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JULIANA LAROCCA DE GEUS

EFEITO DO CIGARRO NOS TECIDOS MOLES, DUROS E NA EFICÁCIA DO CLAREAMENTO DENTAL

PONTA GROSSA
2016

JULIANA LAROCCA DE GEUS

EFEITO DO CIGARRO NOS TECIDOS MOLES, DUROS E NA EFICÁCIA DO CLAREAMENTO DENTAL

Tese apresentada como pré-requisito para obtenção do título de Doutor na Universidade Estadual de Ponta Grossa, no curso de Doutorado em Odontologia – Área de Concentração Dentística Restauradora. Linha de Pesquisa: Pesquisa Clínica em Odontologia.

Orientadora: Profª. Drª. Alessandra Reis.
Co-orientadora: Profª. Drª. Stella Kossatz Pereira.

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Ponta Grossa, 09 de dezembro de 2016.

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Profª. Drª. Alessandra Reis Silva Loguercio
UEPG - **Presidente**

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DADOS CURRICULARES

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RESUMO

De Geus, J.L. **Efeito do cigarro nos tecidos moles, duros e na eficácia do clareamento dental.** [Tese] Doutorado em Dentística Restauradora. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2016.

O objetivo deste estudo foi avaliar clinicamente a longevidade do clareamento dental em pacientes fumantes, quantificar a nicotina em dentes expostos à fumaça de cigarro, bem como avaliar o efeito genotóxico do cigarro através da frequência de micronúcleos. Para tanto foram realizados quatro estudos. Nos estudos 1 e 2, sessenta pacientes, trinta fumantes e trinta não fumantes, foram submetidos ao clareamento com peróxido de carbamida (PC) 10% por três horas diárias durante três semanas. A cor foi avaliada utilizando o espectrofotômetro Vita Easyshade, e as escalas de cor Vita classical organizada por valor e Vita Bleachedguide 3D-MASTER, após 12 e 30 meses do clareamento, antes e depois de profilaxia dental. No estudo 3, sessenta e nove dentes foram expostos à fumaça de 400 cigarros (controle positivo). Em um grupo foi realizada profilaxia dental e em outro, clareamento de consultório com peróxido de hidrogênio (PH) 35%. A cor foi avaliada inicialmente, após a exposição à fumaça do cigarro, e após a profilaxia dental ou o clareamento em consultório. Os dentes de cada grupo foram analisados por cromatografia gasosa e espectrometria de massas, a fim de mensurar a quantidade de nicotina em cada grupo. O estudo 4 consistiu de uma revisão sistemática que avaliou a frequência de micronúcleos em fumantes e em não fumantes. Os dados dos estudos 1 e 2 mostraram que para ambos os grupos, apenas o fator principal tempo foi estatisticamente significativo ($p < 0,001$). Um clareamento eficaz foi observado em ambos os grupos na avaliação de 12 meses, após profilaxia dental, no entanto, um escurecimento dental foi observado após 30 meses para ambos os grupos. No estudo 3 a quantidade de nicotina foi maior no grupo controle positivo (3,3 ± 1,3 µg/g de dente), seguida pelo grupo profilaxia (2,1 ± 1,4 µg/g) e grupo clareamento (0,8 ± 0,3 µg/g). Houve um escurecimento dental visualmente perceptível em todos os grupos expostos à fumaça do cigarro ($p < 0,001$). No estudo 4 foi observada uma diferença significativa na frequência de micronúcleos em fumantes quando comparados aos não fumantes, porém o teste Chi^2 revelou alta heterogeneidade na metodologia dos estudos avaliados ($p < 0,00001$). Pode-se concluir que o clareamento com PC 10% manteve-se estável em ambos os grupos aos 12 meses, quando as manchas extrínsecas da dieta e do cigarro foram removidas por profilaxia dental, o que não ocorreu na avaliação de 30 meses. A fumaça do cigarro penetra na estrutura dental, no entanto

a profilaxia dental e o clareamento com PH 35% removeram parcialmente a nicotina do dente. Foi encontrada uma maior frequência de micronúcleos em células esfoliadas de fumantes em comparação com não fumantes.

