, a uma temperatura de aproximadamente 4 °C. O tempo máximo de armazenamento dos dentes foi de 30 dias após a data da extração¹⁴. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Protocolo 272/07.

Procedimentos laboratoriais

Discos de dentina foram obtidos pela seção transversal da coroa dentária em duas regiões: uma acima da projeção dos cornos pulpaes e outra abaixo da junção amelodentinária, de forma a constituírem discos com a região central composta exclusivamente por dentina^{7,15}. Foram obtidos cinco discos de aproximadamente 1 milímetro de espessura. As seções foram realizadas com discos de diamante adaptados ao mandril e acoplados à peça reta.

Os discos foram submetidos a desgaste complementar para regularização e acabamento de suas superfícies, utilizando lixa de água de granulação 400 seguida de granulação 600 sob pressão digital⁷. Duas canaletas, paralelas entre si, correspondentes aos cornos pulpaes mesiais e distais, respectivamente, foram realizadas com ponta diamantada esférica nº 1014, para orientar a direção de fratura dos espécimes, e, a seguir, foram quebradas em três segmentos iguais com a ajuda de um cinzel dental, originando quinze segmentos de discos de dentina. A parte central de cada um dos discos foi agrupada e considerada grupo controle (G_1). As extremidades formaram dois grupos experimentais (G_2 e G_3), cada um composto por cinco segmentos. Os espécimes foram mantidos em água destilada a 4 °C durante 3 dias, até o momento da realização dos ensaios.

O grupo controle foi tratado com ácido fosfórico a 37% por 15 segundos e depois lavado com água destilada durante 30 segundos, a fim de expor a abertura dos túbulos dentinários¹⁶.

Os grupos experimentais foram condicionados com ácido fosfórico a 37% (Dentsply Ind. Com. LTDA, Petrópolis, Rio de Janeiro) por 15 segundos,

lavados por 30 segundos e imersos em uma solução saturada (5 ml/1 g) à base de cloreto de sódio (sal de cozinha). Posteriormente, foram lavados por 5 segundos para interromper o tratamento.

Os segmentos do grupo G_2 foram submersos em solução à base de cloreto de sódio por 2 minutos, e os do grupo G_3 por 4 minutos.

Os segmentos foram submetidos ao procedimento de secagem em estufa a 37 °C por 12 horas para ser desidratados¹⁷, depois foram armazenados em três placas de Petri, cada uma das quais tendo recebido um grupo definido no estudo (G_1 , G_2 ou G_3). Depois de desidratados, foram selecionados aleatoriamente dois segmentos de discos de dentina de cada um dos grupos nos quais foi realizada a análise microscópica. Os espécimes receberam recobrimento condutor com ouro e foram analisados pelo MEV Jeol JSN 6360LV no Laboratório de Microscopia Eletrônica do Departamento de Engenharia Metalúrgica e de Materiais (DEMET) da Universidade Federal de Minas Gerais (UFMG).

Depois de obtidas as imagens, foram selecionadas, aleatoriamente, sete áreas de cada amostra, e em cada área foi feita a medição de dez túbulos dentinários, realizada manualmente sempre pelo mesmo examinador, utilizando uma régua e tendo centímetro como unidade de medida. Foram realizadas duas medições em cada túbulo, uma transversal e uma longitudinal, que ia de uma periferia a outra do túbulo. Posteriormente, calculou-se a média das medidas de cada túbulo e obteve-se um único valor para o diâmetro de cada. Esse valor, dado em centímetros, foi convertido em micrômetros, tendo por base a referência fornecida nas imagens microscópicas, e teve-se que 3 cm correspondia a 5 μ m. Foram obtidos a média e o desvio-padrão de cada uma das amostras.

Foi selecionada aleatoriamente uma área do grupo experimental, depois feita uma microanálise de raio-X por espectroscopia de energia dispersiva (EDS, NORAN Voyager – Altran Corporation,

Boston, USA) pela emissão dos raios-x a uma voltagem de 15 KV na parede do túbulo dentinário.

Análise estatística

A análise descritiva abordou medidas de tendência central e de variabilidade. Na análise comparativa, utilizou-se o teste t de Student para comparação de médias entre grupos, e, na análise dos dados, o software Epi Info 6.04® (Center of Disease Control – EUA, 2001. Disponível em <http://www.cdc.gov>).

Pela microanálise de raios-x, o software SpectraPlus® (Thermo NORAN) executou a análise qualitativa, identificando os elementos químicos do espécime, e a semiquantitativa, estimando percentualmente o teor desses elementos.

A análise qualitativa, avaliada por inspeção visual, demonstrou que o tratamento com solução à base de NaCl por 2 minutos não alterou, como esperado, o diâmetro dos túbulos dentinários, por isso, foram consideradas para análise quantitativa somente as amostras submetidas ao tratamento com solução à base de NaCl por 4 minutos.

RESULTADOS

Ocorreram modificações estruturais visíveis, como podem ser observadas nas ilustrações

representativas do tratamento de discos de dentina com solução à base de NaCl pela análise qualitativa (Figura. 1).

O diâmetro dos túbulos dentinários do grupo controle (G₁) apresentou amostras-controle 1 e 2 com médias de 2,05 µm e 2,28 µm e desvio-padrão de 0,41µm e 0,47µm, respectivamente.

O diâmetro dos túbulos dentinários do grupo experimental (G₃) apresentou amostras experimentais 1 e 2 com médias de 1,07 µm e 1,21 µm e desvio-padrão de 0,94 µm e 0,95 µm, respectivamente. Por esses resultados, observou-se que, em relação às médias do diâmetro dos túbulos dentinários, comparando o grupo controle A1 com o grupo experimental A1, há uma diferença estatisticamente significativa ($T = 2,703$; $p < 0,05$). O mesmo se verificou quando comparados o grupo controle A2 e o grupo experimental A2 ($T = 16,212$; $p < 0,05$). Essa análise também detectou diferença estatisticamente significativa (Tabela 1) no padrão de obliteração dos túbulos dentinários após tratamento por 4 minutos com solução à base de NaCl quando comparado ao grupo controle.

A microanálise qualitativa de raios-x, realizada nos espécimes dentinários tratados com solução à base de NaCl, indicou presença de sódio e cloreto na parede do túbulo dentinário (Gráfico 1).

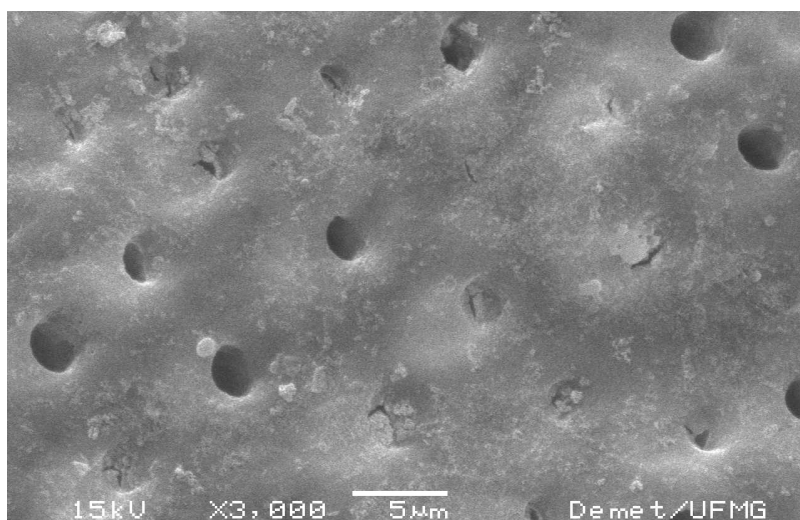


FIGURA 1 | Aparência da dentina após tratamento com solução a base de NaCl por 4 minutos. Observa-se obliteração de túbulos dentinários (setas).

A porcentagem dos elementos também foi fornecida pela microanálise, mostrando valores variáveis para os principais componentes presentes

na amostra. Como mostra a Tabela 2, foram observadas pequenas porcentagens de sódio e cloreto.

TABELA 1 | Comparação entre média (µm) e desvio-padrão dos grupos controle (G1) e experimental (G3).

Grupos	Amostra 1		Amostra 2	
	Média±DP	Teste t* (valor p) **	Média±DP	Teste t* (valor p) **
G1	2,05 ± 0,41	2,70 (p < 0,05)	2,28 ± 0,47	16,21 (p < 0,05)
G3	1,07 ± 0,94		1,21 ± 0,95	

* Teste t de Student

** valor p (nível de significância de 5%)

GRÁFICO 1 | Espectro de raios X mostrando picos característicos dos elementos na parede do túbulo dentinário em níveis específicos de energia, após tratamento da dentina com solução a base de NaCl.

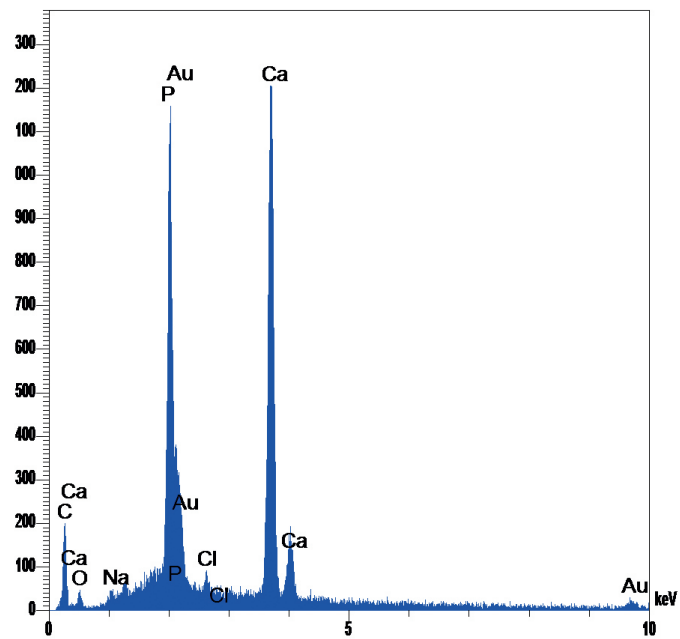


TABELA 2 | Porcentagem dos elementos químicos encontrados na parede do túbulo dentinário após tratamento da dentina com solução a base de NaCl, através da microanálise semiquantitativa de raios X.

Elt	XRay	Int	Error	K	Kratio	W%	A%	ZAF
O	Ka	10.6	0.5947	0.0152	0.0128	9.59	19.32	7.5078
Na	Ka	7.8	0.5105	0.0052	0.0044	0.89	1.25	2.0383
P	Ka	425.4	3.7658	0.3250	0.2731	30.11	31.33	1.1025
Cl	Ka	33.1	1.0509	0.0316	0.0266	3.15	2.86	1.1861
Ca	Ka	474.0	3.9748	0.6229	0.5235	56.25	45.23	1.0746
				1.0000	0.8403	100.00	100.00	

DISCUSSÃO

Após tratamento com solução à base de NaCl por 4 minutos, detectou-se redução significativa no diâmetro dos túbulos dentinários, em grande parte dos quais a obliteração foi total. Resultados semelhantes foram descritos por Arrais¹⁷ (2004), Al-Sabbagh *et al.*¹⁸ (2009) e Sun *et al.*¹⁹ (2010), que avaliaram respectivamente o uso de oxalato de potássio, hidróxido de cálcio e fosfato de cálcio, e, no tratamento da hiperestesia dentinária, o parâmetro de obliteração dos túbulos dentinários.

Segundo Garone Filho²⁰ (1996), a obliteração dos túbulos é obtida pela combinação de um sal solúvel com o líquido dos túbulos dentinários, reagindo com íons cálcio e potássio provenientes da dissolução da hidroxiapatita que constitui as paredes dos túbulos dentinários. Essa reação resulta em compostos insolúveis que se precipitam como cristais e obliteram a embocadura dos túbulos, eliminando a hiperestesia dentinária. Esse é, possivelmente, um dos mecanismos pelos quais a solução à base de NaCl poderia causar a obliteração dos túbulos dentinários. Pela microanálise de raios-x foi avaliada a captação de compostos pela dentina, caracterizando a natureza dos depósitos em sua superfície, observando-se picos de sódio e cloreto presumidamente provenientes da solução à base de NaCl.

Grande variabilidade no diâmetro dos túbulos dentinários tratados com NaCl determinou alto desvio-padrão do grupo experimental, o que talvez se deva a irregularidades na superfície dos discos – dessa forma, áreas mais irregulares aderiram maior quantidade de componentes presentes na solução à base de NaCl. Além disso, o secionamento transversal de túbulos dentinários orientados em vários planos pode proporcionar diferenças em seu padrão de obliteração²¹.

O grupo controle apresentou túbulos dentinários com diâmetros heterogêneos, mas todos os túbulos foram desobstruídos, provavelmente pelo condicionamento ácido realizado. Pesquisas

in vitro, utilizando ácido fosfórico como controle, apresentaram resultados semelhantes a esses^{16,22}, o que demonstra a efetividade da aplicação de ácido fosfórico a 37% por 15 segundos na desobstrução dos túbulos dentinários.

Aranha²³ (2011) descreveu que o ácido fosfórico a 37% por 15 segundos é capaz de remover a camada de *smear layer* e proporcionar um padrão de dentina hiperestésica. Assim, pode-se determinar que as amostras condicionadas com ácido fosfórico possuem padrão de hiperestesia, já que pacientes com superfícies dentinárias com túbulos abertos demonstram sintomatologia dolorosa. As variações na aparência da superfície da dentina, ao longo de um mesmo disco, ocorrem apesar dos túbulos serem de tamanho similar, pois estão orientados em vários planos: oblíqua e paralelamente à superfície²¹. Dessa forma, o condicionamento ácido da dentina é afetado pelo tipo de seção transversal dos túbulos na superfície, pois os túbulos grandes, redondos e verticais são condicionados mais proporcionalmente do que os pequenos, ovais e horizontais. Assim, a heterogeneidade determinada em decorrência do condicionamento ácido dos túbulos dentinários do grupo controle provavelmente se deve à variedade inerente à dentina.

É de se ressaltar que todas as técnicas têm benefícios e limitações, portanto os resultados devem ser sempre tratados com cautela, especialmente quando se refere a estudos *in vitro*.

Uma hipótese ainda a ser estudada é a possibilidade de que a solução à base de NaCl combine as propriedades de oclusão dos túbulos dentinários com a inibição da atividade neural, o que é suportado por Erdemir *et al.*²⁴ (2010), que, estudando a ação do nitrato de potássio no tratamento da dentina hiperestésica, descreveram que o aumento da concentração de potássio extracelular altera o mecanismo da bomba de Na⁺ e K⁺, bloqueando a percepção dos mecanorreceptores aos movimentos do fluido dentinário e, portanto, dificulta a passagem

do estímulo doloroso. Dessa forma, sugere-se que o aumento da concentração de sódio possa atuar também por esse mecanismo.

CONCLUSÃO

Considerando os fatores avaliados e fundamentados nos resultados obtidos por este estudo, pode-se sugerir que o sal de cozinha reduz o diâmetro dos túbulos dentinários, podendo assim diminuir a permeabilidade da dentina.

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Comparison of p16ink4a immunostaining in benign and malignant HPV-related lesions

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ABSTRACT | *Objective:* To analyze the difference between p16ink4a immunostaining in normal epithelium, two benign HPV-related lesions (papilloma and condyloma acuminatum), and one malignant HPV-related lesion (oropharynx carcinoma). *Methods:* Five normal oral mucosas, fifteen papilloma, fifteen condyloma acuminatum, and fifteen HPV-positive oropharynx squamous cell carcinoma were included in this study. The histological sections were stained with anti-p16ink4a by immunohistochemistry. For the positive stain, the score was based on a scale of - to 3+, as follows: - negative stain; 1+ less than 25% of positivity and focal distribution; 2+ 26-50% of positivity and focal distribution; and 3+ 50-75% of positive cells and diffuse distribution. The evaluation of the intensity score was based on: - negative; 1- low intensity; 2- moderate intensity; 3- intensive. *Results:* The results showed no significant differences between the scores (positive x intensity) of p16ink4a in normal epithelium, papilloma, and condyloma acuminatum. All benign lesions and normal epithelium showed significant differences when compared with the oropharynx squamous cell carcinoma. *Relevance:* There are differences in the expression of p16ink4a between benign HPV-lesions and malignant HPV-lesions.

DESCRIPTORS | HPV; Papillomaviridae; p16; Condylomata Acuminata; Neoplasms of Squamous Cell.

RESUMO | **Comparação da marcação de p16ink4a por imuno-histoquímica entre lesões benignas e malignas positivas para HPV** • *Objetivo:* Analisar a diferença da marcação imuno-histoquímica de p16ink4a no epitélio normal, em duas lesões benignas associadas ao HPV (papiloma e condiloma acuminado) e uma lesão maligna relacionada ao HPV (carcinoma espinocelular de orofaringe). *Material e métodos:* Cinco mucosas normais, quinze papilomas, quinze condilomas acuminados e quinze carcinomas espinocelulares (CEC) positivos para HPV foram incluídos neste estudo. Os cortes histológicos foram corados com antip16ink4a por imuno-histoquímica. Para a marcação positiva, o escore foi baseado numa escala de - a 3+, sendo: - marcação negativa; 1+ menos do que 25% de positividade e distribuição focal; 2+ 26-50% de positividade e distribuição focal; 3+ 50-75% de células positivas e distribuição difusa. A avaliação da pontuação da intensidade baseou-se em: - negativo; 1- baixa intensidade; 2- intensidade moderada; 3- intensiva. *Resultados:* Os resultados não mostraram diferenças significativas entre os escores (intensidade x positivo) de p16ink4a do epitélio normal, papiloma e condiloma acuminado. Todas as lesões benignas e o epitélio normal mostraram diferenças significativas quando comparadas com o CEC de orofaringe. *Relevância:* Há diferenças na expressão de p16ink4a entre lesões benignas e malignas positivas para HPV.

DESCRIPTORES | HPV; Papillomaviridae; p16; Condiloma Acuminado; Neoplasias de Células Escamosas.

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INTRODUCTION

Human papilloma virus (HPV) is a DNA virus of the *Papillomaviridae* family with approximately 7900 base pairs. There are over 150 different types divided into low-risk (e.g. HPV6 and HPV11) and high-risk (e.g. HPV16 and HPV18) HPV. Oral HPV transmission is not fully understood and assumptions include a subclinical infection, vertical transmission, and horizontal transmission.¹ Horizontal transmission seems to be the most common route, including sexual transmission (orogenital contact). The self-inoculation virus from other skin sites or anogenital contact is also considered.² The infection occurs in proliferating cells (basal cells) of a stratified squamous epithelium through micro-wounds.³ After the infection, the HPV genome can integrate in the nucleus as episome and replicate in synchrony with the host cellular DNA replication.⁴

The HPV genome has early genes, whose primary function is the episomal replication, and late genes, which encode the proteins of viral capsid. The early genes are divided into E1 to E7 and late ones into L1 / L2. The products of E1 and E2 genes are more specifically related to regulation of transcription and replication of the viral proteins.^{5,6}

When there is integration of viral genome into the host cell genome, the genes may be altered or eliminated, leading to uncontrolled transcription of E6 and E7.¹ Such oncoproteins are capable of interfering with important mechanisms controlling cell cycle and apoptosis and maintenance of chromosomal stability, deregulating the cell cycle control and inducing the cells into the S phase.^{3,7} It is believed that tumor cells result from the deregulation of two major cell cycle control pathways: the p53 pathway and pRB pathway.⁸

The cell cycle is controlled by cyclins, cyclin dependent kinases (CDKs), and their inhibitors. There are two main inhibitor groups: Cip / Kip (p21, p27, and p57) and INK / ARF (p16 and p14).⁹ These groups function as tumor suppressors and

are often altered in tumors. These proteins bind to the complex cyclin / inactivate CDK and thus stop the cell cycle. p16ink4a is a protein encoded by the CDKN2A gene and acts as a tumor suppressor. P16 inhibits CDK4 (cyclin-dependentkinase 4), an important protein kinase during the G1 phase of the cell cycle. This kinase is responsible for the phosphorylation of retinoblastoma (Rb) gene, a tumor suppressor.¹⁰ Mutations in CDK4 and changes of p16 and Rb may be present in carcinomas, particularly cancers associated with HPV. High-risk E7 binds to and inactivates Rb, leading to an upregulation of p16. For this reason, the overexpression of this protein is target for identifying HPV positive cases.¹¹ We aim to analyze the difference between p16inka immunostaining in normal epithelium, two benign HPV-related lesions (papilloma and condyloma acuminatum), and one malignant HPV-related lesion (oropharynx carcinoma).

MATERIAL AND METHODS

Tissue samples

Ethics approval was obtained from the Human Research Ethics Committee (Protocol number: 10641412.2.0000.0075). Oral HPV-related diseases, diagnosed at the Oral Pathology Diagnostic Service of the School of Dentistry of University of São Paulo, were retrieved and fifteen papilloma, fifteen condyloma acuminatum, and fifteen HPV-positive oropharynx squamous cell carcinomas (OSCCs) were included in this study. Five normal oral mucosas were used as control. The HPV-associated OSCCs were chosen based in the intense positivity of p16ink4a, overexpression in more than 70% of the cells¹² and with positive cytoplasm and nucleus. The OSCCs demonstrating weak or no positivity were excluded from this study. The histological sections were first stained with H&E and reviewed by two observers. Then, the material was used for the IHQ.

Immunohistochemistry for p16ink4a

Three-micrometer (3 µm) serial sections from formalin-fixed paraffin-embedded blocks were obtained and mounted on poly-L-lysine-coated glass slides. Immunostaining was performed by the linked streptavidin-biotin horseradish peroxidase technique (LSAB-HRP) (Universal LSAB®+ Kit/HRP, DAKO Carpinteria, CA, USA). The samples were deparaffinized in xylene followed by hydration in descending ethanol grades and then treated with 0.3% H₂O₂ and 100% methanol for 15 minutes to quench endogenous peroxidase. Antigen retrieval was performed by heating specimens for 30 minutes at 95°C in sodium citrate. The sections were incubated overnight at 4°C with primary monoclonal anti-p16ink4a antibody (1:1000; Abcam 54210, clone2D9A12, Cambridge, MA, USA). After three washes with Tris buffer, the slides were treated with biotinylated species-specific secondary antibodies and streptavidin-biotin enzyme reagent (Universal LSAB®+ Kit/HRP, DAKO Carpinteria, CA, USA). The color was developed with 3,3'-diaminobenzidine tetrahydrochloride chromogen solution (Liquid Dab+ Substrate-Chromogen System, Dako, Carpinteria, CA, USA). Sections were counterstained with Mayer's haematoxylin (Sigma-Aldrich, St. Louis, MO, USA). Rat's liver was used as a positive control. The results were evaluated separately by three investigators.

Immunohistochemistry analysis

For the evaluation of the p16INK4a immunostaining, a semi-quantitative analysis was performed using the scoring method for both intensity and positivity. Five randomly chosen areas were selected and analyzed in conventional light microscope (Olympus CH2 Olympus Optical Co. Ltd., Japan) with 400x magnification. An analysis of expression of p16INK4a was made based on the intensity distribution and on the proportion of cells positive for this marker.

Immunoreactivity was classified by a semi-quantitative score for both positivity and intensity of staining and the results were multiplied (positive x intensity).¹³ For the positive stain, the score was based on a scale of - to 3+, as follows: - negative stain; 1+ less than 25% of positivity and focal distribution; 2+ 26-50% of positivity and focal distribution; and 3+ 51-75% of positive cells and diffuse distribution; 4+ ≥76% of positive and diffuse distribution.¹⁴ The evaluation of the intensity score was based on: - negative; 1- low intensity; 2- moderate intensity; 3- intensive. The nuclear and cytoplasm stain analysis were scored as positive or negative stain.

Statistical analysis

The data were analyzed using the GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). The results were analyzed first by the D'Agostino-Pearson omnibus test of normality. Results were not parametric, therefore we used Kruskal-Wallis test and Dunn's post-hoc test.

RESULTS

p16ink4a immunoscore was analyzed according to intensity, immunostain and distribution, and nuclear and cytoplasmic stain. The number of cases showing each score from samples of normal epithelium, papilloma, condyloma acuminatum, and oropharynx squamous cell carcinoma are shown in Table 1.

The reason for intensity and distribution analysis is that the positive staining of p16 is present and diffuse in all sections, but intensity was noticed to be different in each lesion group (Figure 1).

Despite papilloma showing a diffuse stain of p16ink4a, the intensity was low, especially when compared to squamous cell carcinoma of the oropharynx, which also shows scattered positive cells, but with a strong intensity (nucleus and cytoplasm) (Figure 1).

Table 1 | P16ink4a expression patterns in normal mucosa, papilloma, condyloma acuminatum, and oropharynx squamous cell carcinoma.

	P16ink4a Intensity			P16ink4a Stain					Nuclear Stain	Cytoplasmic Stain
	1	2	3	0	+1	+2	+3	+4	+ or -	+ or -
Normal (5)	5	0	0	0	1	2	0	1	+	-
Papilloma (15)	7	7	1	0	6	5	0	4	+	-
Condyloma(15)	2	10	3	0	2	3	1	9	+	-
OPSCC (15)	0	0	15	0	0	0	1	14	+	+

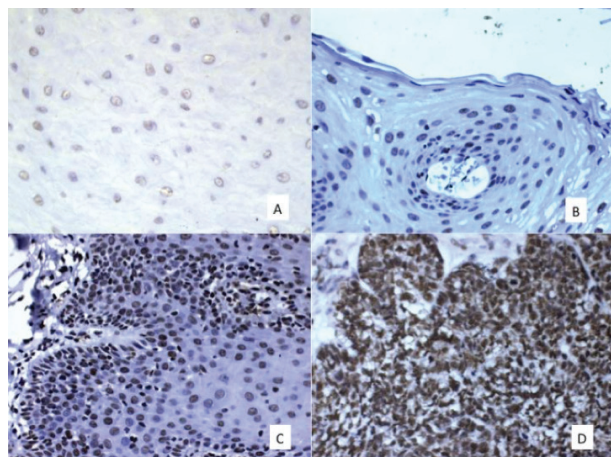


Figure 1 | p16ink4a immunohistochemistry stain. A. Normal epithelium; B. Papilloma; C. Condyloma; D. Oropharynx squamous cell carcinoma.

The results showed no significant differences between the scores (positive x intensity) of p16ink4a in normal epithelium (Figure 1A), papilloma (Figure 1B), and condyloma acuminatum (Figure 1C). Hence, normal mucosa and benign lesions showed significant differences in p16ink4a staining when compared with the oropharynx squamous cell carcinoma (Figure 1D).

The differences of p16ink4a immunostaining (intensity x distribution) between normal mucosa, benign, and malignant HPV-related lesions are shown in Figure 2.

DISCUSSION

The presence of HPV DNA in a tissue, by itself, does not mean that it will induce cancer. There are hundreds of different types of HPV, which are divided into low-risk and high-risk. Only the DNA of high-risk HPV, transcribed, which demonstrates to

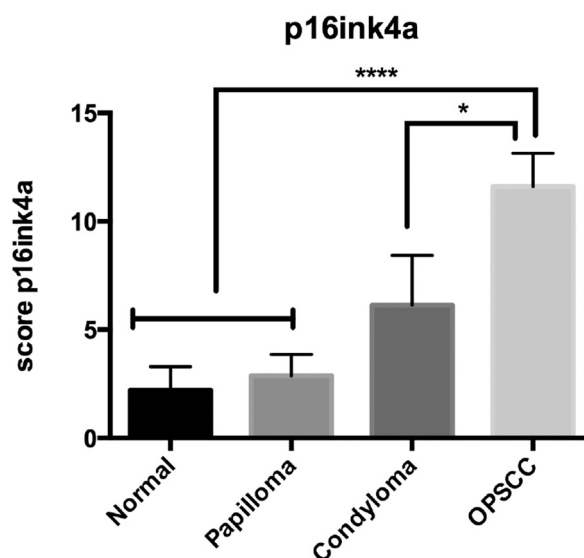


Figure 2 | Comparison of p16ink4a immunostaining between normal mucosa, benign, and malignant HPV-related lesions: OPSCC indicating significant differences when compared to normal mucosa, papilloma, and condyloma (* $p < 0.05$; **** $p < 0.0001$).

be active, has clinical and biological relevance in the origin of HPV-OSCC.¹⁵ This is represented when HPV induces molecular changes resulting in malignant transformation.

p16-IHC has been used to detect high-risk HPV because of its sensitivity and because it does not only indicate the presence of HPV but also its activity, since the overexpression of p16 is caused by HPV oncoproteins.^{12,14} In situ hybridization technique is a less sensitive method than PCR for HPV DNA detection, but more specific than p16 immunostaining in the detection of HPV in carcinomas.⁷ But some authors believe that conventional HPV ISH nonspecific background is common and the test lacks sensitivity,^{14,16} as well as it

is more expensive than IHC. Thus, the use of IHC is preferable.¹⁴

HPV interacts into the host genome and expresses the replication transcription factors E2, E6, and E7 oncoproteins, which repress transcription factors.³

Both low-risk and high-risk express E6 and E7, but, in the low-risk, E7 does not strongly affect the centrosome homeostasis. Still in the high-risk, E6 and E7 sequestrate the cell centrosome and cause mitotic abnormalities.¹⁷ Also, in low-risk HPV, E6 and E7 do not immortalize keratinocytes *in vitro*, as they do in high-risk.¹⁸ Other studies show differences between low and high-risk E6 and E7 and demonstrate that even though both, low and high-risk oncoproteins, are capable of binding to p53 C-terminus, only high-risk E6 oncoproteins are capable of destroying p53.¹⁹

High-risk E6 binds to and inactivates p53, leading to its degradation. The degradation of this important tumor suppressor alters the cell cycle, leading to a malignant transformation. Also, high-risk E7 binds to another tumor suppressor, retinoblastoma (Rb). Once it binds to Rb, it releases the E2F transcription factor, leading it from G1 to S cell cycle phases. Low levels of Rb lead to overexpression of p16. Therefore, the finding of overexpression of p16 has been associated with the presence of HPV in its active form. The use of this marker is very helpful, especially because carcinomas of head and neck related to tobacco showed loss of p16 expression.²⁰⁻²³ However, low-risk E7 binds to Rb but fails to degrade it. Giarrè et al.²⁴ showed that HPV16 E7 subverts G1-S capture and that HPV1 E7 is less efficient in overcoming G1 cell cycle,²⁴ consequently it should not overexpress p16, as showed in this study.

Thus, normal epithelium, negative for HPV, and oral benign low-risk HPV lesions such as papilloma and condyloma, were expected not to show strong expression of p16ink4a, as seen in our results. It

is known that condyloma acuminatum may present low-risk and high-risk HPV,^{25,26} and possibly may release proteins that increase p16 expression, as seen in our cases, which showed a strong positive nucleus but not cytoplasmatic expression. According to this study, there were no significant differences between the two benign lesions used and normal epithelium, even if some samples showed nuclear staining for p16. Thus, the presence of HPV cannot be assured only by the positivity to p16ink4a-IHC in those cases.

The correlation of p16 with high-risk HPV has caused great debates, since different studies used different IHC methods and antibodies, and they could cause false-positive or false-negative results. But, based in a systematic review,¹² we considered the correlation between p16-IHC and HPV in cases showing p16 overexpression in more than 70% of the cells and with cytoplasm and nucleus with positive stain. Thus, our results showed a significant difference between HPV-OSCC and HPV-benign lesions, as well as normal epithelium. OPSCC was the only group that showed both cytoplasmatic and nuclear stain. The other groups showed only nuclear stain.

In conclusion, we observed differences in the expression of p16ink4a between benign HPV-lesions and malignant HPV-lesions.

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Relationship between stylohyoid ligament calcification and systemic osteoporosis by CBCT and panoramic radiography

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ABSTRACT | The purpose of this study was to use panoramic radiography and cone beam computed tomography images to investigate (1) the accuracy of these methods concerning osteopenia and osteoporosis diagnosis, and (2) the correlation between presence of stylohyoid ligament calcification and osteopenia and osteoporosis. A hundred seventy-one images from a digital archive were used in this study. All panoramic radiography and cone beam computed tomography images were obtained using the Veraviewepocs 3D system and observed with the i-VIEW-3DX software. For osteopenia and osteoporosis diagnosis, the mandibular cortex was assessed. Presence of stylohyoid ligament calcification was also assessed and mineralization in all parts of the stylohyoid complex was considered. All recorded data were referred to statistical analysis and the significance level was set at 5%. There is an agreement between the imaging modalities for osteopenia and osteoporosis diagnoses, since the rate of disagreement is 21.6%. The results confirmed the osteoporosis diagnosis in older women and the correlation between osteopenia and osteoporosis diagnoses and the presence of stylohyoid ligament calcification ($p = 0.06$). The present study suggests that (1) panoramic radiography and computed tomography images are accurate enough for osteopenia and osteoporosis diagnoses with low disagreement rate between methods, and (2) there is a significant correlation between presence of stylohyoid ligament calcification and osteopenia and osteoporosis diagnoses in older women.