Palavras-chave: Clareamento dental; Tabaco; Nicotina; Testes para Micronúcleos.

ABSTRACT

De Geus, JL. **Effect of cigarette on the soft and hard tissues and on efficacy of dental bleaching.** [Tese] Doutorado em Dentística Restauradora. Ponta Grossa: Universidade Estadual de Ponta Grossa; 2016.

The aim of this study was to clinically evaluate the longevity of tooth whitening in smokers, quantify nicotine in teeth exposed to cigarette smoke, as well as evaluate the genotoxic effect of cigarettes through the micronucleus frequency. For this study were conducted four studies. In studies 1 and 2, sixty patients, thirty smokers and thirty nonsmokers, were submitted to at-home bleaching with carbamide peroxide (CP) 10% for three hours daily during three weeks. The color was assessed using the Vita Easyshade spectrophotometer, and shade guides Vita classical organized by value and Vita Bleachedguide 3D-MASTER, after 12 and 30 months of bleaching, before and after dental prophylaxis. In study 3, sixty-nine teeth were exposed to smoke of 400 cigarettes (positive control). In one group, the dental prophylaxis was performed and, in another, the in-office bleaching with hydrogen peroxide (HP) 35%. The color was evaluated initially, after exposure to cigarette smoke, and after dental prophylaxis or in-office bleaching. The teeth of each group were analyzed by gas chromatography and mass spectrometry in order to measure the amount of nicotine in each group. The study 4 consisted of a systematic review that evaluated the frequency of micronuclei in smokers and nonsmokers. Data from studies 1 and 2 showed that for both groups, the main factor was statistically significant ($p < 0.001$). An effective bleaching was observed in both groups at 12 months after dental prophylaxis, however, a dental darkening was observed after 30 months for both groups. In study 3, the amount of nicotine was higher in the positive control group (3.3 ± 1.3 mg / g of tooth), then the prophylaxis group (2.1 ± 1.4 mg / g) and bleaching group (0.8 ± 0.3 mg / g). There was a visually perceptible dental darkening in all groups exposed to cigarette smoke ($p < 0.001$). In the study 4 was a significant difference in the frequency of micronuclei in smokers compared to nonsmokers, however the Chi^2 test showed high heterogeneity in the methodology of the assessed studies ($p < 0.00001$). It can be concluded that the bleaching with 10% CP was stable in both groups at 12 months, while the extrinsic stains of diet and smoking were removed by dental prophylaxis, which did not occur in the evaluation of 30 months. Cigarette smoke penetrates in the tooth structure, however the dental prophylaxis and bleaching with PH 35% removed partially the nicotine of tooth. A higher

frequency of micronuclei in exfoliated cells of smokers was found in comparison with non-smokers.

Key words: Tooth bleaching; Tobacco; Nicotine; Micronucleus Tests.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

a*	Vermelho-verde
ADA	*American Dental Association*
ANOVA	Análise de Variância
b*	Azul-amarelo
BBO	Bibliografia Brasileira de Odontologia
CG-MS	Cromatografia Gasosa – Espectrômetro de Massas
$CHCl_2$	Cloreto de metileno
CIE	Comissão Internacional de Iluminação
COEP	Comissão de Ética em Pesquisa
ΔE	Variação de cor
ΔUEV	Variação de Unidades de Escala Vita
EPHPP	*Effective Public Health Practice Project*
g	Grama (s)
h	Hora (s)
IS	*Internal Standard*
ISO	*International Organization for Standardization*
L*	Luminosidade
LILACS	Literatura Latino-Americana e do Caribe em Ciências da Saúde
min	Minuto (s)
mL	Mililitro (s)
MN	Micronúcleos
mm	Milímetro (s)
mm^2	Milímetro quadrado
m/z	*Mass-To-Charge Ratio*
n	Número amostral
N	Newton
NH_4OH	Hidróxido de amônio
PC	Peróxido de carbamida
PH	Peróxido de hidrogênio
PRISMA	*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*
PROSPERO	*International Prospective Register of Systematic Reviews*
rpm	Rotações por minuto
s	Segundo (s)
TCLE	Termo de Consentimento Livre e Esclarecido
UEPG	Universidade Estadual de Ponta Grossa
UEV	Unidades de Escala Vita