DESCRIPTORS | Age-related Osteoporosis; Diagnostic X-Ray; Pathologic Calcification.

RESUMO | **Relação entre calcificação do ligamento estiloide e osteoporose sistêmica a partir de imagens de TCFC e radiografias panorâmicas** • O objetivo deste estudo foi investigar, a partir de imagens de tomografia computadorizada por feixe cônico e radiografias panorâmicas, (1) a acurácia dos métodos no diagnóstico de osteoporose e osteopenia e (2) a correlação entre a calcificação do ligamento estiloide e o diagnóstico de osteopenia e osteoporose. Foi utilizado um arquivo digital composto por 171 imagens. Tanto as imagens tomográficas quanto as radiografias panorâmicas foram obtidas com o aparelho Veraviewepocs 3D e observadas com auxílio do programa i-VIEW-3DX. Para o diagnóstico de osteopenia e osteoporose, a cortical mandibular foi avaliada. A presença de calcificação no ligamento estiloide foi verificada, considerando-se mineralização em todas as partes do complexo estiloide. Foi realizada a análise estatística dos dados tabulados, e o nível de significância foi estabelecido em 5%. O resultado apontou correlação entre os métodos de imagem no diagnóstico de osteopenia e osteoporose. Confirmou-se também a maior frequência de osteoporose em mulheres com idade avançada e a correlação entre os diagnósticos de osteopenia e osteoporose e a presença de calcificação no complexo estiloide ($p = 0,06$). O presente estudo sugere que (1) tanto a radiografia panorâmica quanto as imagens de tomografia computadorizada por feixe cônico podem ser usadas no diagnóstico de osteopenia e osteoporose e (2) há correlação significativa entre a calcificação do ligamento estiloide e o diagnóstico de osteopenia e osteoporose em mulheres com idade avançada.

DESCRIPTORES | Osteoporose; Raios X Diagnósticos; Calcificação Patológica.

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INTRODUCTION

Calcium is an essential nutrient for the human body and has received substantial attention due to its role in osteoporosis and several other chronic diseases. It is the most abundant mineral in the human body and 99% of its stores are found in the skeleton. The remaining stores are in soft tissues (0.9%) and the bloodstream and extracellular fluid (0.1%).¹ Calcium requirements remain stable until menopause, when the bone resorption rate increases in parallel with the decrease in ovarian estrogen production. Adequate calcium intake (combined with adequate vitamin D status) has been shown to reduce bone loss in postmenopausal women, being the key component for patients with osteoporosis. In addition, women at menopause require increased calcium concentration due to their absorption deficiency.¹⁻³

Osteoporosis is a systemic condition characterized by low bone mass, leading to bone fragility.^{1,2} Several factors are related to osteoporotic fractures in postmenopausal women, such as history of fractures in adulthood, smoking, estrogen deficiency at an early age, physical inactivity, little exposure to sunlight, and alcohol abuse, in addition to low calcium intake.^{2,3} Although densitometric techniques are well established for osteoporosis diagnosis, some studies have shown that dentists can assess mandibular bone quality using panoramic radiography (PAN). By analyzing the sparse trabeculation of mandibular cortex, they subjectively determine the risk of osteoporosis.^{4,5}

On the other hand, calcifications of soft tissues in the maxillofacial area are uncommon and usually correspond to radiographic findings in routine imaging examination. Anatomical location, distribution and number, size, and shape of calcifications are the most important diagnostic criteria.⁶ Stylohyoid ligament calcification (SLC) is generally diagnosed as a dystrophic calcification, which ranges from barely perceptible to large radiopaque

particles.^{7,8} Its etiology is still unclear, and some theories include degeneration of the ligament, with deposition of calcium in fibrous tissue or malformation due to direct ossification of the cartilaginous cells remaining in the ligament.⁸ The emergence of cone beam computed tomography (CBCT) has expanded the field of oral and maxillofacial radiology. CBCT imaging provides three-dimensional volumetric data construction with high dimensional accuracy of dental and associated maxillofacial structures.^{9,10} Besides that, some studies have already shown that CBCT images can be used to assess osteoporotic women and also to evaluate size and morphology of the stylohyoid complex.^{11,12}

The purpose of this study was to use PAN and CBCT images to investigate (1) the accuracy of these methods concerning osteopenia and osteoporosis diagnosis, and (2) the correlation between presence of SLC and osteopenia and osteoporosis.

MATERIAL AND METHODS

A hundred seventy-one images from a digital archive of patients from the Radiology Service of the Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Japan) were used in this study. Patients with metabolic bone diseases and pathologies or fractures in the region of the stylohyoid complex were excluded from this study.^{11,12}

All CBCT and PAN images were obtained using the Veraviewepocs 3D system (J. Morita Corporation, Kyoto, Japan; 60-80 Kv, 1-10 mA). The field of view (FOV) protocol was established as 80 × 80 mm (scan time: 9.4 s) for CBCT and 40 × 80 mm (scan time: 15 s) for PAN. All images were observed using the i-VIEW-3DX software (J. Morita Corporation, Kyoto, Japan). One experienced specialist with a PhD in Oral and Maxillofacial Radiology performed image analysis.

For osteopenia and osteoporosis diagnoses, the mandibular cortex was classified as follows:

(C1), if the endosteal margin of the cortex was sharp on both sides; (C2), if the endosteal margin showed semilunar defects (lacunar resorption) and/or presented cortical residues on one or both sides; and (C3), if the endosteal margin was clearly porous.¹³ This classification was used for evaluation of the digital PAN and CBCT images. In the CBCT images, after reconstruction of the acquired data, a panoramic view of the jaw was obtained with 20 mm thickness, and this image was considered for mandibular cortex evaluation.

Presence of SLC was also assessed in PAN and CBCT images, and mineralization in all parts of the stylohyoid complex was considered SLC. Length of the styloid process was measured from the base to the tip of each appendix by using software tools. Processes measuring more than 30 mm were considered elongated.¹⁴ For CBCT data, all measurements were performed at the right and left oblique sagittal planes from 3D multiplanar reconstruction.¹² If there was a segmental SLC, the distance was measured including the non-calcified parts.

All recorded data were referred to statistical analysis by using the software R 3.0.2 (Alcatel-Lucent, Murray Hill, New Jersey, USA). The significance level was set at 5%.

Comparison between PAN and CBCT methods for osteopenia and osteoporosis diagnoses is presented in cross-tabs with relative and absolute

frequencies. McNemar's test was utilized to verify which of them was more sensitive.¹⁵ Continuous variables were described by means of statistical position (minimum, maximum, and average) and scale (standard deviation).

By using Fisher's exact test, osteopenia and osteoporosis diagnoses were associated with SLC presence and patient gender. For correlation between osteopenia and osteoporosis diagnoses and patient age, analysis of variance (ANOVA) with a linear model was used.¹⁶

RESULTS

This study included 171 patients, 55 males (32.2%) and 116 females (67.8%) with a mean age of 59.6 years. Table 1 shows the agreement between the imaging modalities. The rate of disagreement was 21.6%. Neither PAN nor CBCT showed conflicts for normal and osteoporosis diagnoses.

Tables 2, 3, and 4 show the correlation between final diagnosis, age, and gender. Statistical description of patients' age is shown in Table 2. Table 3 shows the estimation of the coefficient linear model for age according to each diagnosis possibility. The mean age for normal patients was estimated to be 49.86 years, and this age is increased in 7.89 and 14.76 years for patients with diagnoses of osteopenia and osteoporosis, respectively. The p value (< 0.001) from the ANOVA test confirmed the osteoporosis diagnosis in older adults.

Table 1 | Correlation between imaging modalities for osteopenia and osteoporosis diagnosis

PAN*	CBCT**							
	Normal		Osteopenia		Osteoporosis		Total	
	N	%	N	%	N	%	N	%
Normal	63	36.8	12	7	0	0	75	43.8
Osteopenia	8	4.7	36	21.1	12	7	56	32.8
Osteoporosis	0	0	5	2.9	35	20.5	40	23.4
Total	71	41.5	53	31	47	27.5	171	100
Rate of disagreement:								21.6

* Panoramic Radiography, ** Cone Beam Computed Tomography

Table 4 shows that presence of osteopenia and osteoporosis is more frequent in female patients, since the p value (Fisher's exact test) is 0.002.

Considering that osteopenia and osteoporosis diagnoses were more frequent in females (Table 4),

the relationship between presence of SLC and the aforementioned diseases was evaluated only in the female group (Table 5). The results, with a p value (Fisher's exact test) of 0.06, confirm that these diagnoses are associated with presence of SLC.

Table 2 | Statistical description of patients' age according to osteopenia and osteoporosis diagnosis

Final diagnosis CBCT and PAN*	N	Minimum	Maximum	Mean	Standard deviation	CI for the mean (95%)**	
						Inf	Sup
All	171	28	91	56.9	12.0	55.1	58.7
Normal	63	28	73	49.9	11.0	47.1	52.6
Osteopenia	56	35	85	57.8	9.9	55.1	60.4
Osteoporosis	52	36	91	64.6	10.1	61.8	67.4

* Final diagnosis including CBCT and PAN evaluation

**Confidence intervals for the mean values

Table 3 | Coefficient linear model for age according to osteopenia and osteoporosis diagnosis

Coefficient	Estimate	Standard error	t value	p value
Intercept	49.86	1.31	38.08	<0.001
Osteopenia	7.89	1.91	4.14	<0.001
Osteoporosis	14.76	1.95	7.58	<0.001

Table 4 | Final diagnosis of osteopenia and osteoporosis according to patient gender

Final Diagnosis CBCT and PAN*	Gender					
	Female		Male		Total	
	N	%	N	%	N	%
Normal	34	29.3	29	52.8	63	36.9
Osteopenia	38	32.8	18	32.7	56	32.7
Osteoporosis	44	37.9	8	14.5	52	30.4
Total	116	100	55	100	171	100
p value from the Fisher's exact test						0.002

* Final diagnosis including CBCT and PAN evaluation

Table 5 | Association of osteopenia and osteoporosis diagnosis and SLC in females

SLC**	Final Diagnosis CBCT and PAN*							
	normal		osteopenia		osteoporosis		Total	
	N	%	N	%	N	%	N	%
NO	33	97.1	30	78.9	37	84.1	100	86.2
YES	1	2.9	8	21.1	7	15.9	16	13.8
RTot	34	100	38	100	44	100	116	100
p value from Fisher's exact test								0.06

* Final diagnosis including CBCT and PAN evaluation

** Stylohyoid ligament calcification

DISCUSSION

Calcium requirements for skeletal maintenance vary throughout women's life. During the teen years, calcium requirements are high because the skeleton grows rapidly. During their 20s, the body requires less calcium for bone health. It remains stable until menopause, when there is a lack of vitamin D and, according to the "Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board", a decrease in intestinal absorption.¹ In general, postmenopausal women should ingest 1,200 mg calcium per day from different sources: foods, calcium-fortified foods, and supplements.¹⁷ The importance of adequate calcium intake for skeletal health is well established.^{1,17} Studies have demonstrated that postmenopausal women receiving calcium supplement for one year had decreased bone loss rate (0.014%) compared with untreated women (1.0%).¹⁸

Osteoporosis is a skeletal disorder that causes bone deterioration and affects the stomatognathic system with the same rate as that of the total skeleton.^{2,3} Disease and associated bone fractures, morbidity, mortality, and health care costs are significant health concerns. Therefore, effective preventive strategies, such as early diagnosis and treatment, must be established.^{2,19,20} Mandibular information regarding cortical thickness and morphology of the inferior cortex are useful, and radiographic examination of the mandible may be effective for the osteoporosis diagnosis.^{3-5,11} Panoramic mandibular index (PMI) has been used to assess mandibular bone quality, and it is well established in the literature worldwide.^{3-5,13,21,22} Although Klemetti *et al.*¹³ stated that the PMI should not be utilized to assess the patient's status regarding osteoporosis, Devlin and Horner²¹ and Arifin *et al.*²² reported that mandibular cortical width below the mental foramen, manually measured on dental panoramic radiographies, was significantly correlated with the T scores recorded for spine and

femur. Koh and Kim¹¹ emphasized the importance of CBCT in mandibular evaluation and showed different results for normal and osteoporotic groups. This study presents imaging data regarding jaw assessment on PAN and CBCT exams, which showed to be useful tools for mandibular cortex evaluation, with low disagreement rate (21.6%), as shown in Table 1. In addition, misdiagnosis did not occur in the normal and osteoporotic groups (Table 1), in agreement with the results of Koh and Kim¹¹.

Although men experience age-related bone loss, possibly resulting in osteoporosis, the condition is most commonly associated with women. Incidence of fractures in men increases 5-10 years later than in women.^{1-3,17,19,20} These statements mentioned above are presented in Tables 2, 3, and 4. Tables 2 and 3 show the correlation between patient age and disease severity, thus confirming that osteoporosis incidence increases with age. Table 4 also confirms prevalence in the female group. Considering age incidence and gender prevalence associated with increasing life expectancy, women tend to be affected by any type of fractures, without correct diagnosis and treatment. About 40% of women aged 50 years are expected to have at least one fracture, and 20% of them are expected to suffer from multiple fractures (wrist, vertebrae, and hip are the most common).²⁰

Since the female group is the most affected by osteoporosis, as confirmed in this study (Tables 2, 3, and 4), the possible association between osteopenia and osteoporosis and presence of SLC was analyzed in this group (Table 5) and confirmed by the results. Only one case of SLC was found in a normal patient, which is in agreement with a previous study.¹⁴ Several theories about SLC etiology have been reported, suggesting degeneration of the ligament with deposition of the calcium salts in the fibrous tissue, or malformation due to direct ossification of the cartilaginous cells remaining in the ligament of adult patients.⁸ Rizzatti-Barbosa

*et al.*²³ used PAN while studying its prevalence in both genders and emphasized endocrine disorders in women at menopause as a possible cause for SLC. These authors concluded that this alteration is more frequent in older adults. Öztaş and Orhan⁸ used PAN and also observed increased SLC in older adults in a study on SLC frequency. A relationship between osteopenia and osteoporosis diagnoses and presence of SLC in the present study was found in older women, since osteoporosis was prevalent in this group (Tables 2, 3, and 4), in agreement with the findings of Rizzatti-Barbosa *et al.*²³ and Öztaş and Orhan.⁸ Okabe *et al.*²⁴ reported a significant association between length of the stylohyoid process and serum calcium concentration. Since calcium intake is the treatment of choice for osteoporotic patients, results from this study are in agreement with those reported by Okabe *et al.*²⁴ Therefore, deposition of calcium salts in the fibrous tissue is the most likely cause of SLC.

The most useful techniques for SLC evaluation include PAN, lateral cephalogram, postero-anterior skull view, lateral oblique mandible view, and Towne's view, computed tomography, and magnetic resonance imaging.⁸ However, Okabe *et al.*²⁴ stated that PAN might be of no value in the diagnosis of Eagle's syndrome. When in presence of a nodular mass on the SLC extension, dentists should use computed tomography to clearly distinguish between them. The CBCT technique provides accurate and reliable linear measurements, 3D information, and multiplanar views from dental and maxillofacial structures.⁹⁻¹² Size and morphology of SLC can be easily assessed.¹²

In conclusion, the present study suggests that (1) panoramic radiography and computed tomography images are accurate enough for osteopenia and osteoporosis diagnoses, with a low disagreement rate between methods, and (2) there is a significant correlation between presence of stylohyoid ligament calcification and osteopenia and osteoporosis diagnoses in older women.

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Use of mouthwashes in patients with oral and oropharyngeal cancer

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ABSTRACT | *Objective:* To evaluate the use of mouthwashes in patients with oral and oropharyngeal cancer. *Materials and methods:* Fifty-three patients were interviewed through a specific questionnaire at two reference centers for diagnosis and treatment of cancer. The Case Group consisted of 33 patients with final diagnosis of epidermoid carcinoma of the mouth and oropharynx. The Control Group consisted of 20 patients attended to by services not connected with oncology. *Results:* In the Case Group, 81.8% did not make use of dental floss, showing statistically significant difference from the Control Group ($p=0.036$). As for toothbrushing, we noticed a contrary behavior in which the Cases brushed more times per day than the Controls. The Control Group made less use of mouthwashes when compared to the Case Group, which used it more times per day ($p=0.028$). Patients in the Case Group smoked more than those in the Control Group, with this difference being significant ($p=0.004$). The same behavior was observed for alcohol consumption – consumption in milliliters per day during the year ($p=0.031$). *Relevance:* Various risk factors have been associated with cancers of the oral cavity, among them the consumption of alcoholic beverages. The mechanism by which alcoholic beverages cause oral cancer is unknown, but probably involves topical exposure. *Conclusion:* From this study, we concluded that even with the small casuistic, by means of a stratified analysis, the use of mouthwashes was four times higher in alcohol consumers; however, no increase of risk in smokers, abstainers from alcohol and non-smokers was observed.