SUMÁRIO

1 INTRODUÇÃO

Existem mais de um bilhão de fumantes no mundo de acordo com a Organização Mundial de Saúde (WHO,[1] 2016), representando uma grande parte da população (De Vita Jr,[2] 2011). A fumaça do cigarro contém mais de 4.000 compostos químicos sendo que mais de 60 destes foram identificados como cancerígenos (IARC,[3] 1986).

Danos no DNA das células da mucosa bucal dos fumantes geralmente representam o potencial genotóxico do hábito de fumar, sendo observados indiretamente pelo aumento na frequência de micronúcleos (MN) em células epiteliais esfoliadas (Belien et al.,[4] 1995; Sivasankari et al.,[5] 2012). A avaliação da frequência de MN é um método viável para a detecção do risco de câncer nos seres humanos, porque a maioria dos tumores possui origem epitelial (Cairns,[6] 1975). Um aumento da frequência de MN nas células esfoliadas da mucosa oral tem tradicionalmente servido como um índice para avaliar a genotoxicidade da exposição a vários agentes cancerígenos (Bhattathiri et al.,[7] 1996; Basu et al.,[8] 2004). No entanto, alguns estudos não encontraram um aumento da frequência de MN em fumantes em comparação com não fumantes (Nersesyan et al.,[9] 2006; Angelieri et al.,[10] 2010).

Os fumantes podem ser considerados bons candidatos para procedimentos odontológicos estéticos, visto que a prevalência de auto avaliação de descoloração dental em fumantes é quase o dobro do que a relatada por não fumantes (Alkhatib et al.,[11] 2005). Entretanto, diversos estudos clínicos de clareamento dental utilizam como um dos critérios de exclusão, o hábito de tabagismo (Krause et al.,[12] 2008; Meireles et al.,[13] 2008; Bernardon et al.,[14] 2010; Grobler et al.,[15] 2010; Turkun et al.,[16] 2010; de Almeida et al.,[17] 2012; Bonafé et al.,[18] 2013; Rezende et al.,[19] 2013; de la Peña e Ratón,[20] 2014). Já foi demonstrado que houve clareamento efetivo em fumantes após uma semana do clareamento (de Geus et al.,[21] 2015), porém essa equivalência não foi observada um mês após o clareamento, onde os fumantes tinham os dentes levemente mais escuros do que os não fumantes, já que a fumaça de cigarro deposita manchas escuras na superfície dental, esta situação pode ser ainda mais evidente após alguns meses (Alkhatib et al.,[11] 2005; Bertoldo et al.,[22] 2011).

Durante a queima do cigarro, componentes como alcatrão, nicotina, açúcares e cacau são transferidos para a fumaça devido ao aquecimento (Wasilewski et al.,[23] 2010). Estes componentes seriam provavelmente os responsáveis pelo manchamento nos dentes, pela sua tonalidade escura e capacidade de se aderir à superfície (Bazzi et al.,[24] 2012).

A nicotina é o componente mais específico do cigarro, sendo responsável pela dependência do tabaco. Encontra-se presente em uma quantidade relativamente grande (1-2

mg por cigarro). É absorvida e mensurável tanto em fumantes ativos como passivos (Benowitz e Jacob,[25] 1993; Robinson et al.,[26] 1992). A técnica de cromatografia gasosa acoplada ao espectrômetro de massas (CG-MS) pode ser utilizada para a determinação da nicotina em dentes (Pascual et al.,[27] 2003) e pode ainda auxiliar na detecção da quantidade de nicotina que permanece aderida na superfície dental e quanto penetra em profundidade na estrutura.