DESCRIPTORS | Mouth Neoplasms; Risk; Oral Hygiene.

RESUMO | **Uso de enxaguatórios em pacientes com câncer de boca e orofaringe** • *Objetivo:* Avaliar o uso de enxaguatórios em pacientes com câncer de boca e orofaringe. *Materiais e método:* Foram entrevistados 53 pacientes por meio de questionário específico aplicado em dois centros de referência para o diagnóstico e tratamento do câncer. O Grupo Caso foi constituído por 33 pacientes com diagnóstico final de carcinoma epidermóide de boca e orofaringe. O Grupo Controle foi composto de 20 pacientes atendidos em serviços não ligados à oncologia. *Resultados:* No Grupo Caso, 81,8% não fazem uso de fio dental, demonstrando diferença estatisticamente significativa em relação ao grupo controle ($p=0,036$). Quanto à escovação dentária, notamos comportamento contrário, onde os Casos escovam mais vezes por dia do que os Controles. O Grupo Controle fazia menos uso de enxaguatórios quando comparado ao Grupo Caso, que utilizava mais vezes ao dia ($p=0,028$). Os pacientes do Grupo Caso fumavam mais que os do grupo controle, sendo tal diferença significativa ($p=0,004$). O mesmo comportamento foi observado no consumo de álcool – consumo em mililitros por dia durante o ano ($p=0,031$). *Relevância:* Vários fatores de risco têm sido associados a cânceres da cavidade oral, entre eles o consumo de bebidas alcoólicas. O mecanismo pelo qual bebidas alcoólicas provocam câncer oral é desconhecido, mas provavelmente envolve exposição tópica. *Conclusão:* Concluímos com este estudo que, mesmo com uma pequena casuística, por uma análise estratificada, o uso de enxaguatório foi quatro vezes maior em etilistas, porém não se observou aumento do risco em tabagistas, abstêmios alcoólicos e não tabagistas.

DESCRITORES | Neoplasias Bucais; Risco; Higiene Bucal

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INTRODUCTION

At present, oral cancer is a serious health problem worldwide.¹ The incidence varies greatly all over the world. In Brazil, the annual incidence is approximately 15,000 new cases per year.¹⁻³ Various risk factors have been associated with cancers of the oral cavity, such as smoking, consuming alcoholic beverages, poor oral health, and infection by the human papillomavirus.³⁻⁵

Although there are many hypotheses for the effect of alcohol on tumor promotion, the pathogenic mechanisms remain obscure, since alcohol itself is not carcinogenic.⁶ Lemos and Villoria⁷ observed that the mechanism by which alcoholic beverages cause oral cancer is unknown, but probably involves topical exposure, perhaps with a solvent action that increases the penetration of tobacco and other carcinogenic agents.

The association between the use of mouthwashes containing alcohol and the development of oral cancer has been the subject of scientific studies since 1970. Oral antiseptics have been used as a complementary means of performing oral hygiene and is frequently recommended for patients whose mechanical plaque control procedures are inadequate.⁸ The majority of mouthwashes with antiplaque properties contain a certain degree of denatured alcohol, used as a vehicle for delivery of the antimicrobial ingredients.⁹ Alcohol has the function of providing solubility, conservation, stabilization, and an adjuvant antimicrobial effect.^{10,11}

Limited and conflicting epidemiological evidence is available about the relationship between the use of alcohol in the oral cavity, in the form of mouthwash solutions, or mouthwashes and oral cancer. Some studies have pointed towards an increased risk of oral cancer, due to the local acetaldehyde production that operates by a mechanism similar to that found after the ingestion of alcoholic beverages¹².

Based on the controversial literature, the aim of this case-control study was to evaluate not only the

association of mouthwashes with oral and oropharyngeal cancer, but also the oral hygiene habits of these patients.

MATERIALS AND METHOD

A total of 53 patients attended at the outpatient clinic of the Center of Oral Diagnosis of the Dental School, University of São Paulo (FOUSP), at the Radiotherapy Service of Santa Casa da Misericórdia de Santos, and at the Head and Neck Surgery Service of the Hospital Municipal de Cubatão Dr. Luiz de Camargo da Fonseca e Silva – Pró-Saúde. The Case Group consisted of 33 patients attended at the outpatient clinics of these services, with final diagnosis of epidermoid carcinoma of the mouth and/or oropharynx, with sites in the following anatomic locations. The Control Group was made up of 20 patients attended at other outpatient clinics of the same hospitals and services, without past or present experience of cancer.

Data were collected through a previously established questionnaire, applied by a calibrated researcher. The questions about oral hygiene asked for information about the following aspects: 1) frequency of daily toothbrushing; 2) possible use of dental floss and frequency; 3) if the person noticed any occurrence of bleeding gums; 4) usage of mouthwashes and, if yes, for what reason and which, who prescribed it or what made the person decide to buy it, if no, if the person ever used a mouthwash, for how long and what is the reason for using it (previous and present).

The distribution of frequencies was used to describe the categorical variables and the measures of central tendency and variability for the numerical or continuous variables. The chi-square frequency test was used to compare the categorical variables regarding the group (Case – Control) in contingency tables, and in 2x2 tables when at least one expected frequency was lower than 5, the Fisher's exact test was adopted. The Mann-Whitney U Test was used

to compare the numerical medians of the results regarding the group (Case – Control), when normality of the data was not identified. The level of significance of 5% was considered in all the statistical tests.

RESULTS

Of the 33 patients in the case group, 23 were men and 10 were women. The mean age was 59.2 years; the oldest patient was 86 years old and the youngest, 26 years old. The tongue was the most affected site, totaling 14 cases out of 33; followed by the oropharynx (8 cases), base of the tongue (4 cases), jugal mucosa (2 cases), and floor, edge, soft palate, retromolar region and maxilla, all with 1 case. The majority of the patients were treated with the association of surgery, radiotherapy, and chemotherapy (13 cases), 11 received radiotherapy and chemotherapy, 5 cases were treated exclusively with surgery, 3 cases with surgery and radiotherapy, and 1 case exclusively with radiotherapy.

The patients who wore dental prosthesis totaled 18 cases, and 1 year was the shortest time of use, and 45 years the longest. Of these cases, 4 reported trauma resulting from the dental prosthesis.

Table 1 shows that in 81.8% of the cases, the patients did not use dental floss, with a similar distribution as for dental floss use in the control group, and this difference was statistically significant ($p=0.036$). With regard to gingival bleeding, 87.9% of the cases did not observe bleeding, and comparison with control cases was shown to be marginally significant ($p=0.079$). Regarding tooth brushing, we noted a contrary behavior in which the cases brushed more times per day than the controls, but this difference in behavior was marginally significant.

The use of dental prostheses showed no association with regard to the groups ($p>0.05$).

With respect to mouthwash, the controls made less use of it compared with the case group that used it more times a day ($p=0.028$), which

was shown to be a statistically significant association (Table 2). Twenty-one of the 33 patients interviewed reported making use of washes. Mouthwashes with alcohol were used by 18 patients, and 3 used the type without alcohol. Eleven patients used mouthwash once a day, 2 patients made use of mouthwashes once a week. Each of the following frequencies of 2x/day, 3x/day, 4x/day, 5x/day, 5x/week, 3x/week, 2x/week, and 1x/month were reported by one patient.

Table 3 shows the distribution of habit of smoking or consuming alcoholic beverages. Smoking showed significant difference between the groups ($p=0.004$), and the quantification of the consumption of tobacco in number of packs per year (quantity of packs of cigarettes and equivalent of other types of cigarettes consumed daily for 1 year) was higher in the case group than in the controls ($p=0.044$), and the same behavior was observed for alcohol consumption (consumption in ml per day during one year) ($p=0.031$).

Twenty-three patients reported making use of alcohol, and 10 did not. Of the alcohol consumers, 5 patients drank exclusively fermented beverages, 9 patients drank exclusively distilled beverages, and 9 patients drank distilled and fermented beverages concomitantly. The one who took the most drinks made use of 5 bottles of fermented and 1 liter of distilled beverage per day, for 30 years. The one who drank the least made use of 3 cans of fermented beverage per week for 30 years.

The control group was made up of 20 patients, of whom 10 were men, and 10 women. The mean age was 59.7 years, the oldest patient was 87 years old and the youngest, 43 years of age. Of the 20 patients, 14 did not wear dental prosthesis, and 6 did. Of these 6 patients, 3 reported trauma resulting from the dental prosthesis. The mean time of denture wearing was 21.8 years.

When asked about smoking, 12 patients denied making use of cigarettes, and 8 said they were

smokers. The mean time of using cigarettes among these patients was 22 years, with 3 years being the shortest and 45 years the longest times. The patient who smoked the least made use of 2 cigarettes per day, and the one who smoked the most used 40 cigarettes/day.

Thirteen patients reported making use of alcohol, and 7 did not. Of the alcohol consumers, 9 patients drank exclusively fermented beverages, 2 patients drank exclusively distilled beverages, and 2 patients drank distilled and fermented beverages concomitantly. The one who took the most drinks made use of 1 bottle of fermented and 1 liter of distilled beverage per day, for 30 years. The one who drank the least made use of 1 can of fermented beverage per week for 10 years.

Eight of the 20 patients interviewed reported making use of mouthwashes. Mouthwashes without alcohol were used by all the patients who made

use of these products. Two patients used mouthwashes twice a week, 2 patients used them once a week, and 2 used them once a day. The frequencies of 1x/fortnight and 1x/month were reported by one patient each. Two patients reported making use of mouthwashes for 15 years, with this being the longest time of use. Two years was the shortest time of use, reported by 2 patients. Ten years was the time reported by 2 other patients, and 5 years by another 2 patients. Seven patients reported having started to use mouthwashes without professional indication, that is, on their own account. The use indicated by a dentist was reported by a single patient. Another question raised in the interviews was the reason for using mouthwashes. The most prevalent reason was cleaning, reported by 6 patients. Improving breath was the reason given by 1 patient, and other patients said they used mouthwashes to mask the odor of cigarettes.

Table 1 | Distribution of variables referring to oral hygiene habits and use of dentures according to group.

Variable	Category / Measures	Group CaseControl Freq. % /Measures		p-value
Do you use dental floss?	No Yes	27 (81.8) 6 (18.2)	11 (55.0) 9 (45.0)	0.036
Do you observe bleeding in gum?	No Yes	29 (87.9) 4 (12.1)	13 (65.0) 7 (35.0)	0.079*
Toothbrushing (freq./day)	1 2 3 4	3 (9.1) 10 (30.3) 13 (39.4) 7 (21.2)	2 (10.0) 11 (55.0) 7 (35.0) 0 (0.0)	NA
Toothbrushing (freq./day)	Up to 2 times a day 3 or 4 times a day	13 (39.4) 20 (60.6)	13 (65.0) 7 (35.0)	0.071
Wears dentures	No Yes	18 (54.6) 15 (45.4)	14 (70.0) 6 (30.0)	0.265
Time of dental prosthesis use (years)	N Variation Median Mean (standard deviation)	15 1 - 45. 15 16.2 (12.1)	6 3 - 50. 20 21.8 (17.1)	0.532*

p-value obtained by the chi-square frequencies test.

* p-value obtained by the Fisher's exact test.

Table 2 | Distribution of variables referring to mouthwash use according to group.

Variable	Category / Measures	Group CaseControl Freq. % /Measures		p-value
Use of mouthwash	No Yes	12 (36.4) 21 (63.6)	12 (60.0) 8 (40.0)	0.094
Frequency of mouthwash use	No Fewer than 1 time per day One or more times/day	12 (36.4) 6 (18.2) 15 (45.4)	12 (60.0) 6 (30.0) 2 (10.0)	0.028
Time of mouthwash use (years)	N Variation Median Mean (standard deviation)	21 0.2 – 18. 5 6.3 (4.8)	8 2 – 15. 7.5 8.0 (5.3)	0.428
Time, use of mouthwash (years)	Does not use ≤ 40 >40	12 (36.4) 12 (36.4) 9 (27.3)	12 (60.0) 4 (20.0) 4 (20.0)	0.233
Who indicated it?	I use it on my own account Dentist Daughter Doctor	13 (61.9) 5 (23.8) 1 (4.8) 2 (9.5)	7 (87.5) 1 (12.5) 0 (0.0) 0 (0.0)	NA
Reason for use	Breath Cleaning Cigarette odor Due to Lesion	3 (14.3) 15 (71.4) 1 (4.8) 2 (9.5)	1 (12.5) 6 (75.0) 0 (0.0) 1 (12.5)	NA

p-value obtained by the chi-square frequencies test

NA= not statistically assessable

Table 3 | Distribution of variables referring to smoking and alcohol consumption according to group.

Variable	Category / Measures	Group Case Control Freq. % /Measures		p-value
Smoker	No Yes	7 (21.2) 26 (78.8)	12 (60.0) 8 (40.0)	0.004
Packs cigarettes/ year	N Variation Median Mean (standard deviation)	26 2 – 150. 45.5 48.0 (34.1)	8 0.3 – 50. 27.5 24.4 (19.1)	0.044*
Packs cigarettes/ year	Does not smoke ≤ 20 >20	7 (21.2) 6 (18.2) 20 (60.6)	12 (60.0) 3 (15.0) 5 (25.0)	0.013
Alcohol consumer	No Yes	10 (30.3) 23 (69.7)	7 (35.0) 13 (65.0)	0.723
Consumes alcohol	N Variation Median Mean (standard deviation)	21 22.5 – 14062.5. 1645.0 3124.4 (3898.6)	12 5.8 – 862.5. 54.8 156.1 (247.2)	0.031*
Alcohol consumption (ml day/year)	Does not consume ≤ 40 >40	12 (36.4) 5 (15.2) 16 (48.5)	8 (40.0) 10 (50.0) 2 (10.0)	0.004

p-value obtained by the chi-square frequencies test.

DISCUSSION

Hooper *et al.*¹³ reported that the majority of cases of oral cancer were related to the use of tobacco and alcohol consumption. Other studies have also put forward different hypotheses, such as those of Winn *et al.*¹⁴, who indicated possible risk factors as being teeth in a poor state of conservation, inadequate oral hygiene, and the use of mouthwashes, particularly among non-users of tobacco and alcohol. In addition, Homann *et al.*⁶ reported that, by means of their epidemiological data, they could support that poor oral hygiene was an independent risk factor for cancer of the mouth, whereas Tsai *et al.*¹⁵ suggested that improvement in oral hygiene practices could bring additional benefits when thinking about the prevention of cancer of the mouth.

It has been put forward that acetaldehyde, the first metabolite of ethanol, has demonstrated multiple mutagenic effects, and is carcinogenic to animals. Furthermore, it has been suggested that acetaldehyde may be produced by the metabolism of microorganisms present in the mouth¹³ and poor oral hygiene may elevate the acetaldehyde level in saliva. Studies have also demonstrated that *Candida albicans* could produce significant quantities of acetaldehyde, carcinogenic in clinically relevant concentrations.^{13,16}

Based on the diversity of information in the literature stating poor oral hygiene and bad state of conservation of the teeth could be predisposing factors for cancer of the mouth, our study evaluated some oral hygiene habits and identified that over 81% of patients with cancer did not make use of dental floss. Thus, these numbers led us to note that those who did not use dental floss were 4 times the number of those who use it, and this datum was statistically significant. Due to the sample size, we could not affirm that whoever does not use dental floss has a higher risk of developing cancer of the mouth and oropharynx; however, it led us to think

that care of oral hygiene and health must not be neglected. The data observed as regarding dental floss are in agreement with another datum observed, in which over 87% of the patients in the case group did not observe bleeding in the gingiva, a datum also observed in the control group. The aspect that most drew attention was a datum inverse to all the previous data in the literature, in which the case group reported brushing their teeth more times a day than the control group, no matter that this difference was marginally significant. We imagined this information was due to the greater concern about oral hygiene as soon as the diagnosis of the lesion was made, and the need for maintaining oral hygiene during antineoplastic treatment.