O manchamento provocado pela nicotina e outros componentes do cigarro parece ser superficial e facilmente removido pela limpeza mecânica e clareamento dental (Bazzi et al.[24] 2012), porém a literatura ainda não fornece evidências científicas sobre este aspecto. A deposição de fumaça de cigarro e de corantes advindos da dieta nos dentes promove um escurecimento dental, que tem sido atribuído ao manchamento extrínseco (Alkhatib et al.,[11] 2005, Téo et al.,[28] 2010), mas que pode também ser advindo da penetração dos componentes da queima do cigarro na estrutura dental. A avaliação do resultado "real" do clareamento em fumantes em longo prazo exigiria uma avaliação da cor antes e após a remoção das manchas extrínsecas por meio de profilaxia dental (Bazzi et al.,[24] 2012).

Desta forma o objetivo deste estudo foi conduzir diferentes estudos clínicos, laboratorial e de revisão sistemática, com o objetivo de avaliar a longevidade do clareamento dental em pacientes fumantes, quantificar a nicotina em dentes expostos à fumaça de cigarro antes e após profilaxia ou clareamento, bem como avaliar o efeito genotóxico do cigarro através da frequência de MN em fumantes.

2 PROPOSIÇÃO

2.1 ESTUDOS 1 E 2

2.1.1 Proposição geral

O propósito do presente estudo foi avaliar clinicamente a longevidade da cor dental de pacientes fumantes e não fumantes, após 12 e 30 meses do clareamento caseiro com peróxido de carbamida (PC) 10%.

2.1.2 Proposição específica

1- avaliar a longevidade da cor dental (12 meses) após o clareamento caseiro com PC 10% utilizando as escalas Vita classical e Vita Bleachedguide 3D-MASTER e o espectrofotômetro Vita Easyshade, nos fumantes e não fumantes, antes e após a realização de profilaxia dental;

2- avaliar a longevidade da cor dental (30 meses) após o clareamento caseiro com PC 10% utilizando as escalas Vita classical e Vita Bleachedguide 3D-MASTER (30 meses), antes e após a realização de profilaxia, para ambos os grupos.

2.2 ESTUDO 3

2.2.1 Proposição geral

O propósito do presente estudo *in vitro* foi quantificar a nicotina em dentes expostos à fumaça de cigarro.

2.2.2 Proposição específica

1- avaliar a cor nos períodos inicial, após exposição à fumaça do cigarro, após profilaxia e após clareamento em consultório com peróxido de hidrogênio (PH) 35% utilizando as escalas Vita classical e Vita Bleachedguide 3D-MASTER e o espectrofotômetro Vita Easyshade, em ambos os grupos;

2- quantificar a nicotina presente em cada grupo de dentes (controle positivo, profilaxia e clareamento) através de cromatografia gasosa e espectrometria de massas.

2.3 ESTUDO 4

2.3.1 Proposição geral

O propósito do presente estudo foi avaliar o efeito genotóxico do cigarro através da frequência de micronúcleos (MN) em fumantes por meio de uma revisão sistemática da literatura.

2.3.2 Proposição específica

1- avaliar a frequência de micronúcleos nas células esfoliadas da mucosa oral de fumantes e não fumantes através de uma revisão sistemática e meta-análise de estudos clínicos transversais.

3 MATERIAL E MÉTODOS

Nesta sessão será descrita a metodologia de forma resumida de cada estudo. As informações detalhadas deste item podem ser encontradas nos artigos referentes a cada estudo.

3.1 ESTUDOS 1 E 2

O projeto deste estudo clínico foi aprovado pela Comissão de Ética em Pesquisa (COEP) da Universidade Estadual de Ponta Grossa através do parecer nº 669.914 (Anexo A), o qual foi protocolado sob o número 16211/2014. O estudo foi registrado no clinicaltrials.gov sob o número de identificação de NCT02017873 (Anexo B). A metodologia detalhada destes experimentos está descrita nos ARTIGOS 1 (pág. 28) e 2 (pág. 47).