Mouthwashes have been used for a long time as antiseptic and to refresh breath,¹⁷ as astringent, calming,^{8,18} and as a method of delivering antimicrobial agents to all the sites of the oral mucosa, thus providing a complementary plaque control mechanism.¹⁹

The present study, seeking to evaluate the use of mouthwashes in patients with cancer of the mouth and oropharynx, and based on a vast and diversified literature regarding methodologies and results, verified that of the 33 patients studied in the case group, 21 reported making use of mouthwashes, totaling over 63%. The patients in the control group made less use of mouthwashes compared with the case group ($p=0.028$), which was shown to be a statistically significant association. This datum corroborates the findings of a large number of studies in which association of the use of mouthwashes and cancer have been evaluated.²⁰⁻²²

The occasion in which the use of mouthwashes began was also questioned during the study, when it was observed that out of a total of 21 patients who made use of them, 15 began to use them before diagnosis of the tumor, with a mean time of 5.5 years, and 6 patients began using them after diagnosis. The longest time of use reported before diagnosis

was 15 years and the shortest time, 6 months. This information appeared to us to be important, considering that in an endeavor to make this correlation, some studies did not suggest the possibility that the use of mouthwashes could have been started after the development of cancer, and thereby would lose their etiological or facilitating nature.

Of the 15 cases that began mouthwash use before diagnosis, 11 were smokers and 4 were not. The mean consumption of cigarettes in these patients was 22.6 cigarettes per day. Six patients reported they did not consume alcohol, and 9 did. This information also corroborates the literature, in which the large majority of mouthwash users have been observed to be smokers and alcohol consumers.

As in the study of Wirth *et al.*²⁰, our study also questioned the reason for using mouthwash. The most prevalent reason was cleaning, reported by 15 patients, whereas improving breath was the reason given by 3 patients; 2 patients said they used mouthwashes to mask the odor of cigarettes. Two patients used mouthwash with the purpose of treating the lesion. On the other hand, for Wirth *et al.*²⁰, the main benefits reported were improving bad breath (75%), elimination of bacteria (68%), and reduction in plaque formation (47%).

Mouthwashes with alcohol were used by 17 patients, and 3 used the type without alcohol. Of the 17 patients, 5 used more than one type of mouthwash with alcohol, one used only Plax®, 2 Malvona®, 3 Periogard®, 5 Listerine® and one was unable to report the brand. This datum, neglected by older articles in the literature, could not be statistically evaluated in our study due to the small casuistic.

Even in view of this casuistic, we may suggest that the risk of developing cancer for the group that made use of oral mouth is almost 3 times higher than for those who do not use it, and when we stratified for the main risk factors, only when we considered individually who drank, this risk rose

to being 4 times higher. Therefore, the use of oral mouthwash appears to be a risk factor. We reiterate that a larger casuistic would be necessary to affirm this datum.

One of the failures most perceived in the studies with the object of associating mouthwashes containing alcohol with cancer was the lack of standardization regarding the type of mouthwash, presence of alcohol, and its concentration. This datum was questioned and evaluated by us; however, our casuistic did not allow for statistical tests to be performed. The data found in this study certainly do not allow us to affirm that alcohol in mouthwashes plays any role in the development of cancer, but, once again, it made us think that in the same way as this association cannot be affirmed, it cannot be completely denied.

From the results of this study, we concluded that although we used a small sample of cases and controls, by means of stratified analysis the use of mouthwashes was found to be approximately 4 times higher in patients who consumed alcoholic beverages. Nonetheless, no increase in risk was observed with the use of mouthwashes in patients who did not drink, and who smoked or not. The studied patients with cancer of the mouth and oropharynx brushed their teeth more than once a day, used more mouthwashes and less dental floss when compared with the control patients.

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Systematic review of Lamendin's dental age estimation method

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ABSTRACT | *Objective:* to determine the effectiveness of Lamendin's dental age estimation, by conducting a systematic review. *Methods:* for the meta-analysis, we searched for studies with the aim of showing the effectiveness of the method by comparing the chronological and estimated ages of the sample. Three databases were consulted (MEDLINE, LILACS, and EMBASE). We excluded studies with historical samples and those that changed the technique, analyzed other aspects of the method without presenting results on effectiveness, and that presented the sample in a single group with individuals under 25 years of age. The quality evidence scale QUADAS was used with some modifications. The final sample consisted of eight studies. *Results:* our results showed that the identified studies were methodologically careless and that the method was ineffective in older people, but produced good estimates for adults in other age-ranges. There are discrepancies in the literature regarding the age at which the method works best. *Relevance:* Studies on Lamendin's technique should have a stricter methodological approach and the technique should be tested before being used in a given population to verify the age at which the results are more accurate.

DESCRIPTORS | Forensic Dentistry; Age Determination by Teeth; Evidence-Based Dentistry; Meta-Analysis.

RESUMO | **Revisão sistemática do método Lamedin para estimativa da idade pelos dentes** • *Objetivo:* verificar a eficácia do método de estimativa de idade de Lamendin por meio de uma revisão sistemática. *Métodos:* para a metanálise foram pesquisados estudos que tinham como objetivo mostrar a eficácia do método comparando as idades cronológica e estimada da amostra. Foram consultadas as bases de dados MEDLINE, LILACS e EMBASE. Excluímos os artigos com amostra histórica, os que modificaram a técnica, os que analisaram outros aspectos do método sem retratar sua eficácia e os que apresentaram a amostra em grupo único com indivíduos de menos de 25 anos de idade. Em seguida, a escala de qualidade de evidência QUADAS foi utilizada com modificações. A amostra final foi constituída de oito estudos. *Resultados:* os resultados mostraram que os estudos identificados descuidam de alguns aspectos metodológicos e que o método é ineficaz em idosos, mas que produz boas estimativas nos outros adultos. Existem discordâncias na literatura quanto à faixa etária em que o método funciona melhor. *Relevância:* os estudos que utilizam essa técnica necessitam de mais rigor metodológico, e a técnica deve ser testada antes de ser usada numa determinada população para que seja possível constatar em qual faixa etária os resultados são mais precisos..

DESCRITORES | Odontologia legal; Determinação da idade pelos dentes; Odontologia Baseada em Evidências; Metanálise.

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INTRODUCTION

Posing the question

Age determination by teeth is an important part of Forensic Dentistry and Anthropology, since this method provides valuable assistance in cases of identification. For many years and up to now, the scientific community has produced a large number of researches on this topic. Several of them¹⁻³ are based on the developmental stages of teeth, which makes them suitable for estimating the age of children and adolescents. For adults, methods are often based on degenerative modifications, such as attrition, periodontosis, transparency of the root, secondary dentin, cementum apposition, and root resorption.

Since Gustafson⁴ first published his work on age determination by degenerative changes in teeth, several authors⁵⁻⁸ have developed other methods based on the characteristics he described, including Lamendin et al.⁹ The technique was developed to be simple, and its purpose was to estimate the age of adults at the time of death. The method consists of analyzing only two dental variables (periodontosis and transparency) and applying the numbers to a given formula, and it is a good option for use in forensic cases.

In view of the doubt about the real efficacy of the methods, the precepts of Evidence-Based Dentistry were considered for evaluating the quality of the results shown by the studies of estimating age in adults.

However, it is a challenge to show its application to a specialty that is not part of the clinical routine in dentistry, such as Forensic Dentistry. Thus, in spite of this type of review integrating existent information in an efficient format and providing data for rational decision-making, such studies are rarely observed within Forensic Dentistry.

Thus, considering the importance to society of estimating age, and that a simple and efficient method for use in adults would facilitate the process of identification in Brazil, we chose to evaluate the efficiency of Lamendin's technique.

MATERIAL AND METHODS

Finding the studies

We collected diagnostic studies of Lamendin's method for estimating age, alone or in comparison with another method, provided that they presented the results of each method individually.

The data of the results of interest include the comparison of dental age obtained as a result of the application of the method with the previously known chronological age.

The identification was performed by searching for articles in the MEDLINE, LILACS, and EMBASE databases, using the terms "Lamendin", "age estimation", "dental age estimation", "adult dental aging technique", "age determination", and "age determination by teeth".

At first, we carried out the so-called selection stage, in which the articles identified by the inclusion criteria were once again evaluated, but this time in accordance with the exclusion criteria of the systematic review. In this stage, we observed whether the studies published the results of the application of Lamendin's method, comparing chronological and estimated ages; whether there had been any change in the original technique; and whether individuals under 25 years old had been included in the sample.

To reach the final sample, the selected articles went through the process of classifying the quality of evidence, with the help of the QUADAS scale¹⁰ for diagnostic studies of accuracy, adapted to the studied method. Two examiners performed this stage, so that the studies were only included if there was agreement between the two examiners.

CRITICAL EVALUATION

Since the efficiency of the age estimation methods were analyzed in a different way from that of conventional diagnostic tests, the data collection table created for this study deviated from the tables more

commonly observed in systematic reviews of diagnostic test studies. In this case, the most important issue was to verify to what extent the estimated age approximated the chronological age, which were the factors that could influence the process of estimation, and whether the methodology applied was reliable.

For this purpose, the following data were recorded:

- Population: The country of origin of the sample, or whether it belonged to some collection or presented some particularity.
- Burial: Whether this occurred and how long ago.
- Exposure: Whether the sample was exposed to any substance or element, and what it was.
- Dental Condition: The state in which the studied teeth were found.
- Size of the Tooth/Skull sample (T/S): The number of teeth and skulls of which the sample was composed.
- Standard Reference: Source of obtaining the chronological age.
- Doubt in the standard reference: Whether the age obtained as standard reference left room for doubt.
- Blind: Whether the specific study had been conducted in this manner or not.
- Intra-examiner: Whether the intra-examiner variance was verified.
- Inter-examiner: Whether the inter-examiner variance was verified.
- Groups: Into which types of groups the sample was divided.
- Best result (group): Which groups presented the best results of the study in question.
- Mean value of differences: What was the mean value of differences calculated for the groups of age-ranges.
- *P* (estimated x chronological age): Whether *P* was presented for the comparisons between estimated age and chronological age.

- Specific formula: Whether the study developed a specific formula for the studied population.
- Best result (formula): When a new formula was developed, was there any record of whether it presented better results than the original formula of Lamendin's method.
- Statistical proof (formula): Whether there was statistical comparison between the results of the original and specific formula.

After gathering and tabulation of the data, the information obtained was descriptively analyzed and examined in the form of a meta-analysis as well. Statistical treatment was performed with the software program BioEstat® 5.0, with 95% confidence interval

The study was properly approved by the Research Ethics Committee of the School of Dentistry of University of São Paulo, with protocol number 76/11 and CAAE 0086.0.017.000-11.

RESULTS

Data gathering

The process resulted in 13 identified articles which met the inclusion criteria, all being products of searches in the online databases. The chosen articles were the following:

1. Baccino E, Ubelaker DH, Hayek LAC, Zerilli A. Evaluation of seven methods of estimating age at death from mature human skeletal remains. *J Forensic Sci.* 1999 Sept;44(5):931-6.
2. Foti B, Adalian P, Signoli M, Ardagna Y, Dutour O, Leonetti G. Limits of the Lamendin method in age determination. *Forensic Sci Int.* 2001 Nov 1;122(2-3):101-6. doi:10.1016/S0379-0738(01)00472-8.
3. González-Colmenares G, Botella-López MC, Moreno-Rueda G, Fernández-Cardenete JR. Age estimation by a dental method: A comparison of Lamendin's and Prince & Ubelaker's

- technique. *J Forensic Sci.* 2007 Sept;52(5):1156-60. doi: 10.1111/j.1556-4029.2007.00508.x.
4. Kimmerle EH, Prince DA, Berg GE. Inter-observer variation in methodologies involving the pubic symphysis, sternal ribs, and teeth. *J Forensic Sci.* 2008 May;53(3):594-600. doi: 10.1111/j.1556-4029.2008.00715.x.
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11. Schmitt A, Saliba-Serre B, Tremblay M, Martrille L. An evaluation of statistical methods for the determination of age of death using dental root translucency and periodontosis. *J Forensic Sci.* 2010 May;55(3):590-6. doi: 10.1111/j.1556-4029.2010.01341.x.
12. Soomer H, Ranta H, Lincoln MJ, Penttilä A, Leibur E. Reliability and validity of eight dental age estimation methods for adults. *J Forensic Sci.* 2003 Jan;48(1):149-52.
13. Ubelaker DH, Parra RC. Application of three dental methods of adult age estimation from intact single rooted teeth to a Peruvian sample. *J Forensic Sci.* 2008 May;53(3):608-11. doi: 10.1111/j.1556-4029.2008.00699.x.

For the effect of practicality, these articles will be referred to by their corresponding numbers in the abovementioned description of the selection process.

Analysis and presentation of data

After identification of the studies, we conducted the selection stage. At this point, the studies that fitted into the exclusion criteria of the systematic review were discarded. Thus, those that changed the original technique in an unacceptable manner or failed to present statistical results regarding the efficacy of the method were excluded. An example of acceptable change is the use of a pachymeter to replace the millimetric ruler, and the dry point compass to measure the variables. Therefore, article 3 was excluded because it measured the root transparency on the distal surface, in contrast to the original method, while articles 4 and 11 were excluded because they only compared the variance of the examiners when making measurements and only performed statistical tests to confirm whether there was a relationship between the variables and age, respectively, both without worrying about reflecting the efficacy of the method.

Since the used formula does not allow age estimation of those under 25 years old, the studies with samples that included individuals of up to 24

years were only accepted when they presented independent results for the age groups. Thus, it was possible to evaluate only the results of the groups over 25 years old, with the intention of avoiding this bias. In articles 7, 8, and 13, the results were presented in age groups, and therefore, in spite of including individuals under 25 years old in their samples, they were not excluded. Whereas in studies 1 and 12 the samples were analyzed as a single group, and were thus discarded. Afterwards, the texts of the selected studies – 2, 5, 6, 7, 8, 9, 10, and 13 – were fully evaluated in accordance with the level of scientific evidence. At this stage, the QUADAS evaluation table was used because it has been specifically developed for diagnostic accuracy tests.¹⁰

Originally, the QUADAS evaluation table consisted of 14 questions that could be answered with “Yes”, “No”, and “Uncertain” (Table 1). However, as an age estimation method, it does not constitute a typical diagnostic test, and some adaptations to the evaluation table were necessary, so that it could be suited to the technique. Ages at death varied from 30 to 81 years, with mean of 49.08 years.

Items 3, 4, 6, 7, 9, 11, 12, and 13 were excluded, mainly because they dealt with the standard reference that, because it was chronological age, did not allow interpretations and description of the method, in addition to not undergoing alterations with time, with these characteristics being questioned in the items excluded.

Table 1 | The QUADAS tool.

Item	Yes	() No ()	Unclear
1. Was the spectrum of patients representative of the patients who will receive the test in practice?	()	()	()
2. Were selection criteria clearly described?	()	()	()
3. Is the reference standard likely to correctly classify the target condition?	()	()	()
4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	()	()	()
5. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?	()	()	()
6. Did patients receive the same reference standard regardless of the index test result?	()	()	()
7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	()	()	()
8. Was the execution of the index test described in sufficient detail to permit replication of the test?	()	()	()
9. Was the execution of the reference standard described in sufficient detail to permit its replication?	()	()	()
10. Were the index test results interpreted without knowledge of the results of the reference standard?	()	()	()
11. Were the reference standard results interpreted without knowledge of the results of the index test?	()	()	()
12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	()	()	()
13. Were uninterpretable/ intermediate test results reported?	()	()	()
14. Were withdrawals from the study explained?	()	()	()

Source: Whiting et al.¹⁷

At the end, there were remaining items in numbers 1, 2, 5, 8, 10, and 14. The articles that were approved in the selection stage were then evaluated by two examiners in this exclusion stage. The items were responded in the following manner:

1. *Was the spectrum of patients representative of the patients who will undergo the test in practice?*

The answer to this question is “Yes” when the sample had demographic and clinical characteristics similar to those of the group that will undergo the test in practice. Therefore, balance between the number of individuals of both sexes and age groups was considered. In forensic cases, it is common for there to be more male than female individuals, but, when the discrepancy was very large, the sample could be considered representative if the authors mentioned the choice of cases to balance them, for example, producing the mean of similar ages. If the sample was made up of part of a collection, then it was necessary for the authors to explain the choice of included cases to which the answer would be “Yes”.

2. *Were the selection criteria clearly described?*

To answer “Yes” to this question, it is necessary for the inclusion and exclusion criteria to be clearly explained in the study. It was considered sufficient for the studies to have stated the origin of the individuals, and the type and conditions of the teeth used in the sample.

5. *Was the entire sample or a random part of it verified in accordance with the standard reference?*

The intention in this item is to know whether the results obtained by the test under study were verified, in all or in part of the sample, by the standard reference. In the case of this study, the answer was “Yes” if the estimated ages were compared with the individuals’ chronological ages.

8. *Was the performance of the test under study described in sufficient detail to allow replication of the test?*

If the explanation of the technique of the test under study left no doubts about how it was performed, the answer given was “Yes”.

10. *Were the results of the test under study interpreted without there being any knowledge of the standard reference?*

To exclude the possibility of influencing the results of the test under study, it is necessary for it to be performed without one knowing the result of the standard reference. Therefore, if the researchers did not know the chronological age of the individuals when they applied the test under study, the answer given was “Yes”.

14. *Were the desistances/exclusions from the study explained?*

When studies are conducted with patients, it is possible that some of them quit or simply do not continue with the necessary follow-up; when this occurs, these desistances must be explained by the authors. In the case of the studies in question, it is possible that after application of the test under study, some characteristics of the individuals may be perceived, which prevents them from remaining in the sample. If these exclusions were explained, or if there were no

reasons to suspect that there were exclusions, the item was marked as “Yes”.¹⁰

As a parameter for inclusion, it was considered that at least 50% of “Yes” answers would be adequate. Since the eight questions excluded could also be answered as “Yes”, with over 50% positive, the studies would have a minimum of 11 affirmative responses. In cases in which the evaluation differed between the two examiners, the most negative result was considered, so that there would be no doubt about the quality of the study in question. The results were grouped in Table 2.

As may be observed in the chart, articles 6 and 10 had four “Yes” answers, that is, the lowest number of positive results. Articles 2, 5, 9, and 13 had five “Yes” answers, while numbers 7 and 8 had all positive responses. Thus, all the articles fulfilled the requisite and were accepted for the systematic review.

The data of each of the studies was collected, as shown in Table 3.

It may be observed that many data referring to the conditions of the sample, such as burial, exposure to substances, or status of dental units are not specified by the authors.^{9,11-15}

Table 2 | Quality of evidence of articles with modified QUADAS.

Item \ Study	Foti et al. ¹⁶	Lamendin et al. ⁹	Martrille et al. ¹¹	Meinl et al. ¹⁸	Prince and Konigsberg ¹²	Prince and Ubelaker ¹³	Sarajic et al. ¹⁴	Ubelaker and Parra ¹⁵
1. Was the spectrum of patients representative of the patients who will undergo the test in practice?	Yes	Yes	Uncertain	Yes	Yes	Yes	No	Yes
2. Were the selection criteria clearly described?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5. Was the entire sample or a random part of it verified in accordance with the standard reference?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Was the performance of the test under study described in sufficient detail to allow replication of the test?	Yes	Yes	No	Yes	Yes	Yes	Yes	No
10. Were the results of the test under study interpreted without there being any knowledge of the standard reference?	Uncertain	Uncertain	Yes	Yes	Yes	Uncertain	Uncertain	Yes
14. Were the desistances/exclusions from the study explained?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

It was not always explicit how the chronological age was obtained, mainly in cases in which the sample formed part of a collection. It is known that age is identified due to the register of the collection, but it is not known how the information for the register was first collected.

We also noted that most studies^{9,11,13,15,16} did not make it clear whether they were blind studies or not, but at least half of them provided intra- and

inter-examiner statistics, while one was imprecise regarding this.

All the studies divided the sample into age groups and proved that the method presents divergent results in different age-ranges. Four studies^{12,14-16} observed that the groups with more precise age estimations were those of up to 50 years of age, differently from the remainder of the researches.

Table 3 | Qualitative Data Gathering (conclu

	Foti et al. ¹⁶	Lamendin et al. ⁹	Martrille et al. ¹¹	Meinl et al. ¹⁸	Prince e Königsberg ¹²	Prince e Ubelaker ¹³	Sarajlić et al. ¹⁴	Ubelaker e Parra ¹⁵
Population	France	France	Terry Collection/ USA	Austria (bodies donated/ collection)	Kosovo	Terry Collection/ USA	Bosnia and Herzegovina (War Victims)	Peru
Burial	No (Live)	NE	NE	NE	NE	NE	6 to 10 years	NE
Exposure	Not applicable	NE	NE	37 teeth to formol	NE	NE	NE	NE
Dental Condition	Periodontal Disease	NE	NE	Without trauma, caries, or resorption	NE	NE	NE	NE
Sample size T/S	71/71	45/24	218/218	67/37	401/401	400/359	847/200	100/100
Standard Reference	Clinical chart	NE	Register - NE	Clinical chart, Register	Expert Identifications	Register - NE	Identifications	NE
Doubt in standard reference	No	NE	NE	No, NE	No ¹	NE	No	NE
Blind	No	NE	No	Yes	Yes	No	No	Yes
Intra-examiner	Yes	NE	No	Yes	Yes	Yes	No	No
Inter-examiner	Yes	NE	No	Yes	Yes	Yes	No	No
Groups	Age, sex, tooth, maxilla	Age	Age, sex, ancestry	Age	Age	Age, sex, ancestry	Age, tooth	Age, sex
Best result	<49; male; canines; mandible	50-59	41-60; female; Caucasians	20-40	40-49	50-59; There is no difference; there is difference, Did not say which	30-39, mandibular central incisors	30-39, male

1. Information in Kimmerle et al.¹⁹

Continuation...

	Foti et al. ¹⁶	Lamendin et al. ⁹	Martrille et al. ¹¹	Meiml et al. ¹⁸	Prince e Konigsberg ¹²	Prince e Ubelaker ¹³	Sarajlić et al. ¹⁴	Ubelaker e Parra ¹⁵
Mean value of differences	<49 – 2.95 49-59 – 6.71 59-69 – 13.54 >69 – 18.68	30-39 – 13.1 40-49 – 6.3 50-59 – 3.3 60-69 – 9.8	25-40 – 11.3 41-60 – 6.1 >60 – 16.6	20-40 – 6.8 41-60 – 11.9 >60 – 26.3	18-29 – ≈12 ² 30-39 – ≈4 40-49 – ≈3 50-59 – ≈10 60-69 – ≈15 70-79 – ≈21 +80 – ≈28	25-29 – 13.2 30-39 – 15.5 40-49 – 5.6 50-59 – 5.2 60-69 – 7.2 70-79 – 12.3 80-89 – 20.3 90-99 – 32.6	23-29 – 6.64 30-39 – 4.35 40-49 – 6.09 50-59 – 10.95 60-69 – 17.51	20-29 – 6.58 30-39 – 3.3 40-49 – 5.45 50-59 – 6.86 >60 – 17.76
P (estimated x chronological age)	Yes	No	No	No	No	No	No	No
Specific formula	Not applicable	Not applicable	Not applicable	No	No	Yes	Yes (also for each group of teeth)	Yes
Best result (formula)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Yes	The specific formulas, Yes. The total formula provided worse results for two groups of teeth. The specifics always provided better results than the total.	Yes, except for the 30-39 group
Statistical proof (formula)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Yes	Yes	No

NE: Not specified

2. Values obtained from Prince and Konigsberg¹² graphs.

In addition, some authors divided their samples according to sex, ancestry, type of tooth, and maxilla, but the results of these groups were also not constant. Studies showed better results for males^{15,16}, females¹¹, and no differences observed between the sexes¹³.

Of the two articles that compared the types of teeth, one¹⁶ observed more precise results in canines, while the other¹⁴ found greater agreement between mandibular central incisors.

On the other hand, Prince and Ubelaker¹³ observed differences between Caucasians and Afro-descendants, but did not go into the subject in-depth, and did not specify whether one group would be more precise than the other, in disagreement with Martrille et al.,¹¹ who observed better results in Caucasians. Foti et al.¹⁶ also found greater precision in the mandible than in the maxilla.

The means of the differences in the age groups ranged from 2.95 to 32.6. It was possible to observe that the very high values occur only in older individuals. The best mean values of each study ranged from 2.95 to 6.8; it is worth pointing out that this latter value belongs to a group that included individuals under 25 years old. However, only Foti et al.¹⁶ calculated the *P* to verify whether the differences found would be statistically significant.

Lastly, the data regarding the development of a specific formula for the researched population showed that, of the five articles that could develop one, three¹³⁻¹⁵ did so. Moreover, it was found that in general, these formulas provided better results than the original formula, but Ubelaker and Parra¹⁵ did not use statistics to show that the new formula produced significantly better results.

The meta-analysis show by age groups 30-39, 40-49, 50-59, and 60-69, used by the studies¹⁴⁻¹⁶ that the real and estimated ages have the following differences: 4.30 years ($p < 0,01$), 5.27 years ($p < 0,01$), 9.07 years ($p < 0,01$), and 16.62 years ($p < 0,01$), respectively.

The group of up to 29 years old was excluded because it comprised individuals under 25 years old.

DISCUSSION

Enhancing and updating the review

The studies could have had some more methodological rigor regarding the balance of the sample and its particular characteristics. However, the methodologies used were sufficient to provide the results with quality evidence and verify that Lamendin's method is efficient. Nevertheless, due to the different results presented by the diverse samples, we suggest that the method should be tested in the population in which one intends to apply it, to verify in which age range it produces the best results, and thus, diminish the possibility of error. A constant factor in the researches is the observation of overestimations in young individuals and underestimations in older subjects. In addition, the technique loses its efficacy when applied to individuals of over 60 years of age.

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Idiopathic sialadenosis involving parotid and submandibular glands: a case report

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ABSTRACT | This article reports a case in which an adult black male patient was diagnosed as having sialadenosis of idiopathic type, since computed tomography, clinical, and laboratory examinations did not disclose any other abnormalities that could be associated with the glandular swelling observed in the patient. As this condition is quite harmless, requiring no intervention, unless for aesthetic reason, the patient was dismissed, being monitored sporadically. But after 8 months since the first consultation the patient was diagnosed as having an advanced esophageal squamous cell carcinoma, and eventually died of this disease. Therefore, this report raises the question whether there was any relation with the sialadenosis and the esophageal carcinoma. This question is very speculative, but it stands as a notice for clinicians in future cases of idiopathic sialadenosis to evaluate the patient for an underlying malignant disease.

DESCRIPTORS | Parotid Gland; Pathology; Salivary Glands; Salivary Gland Diseases; Sialadenosis.

RESUMO | **Sialoadenose do tipo idiopático envolvendo as glândulas parótida e submandibular: relato de caso** • Este relato descreve o caso de um paciente adulto negro diagnosticado com uma sialoadenose do tipo idiopático, uma vez que exames clínicos, de tomografia computadorizada e testes laboratoriais não revelaram qualquer outra anormalidade que pudesse ser associada ao aumento de volume glandular por ele apresentado. Considerando que essa condição é bastante benigna, não requerendo qualquer outra intervenção exceto por razões estéticas, o paciente foi dispensado e orientado a retornar para controles esporádicos. Entretanto, oito meses após sua consulta inicial, o paciente recebeu um diagnóstico de carcinoma de células escamosas avançado no esôfago, que o levou a óbito. Esse relato, então, levantou o questionamento de alguma relação entre a sialoadenose e o carcinoma esofágico. Essa questão é obviamente especulativa, mas permanece como um aviso aos clínicos para, em futuros casos de sialoadenose idiopática, avaliar o paciente para uma doença maligna subjacente.

DESCRIPTORES | Glândula Parótida; Patologia; Glândulas Salivares; Doenças das Glândulas Salivares; Sialoadenose.

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INTRODUCTION

Sialadenosis has been defined as a persistent, soft, and painless non-inflammatory and non-neoplastic disease of the salivary gland, usually involving the parotid glands, and less frequently the submandibular glands.¹ The disease occurs more frequently in adults, affecting male and female in equal frequency.^{1,2}

The etiology of sialadenosis is unknown; however, the reports have described this condition in association with systemic diseases such as diabetes mellitus, alcoholism, chronic malnutrition, antihypertensive therapy, bulimia, and HIV infection.¹⁻⁶

Diagnosis is based mainly on the clinical findings.² Ultrasound and computed tomography are very useful tools not only to confirm a clinical diagnosis of sialadenosis, but also to rule out other diseases, specifically neoplasias.⁵⁻⁷ Fine needle aspiration biopsy may be useful in some cases to differentiate an inflammatory mass from a neoplasia.⁸ The aim of this report is to show a case of sialadenosis of unknown cause, with a subtle speculation whether this condition could possibly have been a prodromic sign of an underlying esophagus carcinoma in advanced stage.

CASE REPORT

A 48-year-old black male was referred to our clinic for a diagnosis of a bilateral, painless swelling of the parotid and submandibular glands with 6-month duration (Figures 1A and B). The swollen areas were soft on palpation. The patient was a smoker of 20 cigarettes/day for 30 years, but not a habitual alcohol drinker. He was not taking any medication nor suffering of any systemic conditions. He had undergone a treatment for pneumonia 3 years before. Oral examination showed normal mucosa; both the parotid and submandibular glands were milking normally. The measuring of resting whole saliva flow rate was 0.6 mL/min,

indicating a normal output of saliva. Neck palpation revealed no sign of enlargement of the lymph nodes.

A diagnosis of sialadenosis was made and the patient underwent a laboratory examination in order to disclose any possible underlying disease. The exams included complete blood count, blood glucose level, liver function tests, and thyroid hormones. The results of all these tests were within normal limits. The patient was also tested for HIV infection, which was negative.

Although no signs of either a neoplasia or an inflammatory process occurring inside the salivary glands, a biopsy of minor salivary gland of the labial mucosa was provided. Histologic examination revealed normal arrangement of the glandular tissues. Further examination using computed tomography showed enlargement of the parotid glands. No intraglandular lesions or calcification were detected (Figure 1C).

Since nothing was found regarding the patient's general health, and considering that no treatment would be necessary for his condition, he was placed on periodic clinical evaluation under the clinical diagnosis of idiopathic sialadenosis. However, after 8 months he sought a medical consultation complaining of dysphagia. He was then submitted to an upper digestive tract endoscopy, which revealed an obstructive lesion on the esophageal tract. The histopathologic diagnosis was an invasive squamous cell carcinoma. The patient underwent surgical treatment but died 3 months later due to the disease.

DISCUSSION

Bilateral salivary gland swelling may reflect an autoimmune disease, viral infection, or a systemic condition. A complete diagnosis usually requires clinical, laboratory, and radiological investigations.^{2,9} In this case, the clinical evaluation ruled out both autoimmune and infective disease. Laboratory

investigation showed all exams within normal limits; blood test for HIV was negative. Computed tomography showed only enlargement of both parotid glands without any intraglandular lesion. Based on the radiography, clinical, and laboratory examinations, the final diagnosis was an idiopathic sialadenosis.

In this case, the use of needle-aspiration biopsy as an additional resource for a diagnosis of sialadenosis was deemed unnecessary since the clinical examination, coupled with laboratory investigation

and computed tomography analysis, did not show signs of inflammation on the glandular parenchyma, presence of intraglandular mass or nodules, or involvement of any systemic disease.

Sialadenosis usually does not require treatment, unless for aesthetic reasons, in which a total or partial parotidectomy remains the main treatment.^{1,5} In this case, no treatment was thought to be necessary. Therefore, the patient was monthly monitored by clinical examinations.



Figures 1A and B | Diffuse, bilateral swelling of the parotid and submandibular glands. There were no inflammatory signs; the swollen areas were soft on palpation.

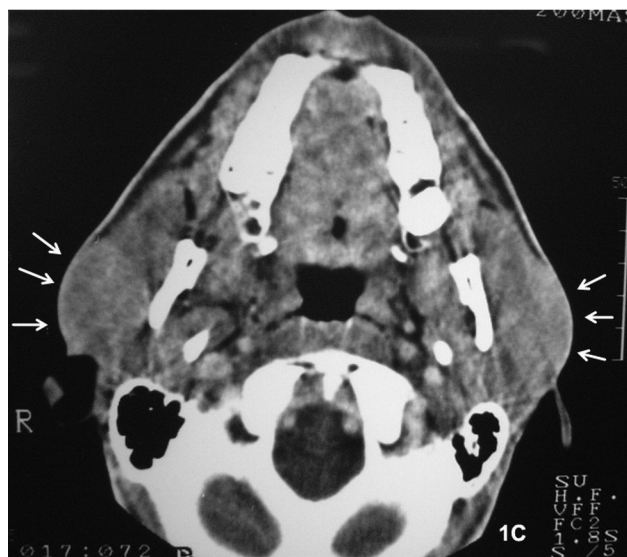


Figure 1C | Helical computed tomography of the soft tissues viewed with window scans clearly shows enlargement of the parotid glands (arrows). There is increased density in both glands, but no sign of intraglandular lesions.

In the literature, most of the cases of sialadenosis are related to chronic alcohol intake, although there have also been case reports related to anorexia nervosa and bulimia.^{2,6} Idiopathic sialadenosis is rarely seen or reported. According to our knowledge, there has been only one case of this condition published worldwide over the last 30 years.⁹

For this case, the question was raised whether an underlying squamous cell carcinoma on the esophageal tract, later diagnosed as an invasive tumor, could be considered the sole cause for triggering the salivary gland swelling.