3.1.1 Seleção dos pacientes

Foram selecionados 60 voluntários (30 fumantes e 30 não fumantes) que procuraram atendimento nas clínicas odontológicas da Universidade Estadual de Ponta Grossa (UEPG), que tiveram interesse em realizar o clareamento dental e que se enquadraram nos critérios de inclusão e exclusão do estudo. O dente incisivo central superior direito deveria ser classificado como cor A2 ou de menor valor, por comparação com a escala Vita classical (Vita Zahnfabrik, Bad Säckingen, Alemanha), Figura 1. Os fumantes deveriam fumar pelo menos 10 cigarros ao dia.

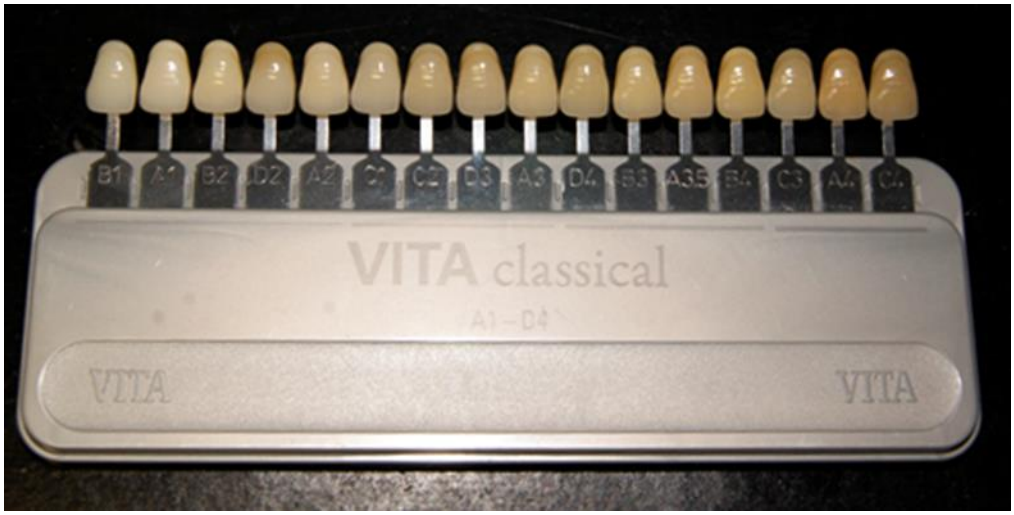

Figura 1 - Escala de cor Vita classical (Vita Zahnfabrik, Bad Säckingen, Alemanha).

3.1.2 Procedimento clareador

A técnica utilizada para os dois grupos foi do clareamento dental caseiro, com gel de PC 10% (Whiteness Perfect 10% – FGM, Joinville, SC, Brasil). Os pacientes foram instruídos a utilizar o gel clareador, pelo período de três horas diariamente, durante três semanas.

3.1.3 Avaliação da cor

A cor foi avaliada nos períodos de 12 e 30 meses após o término do clareamento dental, antes e após a realização de profilaxia dental, através das escalas de cor Vita classical e Vita Bleachedguide 3D-MASTER (Figura 2). Aos 12 meses, a cor também foi avaliada com o espectrofotômetro Vita Easyshade (Vita Zahnfabrik), de acordo com o sistema Vita e CIEL*a*b*, Figura 3.