This question is very speculative since the pathophysiology of sialadenosis is related to an autonomic neuropathy resulting in cytoplasmatic swelling and enlargement of the salivary glands (mainly the parotids). On histopathological grounds, acinar cell hypertrophy with latter fatty infiltration is the prominent feature of the glandular parenchyma. Therefore, the role of a malignant disease in bringing about this whole process could be only acceptable if it was somehow affecting the autonomic nervous system. The malignancy observed in this case (squamous cell carcinoma on the esophageal tract) cannot be easily connected with the pathophysiology of sialadenosis. There is no report of salivary glands enlargement associated with head and neck malignancy as a distinct clinical feature so far. In addition, the usual history in relation to esophageal carcinoma is of a diagnosis in an advanced stage and poor prognosis due to the lack of symptoms, as was the case of the patient here reported.^{10,11} Dysphagia, weight loss, and blood loss are signs associated to esophageal carcinoma, and the patient showed dysphagia only eight months after his last consultation at our clinic.^{10,11}

On the other hand, one of the leading causes in sialadenosis is malnutrition, with bulimia and alcoholism being the most important leading factors in producing malnutrition.⁵

If one is to connect the malignancy seen in the patient as the trigger factor in the development of the glandular swellings, this would only be possible in association with some sort of lack of food absorption induced by the malignancy (given the location of the neoplasia, the esophageal tract), and consequently producing some degree of malnutrition.

CONCLUSIONS

The question raised in this report is so far merely speculative since there is no scientific evidence of association between sialadenosis and underlying malignant disease. The diagnosis that stands is of an idiopathic sialadenosis of parotid and submandibular glands.

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Medical considerations in dental treatment of patients with Williams-Beuren syndrome: report of four clinical cases

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ABSTRACT | Williams-Beuren syndrome (WBS) is a rare genetic disorder characterized by peculiar facies, eyes, dental, cardiovascular, renal, and skeletal abnormalities, mental retardation, friendly and loquacious personality, and occasionally hypercalcemia in infancy. The orofacial aspects and especially the dental management of these patients have received little attention in the literature. The aim of this manuscript is to describe four cases of patients with WBS attended at Special Care Dentistry Center, School of Dentistry, University of São Paulo, with emphasis on the oral findings and systemic conditions of dental interest. The clinical management of patients with WBS is discussed. We conclude that congenital heart defects and behavioral changes are the aspects that most interfere on dental treatment.

DESCRIPTORS | Williams Syndrome; Dental Treatment; Heart Diseases.

RESUMO | **Considerações médicas no tratamento odontológico de pacientes com síndrome de Williams-Beuren: relato de quatro casos clínicos** • Síndrome de Williams-Beuren (SWB) é uma doença genética rara, caracterizada por anomalias peculiares em face e olho, odontológicas, cardiovasculares, renais e esqueléticas, retardo mental, personalidade amigável e comunicativa e, ocasionalmente, hipercalcemia na infância. Os aspectos orofaciais e, especialmente, o manejo odontológico desses pacientes têm recebido pouca atenção na literatura. O objetivo deste artigo é descrever quatro casos de pacientes com SWB atendidos no Centro de Atendimento a Pacientes Especiais da Faculdade de Odontologia da Universidade de São Paulo, com ênfase nos achados bucais e condições médicas de interesse para odontologia. O manejo clínico de pacientes com SWB é discutido. Conclui-se que os defeitos cardíacos congênitos e mudanças de comportamento são os aspectos que mais interferem no tratamento odontológico de paciente com SWB.

DESCRITORES | Síndrome de Williams; Tratamento Odontológico; Cardiopatias

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INTRODUCTION

The Williams-Beuren syndrome (WBS) is a rare genetic disorder that occurs sporadically, with an incidence of 1:20,000 to 1:50,000 live births. It was firstly described in 1961 by the cardiologist John Williams, and in 1962 Beuren expanded the syndrome's features. It affects both sexes equally and seems to occur in all ethnic groups around the world. Individuals with this syndrome have peculiar facies, dental development disorders, eyes, heart, kidney, and skeletal abnormalities, mental retardation, friendly and loquacious personality, and occasionally hypercalcemia during infancy.^{1,2}

Individuals with WBS present hemizygous microdeletion or deletion of contiguous gene in 7q11.23 region that alter the function of several genes, including those responsible for the production of elastin.³ The diagnosis of the syndrome is clinical, but the confirmation occurs through identification of the gene deletion by fluorescent in situ hybridization (FISH) test.⁴

Elastin is a structural protein of the extracellular matrix which composes 90% of elastic fibers and it is responsible for reestablishment of various tissues after distention. *Elastin* is organized into *elastic fibers* in the walls of arteries, skin, lungs, gut, cartilage, ligaments and joints as well as alterations in its synthesis can lead to cardiovascular problems, ligamentous laxity, and joint contractures.⁵

Cardiovascular changes are very important due to their high frequency and clinical impact. The most common finding is the supravalvular aortic stenosis (SVAS), followed by pulmonary artery stenosis and mitral valve prolapsed.² Hypercalcemia is the presence of high levels of calcium in blood, and only 15% of individuals with WBS present this idiopathic condition that usually regresses until 4 years old.⁶

The typical facial appearance of individuals with WBS is described by several authors as "elfin face" and is characterized by periorbital fat

accumulation, low nasal bridge, prominent cheeks, long nasal filter, thick lips, bitemporal depression, small and upturned nose, small chin, stellate *iris pattern* (observed mainly in individuals with light eyes), strabismus, malocclusion, and disorders of dental development.²

Among the dental development disorders, the most mentioned ones in literature are microdontia, hypodontia, small roots, fused tooth, twinned tooth, taurodontism, enamel hypoplasia, and presence of generalized diastemas.⁷ Occlusal changes have also been described, including predominantly Angle class III malocclusion.

Considering that many dental development disorders may require careful treatment plan, it is important for the dentist to recognize and to search for these changes in people with WBS. Thus, the dentist can anticipate the dental needs and know the systemic implications to provide an efficient and safe dental care to the patient.

CASE REPORTS

CASE 1 – Patient P.L.G.O., male, 8 years old, diagnosed with WBS at 7 years old through the FISH test, went to the Special Care Dentistry Center (SCDC), School of Dentistry, University of São Paulo for dental treatment. He had thick lips, prominent cheeks, depressed nasal bridge, small nose, and accumulation of periorbital fat (Figure 1A). Medical history revealed SVAS, pulmonary stenosis, heart murmur, arterial and pulmonary hypertension, renal failure, severe mental retardation, skeletal growth delay, hyperactivity, hyperacusis, pulmonary fibrosis, and gastroesophageal reflux. During the first two years of life, the patient had suffered a stroke and three acute myocardial infarctions. Since then, the patient has been treated with two types of diuretics, association of three antihypertensive drugs, anticonvulsant, anti-thrombotic, and gastric protector. He is in the *waiting* list for a heart transplant.



FIGURA 1 | Orofacial and dental characteristics of patients with Williams-Beuren syndrome. A) Case 1 showing thick lips, prominent cheeks, depressed nasal bridge, small nose, and accumulation of periorbital fat. Generalized diastemas and gingival hyperplasia can also be seen. B) Case 2 showing thick lips, prominent cheeks, depressed nasal bridge, small nose, small chin, generalized diastemas, and malocclusion. C) Case 3 showing thick lips, prominent cheeks, and long nasal filter. D) Case 4 showing features like thick lips, long nasal filter, and small chin.

Clinical examination revealed mixed dentition, some deciduous teeth with prolonged retention, generalized diastemas, three teeth with caries, thick biofilm, spontaneous gingival bleeding, and gingival hyperplasia. The boy breathed through the mouth, remained with his tongue interposed between the front teeth, and had the habit of grinding the teeth at night. His bite was open and classified as Angle class III malocclusion. Due to lack of cooperation and to

the large amount of required dental procedures, it was decided with the cardiologist to perform the treatment under general anesthesia. The treatment plan included restoration of decayed teeth, tooth extractions, periodontal scaling, and topical application of fluoride varnish. The mother was instructed on the importance of proper oral hygiene and the maintenance of good oral health, especially facing the possibility of heart transplantation.

CASE 2 – Patient B.E.M.S., female adolescent, 15 years old, positive for FISH test at the age of four, sought the SCDC for routine dental treatment. She had typical facies, showing thick lips, prominent cheeks, depressed nasal bridge, small nose, small chin, and starry iris (Figure 1B). Medical history revealed that the SVAS was surgically corrected in early childhood, but the heart murmur persisted, as well as high blood pressure, kidney failure, severe mental retardation, growth retardation, hyperactivity, and hyperacusis. She has been regularly taking two antihypertensive medications, two types of diuretics, vasodilators, intestinal regulator, and gastric protector.

The patient had permanent dentition with generalized diastemas, three teeth with caries, generalized gingival bleeding, and drug-induced gingival enlargement grade 2. She was breathing through the mouth and interposing the tongue between the anterior teeth. She showed anterior crossbite, open bite, and malocclusion type II – division 1, according to the Angle classification. Dental treatment was carried out in dental clinic without sedation although she did not wholly cooperate with the oral management. Sometimes certain degree of passive physical restraint was needed. We noticed the sensitivity of patient to the sounds of sucking and high-revving engine. Supra and sub gingival scraping and restoration of teeth with dental caries were performed. We have decided not to perform gingivoplasty due to poor cooperation of the patient, and the switch to hypotensive drug was not considered by cardiologists. We followed the recommendations of the American Heart Association⁸ and since her congenital cardiopathy had been corrected surgically we did not perform prophylactic antibiotics before sub gingival scraping. Returns for preventive therapy were recommended every 6 months.

CASE 3 – Patient P.V.C., male, 11 years old, diagnosed with WBS at 9 years old by FISH test,

presented to the SCDC complaining about caries and gingival bleeding. He showed thick lips, prominent cheeks, and long nasal filter (Figure 1C), mild mental retardation, hypothyroidism, and hyperacusis. He did not exhibit any congenital heart disease. He was using thyroid hormone for treating hypothyroidism since 10 years old. He breathed through the mouth, reported sleep snoring and bruxism. The occlusion examination revealed crossed and open bite, class II Angle malocclusion, and atypical swallowing.

He presented mixed dentition with prolonged retention of primary teeth, generalized diastemas, several teeth with dental caries, gingival bleeding on probing, hypertrophic labial frenulum, and enamel hypoplasia in several teeth. *Panoramic radiograph* confirms *hypodontia* of maxillary lateral incisors teeth. The patient cooperated entirely with treatment, which included restorations with composite resin, scraping, and periodontal polishing. Returns for preventive therapy were recommended every 6 months and orthodontic treatment was nominated.

CASE 4 – Patient F.S.S., male, 22 years old, tested positive for FISH at 10 years old, presented for dental treatment, exhibiting typical WBS features like thick lips, long nasal filter, and small chin (Figure 1D). He presented mild mental retardation, supralvalvar aortic stenosis, surgically corrected in childhood, and persistent murmur heart.

His teeth were free of caries, but gingivitis was present since he showed gingival bleeding on probing. His orthodontic treatment was carried out at SCDC for 10 years. He had deep bite and hypodontia of the upper right second premolar. The supragingival scraping and extraction of all third molars, which were misplaced, were carried out without any resistance of the patient. Prophylactic antibiotics were not prescribed since he had his congenital cardiopathy surgically corrected during his childhood.

DISCUSSION

We presented four clinical cases of three children and a young man with WBS with oral changes and different systemic disorders linked to the syndrome, which illustrate the wide clinical spectrum of this syndrome.

The main systemic disorders that interfere directly in the dental clinical management of patients with WBS are heart disease and behavior alterations. Cardiovascular abnormalities caused by deficiency of elastin occur in 80% of WBS individuals, being the SVAS the most frequent heart defect. Cardiovascular diseases are significant causes of morbidity and mortality in WBS with risk of sudden death. The literature shows that when the correction is possible, the immediate surgical results are low mortality and good results with stable pressure gradient and without restenosis.¹

Among our cases, three had a history of significant heart diseases, which had been surgically corrected in the patients 2 and 4. The patient 1 was awaiting heart transplantation, and his severe and unstable cardiovascular condition contraindicated dental treatment at that time. Since the removal of oral infection foci is important to the patient, the cardiologist was trying to compensate him before heart transplantation.

In Case 2, neither heart disease nor hypertension limited dental intervention, since the patient was stable. She was taking amlodipine (calcium channel blocker) 5 mg two times a day, causing gingival overgrowth. Proper conduct for patients with hypertension is to measure the blood pressure (BP) before starting all dental visits. She was stable and the hypertension was under control. For this reason, the cardiologist decided to maintain the current medication even with it causing gingival overgrowth. The gingivoplasty was not performed due to poor cooperation of the patient. The stress caused by this surgery, as well as the slight discomfort after surgery, could contribute to increase BP

of the patient. Considering that dental treatment should not interfere on the patient's hemodynamics, we avoided dental procedures that generate rapid changes in BP, heart rate, and myocardial oxygen demand. Therefore the dentist should minimize this stress by scheduling the patient in calm times, performing short visits, properly controlling the operative pain, and avoiding elective procedures.

Hypertension is described in 40-70% of children with WBS and is associated with aortic coarctation, nephrocalcinosis, nephropathy or renal failure, and diffuse arterial disease with few cases refractory to medication. It can be manifested later at school age and should be important to perform hypertension research in patients with WBS.⁹

Another systemic change that interfered with dental treatment was hyperacusis, which affects the patient's behavior during the consultation. Approximately 94% of subjects with WBS⁴ have heightened sensitivity to sounds. The implication in dental treatment may be directly related to the sounds of high revving engine and sucking, which can be very uncomfortable for people with WBS.

In the cases 1, 2 and 3, the individuals exhibited caries. The literature provides conflicting data on the incidence of dental caries in people with WBS. Mozkovits et al.² reports that there is no difference in the decay rate in the general population comparing to WBS individuals. Axelsson et al.⁷ commented that dental caries in WBS may be the result of an association between enamel hypoplasia, hypomineralization and poor oral hygiene. Our patients had poor oral hygiene and gingivitis. The malocclusion, mental retardation, and poor control of plaque put them in a high risk category for caries.

The microdeletion of chromosome 7, which determines the establishment of WBS, may include some genes directly involved in odontogenesis as the genes clustered family TFII-I (Gtf2i, Gtf2ird1 and Gtf2ird2) and its absence of expression seems

to be related with some tooth development disturbances such as changes in shape, number, hypodontia and enamel hypoplasia.¹⁰ Development disorders of teeth observed in the cases described herein included hypodontia, generalized diastemas, prolonged retention of deciduous teeth, teeth with altered morphology and enamel hypoplasia. Early diagnosis and treatment of these disorders involve better oral health conditions in adulthood.

The hypodontia can be managed with prostheses or implants and the tooth shape changes can be corrected with direct (composite resin) or indirect (porcelain) restorations. Orthopedic and orthodontic treatment will correct the occlusal alterations, and should be performed when WBS individuals cooperate, as seen in Cases 3 and 4.

Although the behavioral aspects of individuals with WBS is predominantly hyperactive and most of them show significant mental retardation, exacerbated sympathy and talkativeness may give the false impression to the dentist that the management will be easy and the cooperation of the patient is guaranteed.

Moskovits et al.² point out that dentists should consider the hyperactive behavior of patients with WBS as an obstacle and use sedation orally or inhaled, as well as general anesthesia to reduce anxiety and overcome lack of cooperation of young patients. However, treatments under general anesthesia may have serious consequences in patients with WBS because of their heart problems. The findings of Olsen et al.¹¹ suggested high rates of cardiac complications, so they recommend a serious analysis of the benefit-risk for general anesthesia, as there are reports of cases of sudden death related to the procedure. Our patient 1 waits for future release of the cardiologist for general anesthesia running for dental treatment.

CONCLUSION

Before starting the dental treatment, the dentist should be aware about the cardiac condition of the patient with WBS, and identify their behavior in the doctor's office. The disturbance of dental development, especially hypodontia, should be considered in the dental treatment plan.