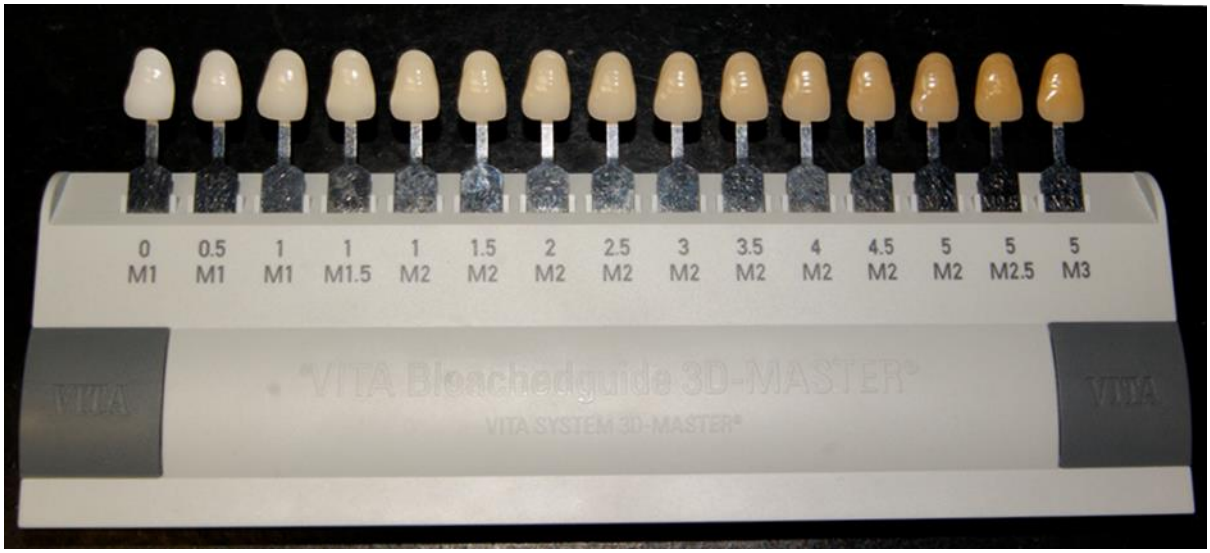

Figura 2 - Escala Vita Bleachedguide 3D-MASTER (Vita Zahnfabrik).

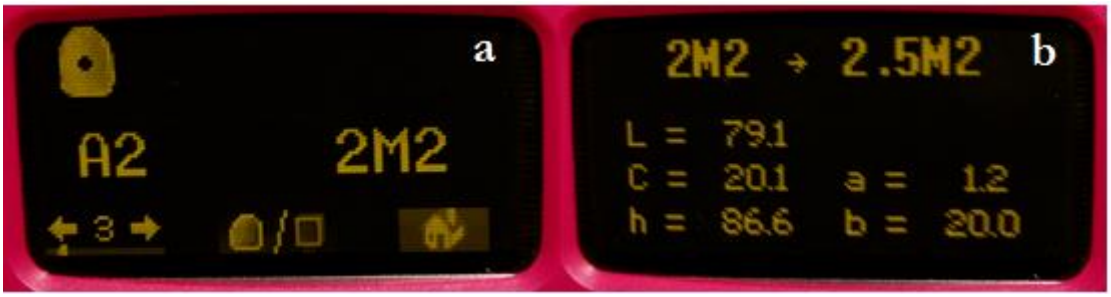

Figura 3 – Valores apresentados pelo Espectrofotômetro Vita Easyshade a) cores da escala Vita b) valores CIEL*a*b*.

A área de mensuração da cor foi o terço médio da face vestibular do incisivo central superior direito, de acordo com as especificações da American Dental Association (ADA) (Armênio et al.[29] 2008, Reis et al.[30] 2011, Bonafé et al.[18] 2013).

3.1.4 Análise estatística

Os dados de cor foram tabulados no Programa Sigma Plot for Windows (Systat Software Inc., San Jose, CA, USA) e foram avaliados por análise de variância de dois fatores (ANOVA) de medidas repetidas (Grupo *vs* Tempo de tratamento), sendo o Tempo a medida repetida ($\alpha = 0,05$). Foi realizado o teste de Tukey para o contraste das médias ($\alpha = 0,05$).

3.2 ESTUDO 3

O projeto deste estudo *in vitro* foi aprovado pela Comissão de Ética em Pesquisa (COEP) da Universidade Estadual de Ponta Grossa através do parecer nº 1.065.444 (Anexo C), o qual foi protocolado sob o número 11265/2015. A metodologia detalhada deste experimento está descrita no ARTIGO 3 (pág. 66).