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Oral lipoma of unusual size and location: a case report

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ABSTRACT | A lipoma is a slow-growth benign tumor of the adipocytes. In the oral cavity, it is more commonly observed in the buccal mucosa; other sites less affected are the tongue, floor of the mouth, and lips. The objective of this report is to describe an extensive tumor affecting the dorsum of the tongue and the therapeutic maneuver performed. A 63-year-old man was referred to our clinic for evaluation of a tumor in the dorsum of the tongue with 6 months of duration. The physical examination revealed an extensive nodule, asymptomatic, submucosal, in the dorsum of the tongue at the left that measured approximately 4 cm. On palpation, the lesion was mobile and had rubbery consistency. According to these findings, the diagnostic hypotheses were lipoma and neurofibroma. Hence, surgical excision was performed and during the procedure a yellowish and well-delimited lesion was observed. The surgical specimen floated in 10% formalin. The histopathological examination confirmed the diagnosis of lipoma. The patient is asymptomatic after 40 months of follow-up. This report describes an unusual presentation of lipoma on the dorsum of the tongue and demonstrates that this lesion can reach large proportions.

DESCRIPTORS | Lipoma; Tongue Diseases; Mouth Neoplasms; Tongue Neoplasms.

RESUMO | **Resumo Lipoma oral de tamanho e localização incomuns: relato de caso** • Lipoma é um tumor benigno de crescimento lento dos adipócitos. Na cavidade oral, é mais comumente observado na mucosa jugal; outros sítios menos afetados são a língua, soalho oral e lábios. O objetivo deste relato é descrever um extenso tumor afetando o dorso da língua e a manobra terapêutica realizada. Um homem de 63 anos de idade foi encaminhado à nossa clínica para avaliação de um tumor no dorso da língua com 6 meses de duração. O exame físico revelou um nódulo extenso, assintomático, submucoso, no dorso da língua à esquerda, medindo aproximadamente 4 cm. À palpação, a lesão era móvel e tinha consistência borrachoide. De acordo com esses achados, as hipóteses diagnósticas foram lipoma e neurofibroma. Portanto, foi realizada excisão cirúrgica e, durante o procedimento, foi observada uma lesão amarelada e bem delimitada. O espécime cirúrgico flutuou em formalina a 10%. O exame histopatológico confirmou o diagnóstico de lipoma. O paciente encontra-se assintomático após 40 meses de acompanhamento. Este relato descreve uma apresentação incomum de lipoma no dorso da língua e demonstra que essa lesão pode atingir grandes proporções.

DESCRIPTORES | Lipoma; Doenças da Língua; Neoplasias Bucais; Neoplasias da Língua.

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INTRODUCTION

Lipoma is a benign tumor of mature adipocytes that presents a slow-growing and is usually asymptomatic. The tumor's consistency may vary from rubbery to flaccid, according to fibrous tissue amount.^{1,2} It usually has intact surface, but ulceration can occur in case of secondary trauma.³ Although being considered the most common mesenchymal neoplasm, this tumor is relatively uncommon on the mouth and the main sites affected are buccal mucosa, tongue, floor of the mouth, and lips.⁴

Regarding the size, the tumor may vary from few centimeters to large proportions⁵ and the latter can cause significant alteration in the organ function, such as difficulty to speak, chew, and swallow.⁶ The aim of this study is to report an extensive lipoma involving the dorsum of the tongue. In addition, its clinical implications and treatment are also emphasized.

CASE REPORT

A 63-year-old man was referred to the Stomatology Department due to an extensive enlargement on the dorsum of the tongue with 6 months of duration: the medical history showed controlled diabetes; the intraoral examination revealed a well-delimited asymptomatic nodule, located on the dorsum of the tongue, measuring around 4.0 cm, and covered by normal mucosa (Figure 1A and 1B); on palpation, the lesion was mobile and rubbery. According to these findings, we raised the diagnostic hypotheses of lipoma and neurofibroma. A surgical excision was proposed and during the procedure a well-circumscribed yellowish lesion with a smooth surface was totally removed (Figure 2 and 3). The lesion floated in formalin solution (Figure 4). The histopathological examination exhibited a large number of mature adipocytes interspersed with bands of connective tissue. It also presented some small caliber congested blood vessels

(Figures 5 and 6). Hence, the diagnosis of lipoma was confirmed. The patient is asymptomatic for 40 months (Figure 7).

DISCUSSION

Lipomas are mesenchymal neoplasms that are uncommon in the oral cavity. Usually, oral lipomas manifest as asymptomatic, slow-growing, and submucosal nodules.⁷ They occur mainly in the buccal mucosa, followed by tongue, floor of the mouth, and lips.³ However, considering the tongue neoplasms, lipomas account for only 0.3% of them.⁸ A recent review about tongue lipomas showed that most of the cases had less than 2.0 cm of diameter (average of 2.1 cm), and the largest tumor had 6.0 x 6.0 x 5.0 cm. Furthermore, the lateral region of the tongue was the main site affected.⁹ In general, it has soft to rubbery consistency and floats.⁵ However, an atypical form of lipoma called infiltrative lipoma can invade muscle tissue and recur after excision.¹⁰ A Brazilian study evaluating 41 oral lipomas showed a predominance of women (ratio 2.4:1) and a peak incidence in the sixth and seventh decades.¹¹ However, another study performed in the Brazilian population¹² did not find gender predilection. Recently, it was proposed that diabetes is associated with the incidence of oral lipoma, as well as trauma, chronic stimulation and heredity.² Disruption of adipocyte maturation process can be caused by mutations in mitochondrial DNA, and diabetic patients are exposed to such mutations.¹³ Our patient reported to have controlled diabetes.

Although microscopical lipoma can be classified into several subtypes, the histopathological aspect of the lesion is characteristic. A classic lipoma usually has a circumscribed and non-encapsulated aggregate of mature adipocytes, with an abundant and clear cytoplasm and absence of cellular atypia and metaplasia.¹⁴ This description is consistent with the histopathological findings of our study.

The treatment of choice is surgical resection,⁹ since the tumor is easily excised and usually do not recur. However, for infiltrative lipomas, the recommendation is the removal with healthy tissue margin to prevent recurrence.^{1,12} The delay in the treatment of major tongue lipomas can lead to difficulty in speech, breathing, and swallowing, as well as atrophy of the lingual muscles and dental alterations.¹⁵ The treatment in this case consisted

of surgical excision and the patient is asymptomatic after 40 months.

CONCLUSION

This report describes an unusual presentation of lipoma due to its clinical dimension and location. Therefore, it is important to include lipoma in the differential diagnosis of extensive lesions involving the tongue.



Figure 1A and 1B | Extensive swelling on the dorsum of the tongue with intact surface. On palpation, the lesion was mobile with rubbery consistency.

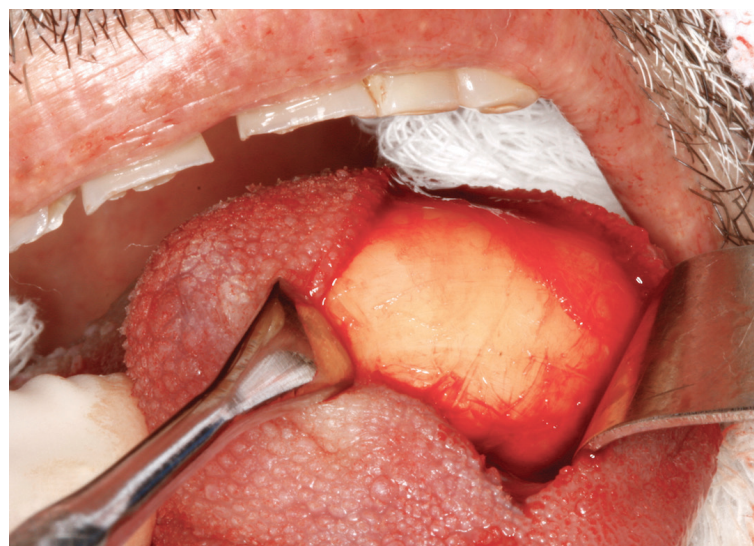


Figure 2 | Trans-surgical showing a yellowish and well-delimited lesion not adhered to the deep plans.

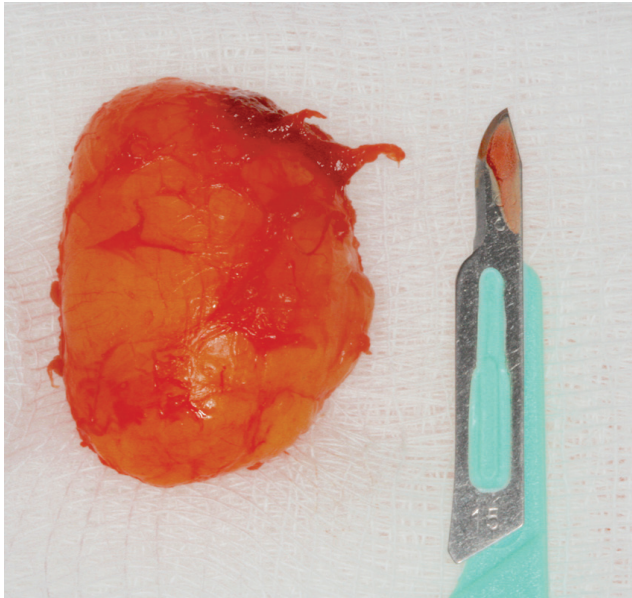


Figure 3 | Specimen measuring 3.7 cm x 2.4 cm x 1.8 cm.

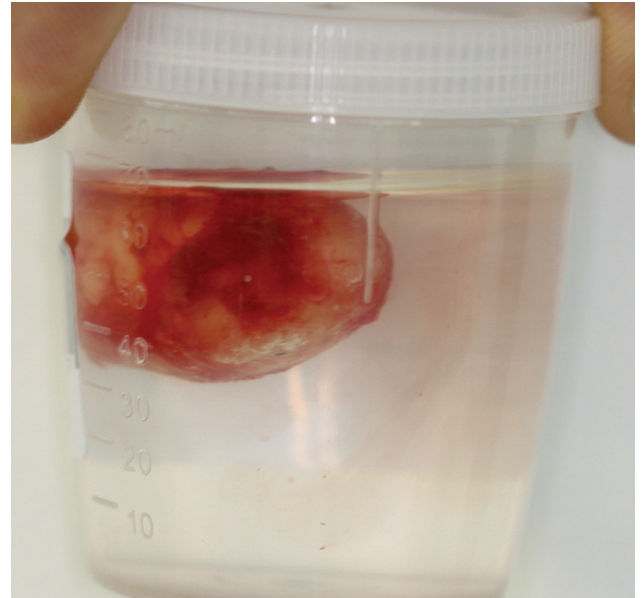


Figure 4 | Specimen floating in formalin solution.

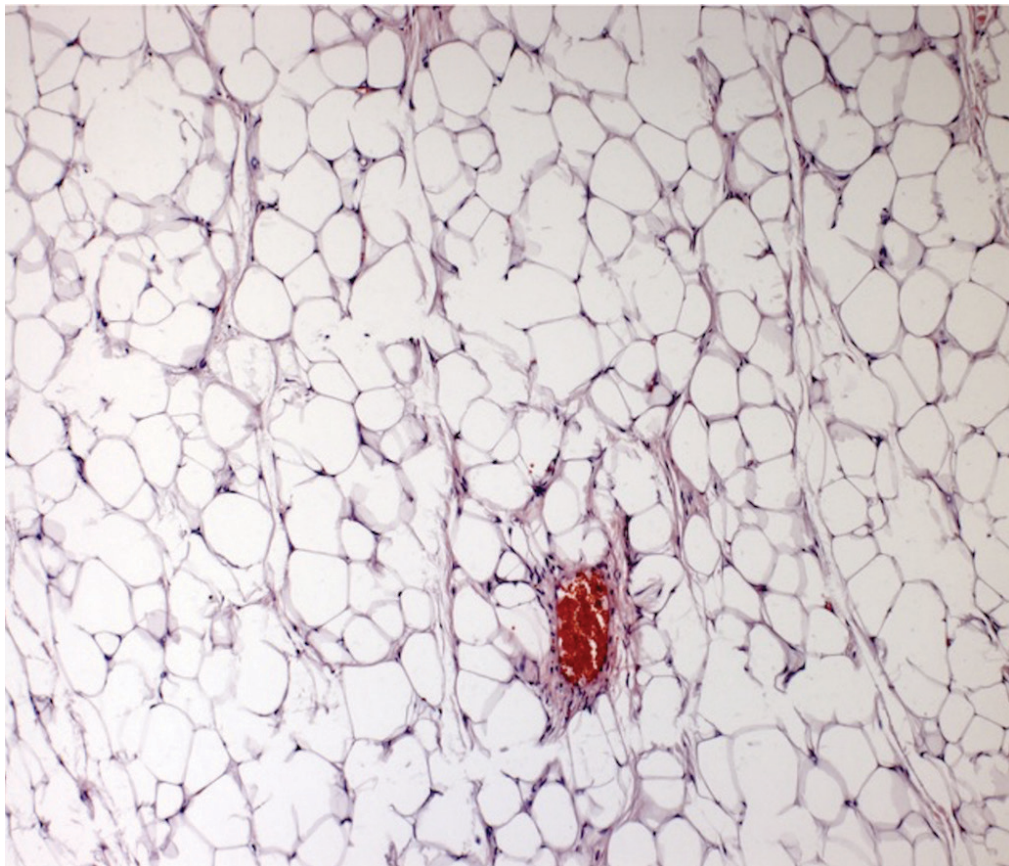


Figure 5 | Mesenchymal neoplasm fragment exhibiting large number of mature fat cells, some congested blood vessels of small caliber, and some muscle fiber bundles.

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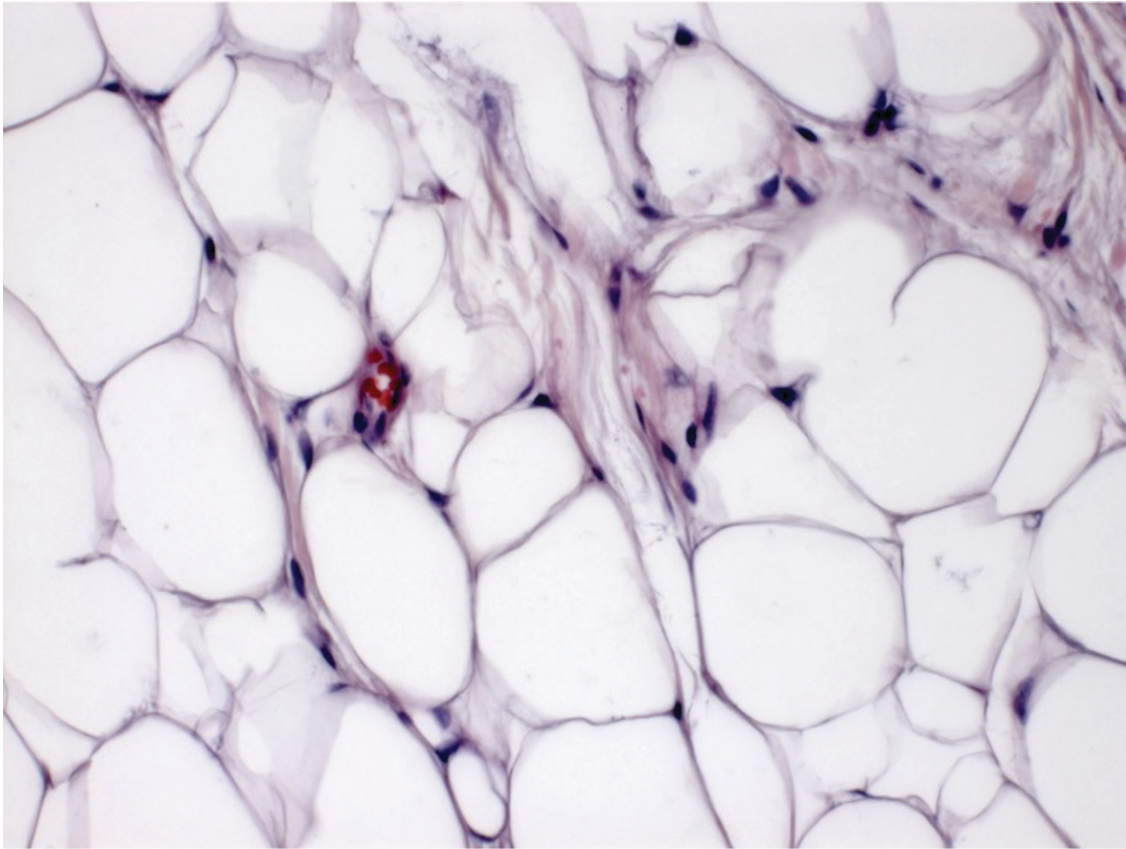


Figure 6 | Mature fat cells, arranged in a lobular arrangement, interspersed with bands of connective tissue.



Figure 7 | Complete healing and no signs of recurrence. Patient is asymptomatic after 40 months of follow-up.

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