3.2.1 Exposição à fumaça de cigarro

Sessenta e nove molares foram fixados em um isopor (Figura 4) e colocados em uma máquina de cigarros (Figura 5) para a impregnação de fumaça nas estruturas dentais. Foram realizados quarenta ciclos (15 minutos/ciclo) com 10 cigarros (Marlboro Red, Philip Morris Brazil Ind. e Com. Ltda., Santa Cruz do Sul, RS, Brasil), totalizando 400 cigarros.

Figura 4 – Dentes fixados em isopor.

Figura 5 – Máquina de cigarros.

3.2.2 Procedimento de remoção de manchas

Após a exposição à fumaça do cigarro, os dentes foram divididos em três grupos. Nos espécimes do grupo controle positivo não foi realizado nenhum tratamento, para remover as manchas produzidas pela fumaça do cigarro. Nos espécimes do grupo profilaxia, os dentes foram submetidos a uma limpeza com escova rotativa e pasta profilática. Nos espécimes do grupo clareamento, foi realizado clareamento em consultório com PH 35% (Whiteness HP

MAXX, FGM), de acordo com as instruções do fabricante. Foram realizadas duas sessões, onde em cada sessão o gel foi aplicado, sobre toda a superfície dental, três vezes de 15 minutos.

3.2.3 Avaliação da cor

A cor foi avaliada através das escalas de cor Vita classical, Vita Bleachedguide 3D-MASTER e do espectrofotômetro Vita Easyshade (Vita Zahnfabrik), antes da exposição à fumaça do cigarro (*baseline*), após a exposição à fumaça do cigarro, e após o tratamento para a remoção de manchas (profilaxia dental ou clareamento em consultório). Para a avaliação da cor com o espectrofotômetro, foi confeccionada uma matriz de silicone (Perfil, Coltene, Vigodent SA Industria e Comércio) para o local de mensuração (Figura 6).

Figura 6 – Matriz de silicone perfurada com bisturi tipo Punch.

3.2.4 Quantificação da nicotina por CG-MS

Após a avaliação da cor, os dentes foram martelados e quebrados em pedaços (Figura 7) e cada 5 g de dentes foram unidos a 30 g de bolas de aço e pulverizados por uma máquina moinho de bolas (SPEX SamplePrep 8000M Mixer/Mill, Metuchen, NJ, EUA), mostrado na Figura 8, durante 60 min a 1.725 rpm.

Figura 7 – Dentes quebrados em pedaços para realização da moagem.

Figura 8 – Moinho de bolas SPEX SamplePrep 8000M Mixer/Mill.

Quinze miligramas do pó dos dentes (Figura 9) foram adicionadas a 1 mL de hidróxido de amônio (NH_4OH) a 2%, incubadas num banho de sonicação (Aquasonic 50HT, VWR Scientific, Radnor, PA, USA) e, em seguida, 1 mL de cloreto de metileno ($CHCl_2$) foi adicionado à solução, a qual foi centrifugada (NF 800, Nuve, Ankara, CA, Turkey) durante 15 min a 9000 rpm. A camada orgânica foi transferida para um tubo de ensaio para a realização da cromatografia gasosa e espectrometria de massas (CG-MS).

Figura 9 – Dentes em pó após moagem.

A análise foi realizada usando um cromatógrafo em fase gasosa Agilent 7898B (Santa Clara, CA, EUA), acoplado a um detector específico de massas, Agilent 5977 (Figura 10). A identificação da nicotina baseou-se no índice de retenção relativo em comparação com os padrões da literatura.

Figura 10 - Cromatógrafo Gasoso-Espectrômetro de Massas.

3.2.5 Análise estatística

Todas as análises foram realizadas com software (Statistica para Windows, Stat Soft Inc.) e um nível de significância de 5%. A quantificação da nicotina e alteração de cor em unidades de escala Vita e em ΔE foram submetidas a uma análise de variância de um fator e teste de Tukey para comparações de pares. No caso onde os dados não apresentaram distribuição normal, as estatísticas não paramétricas (Kruskall-Wallis e teste de Dunn) foram realizadas.

3.3 ESTUDO 4

Este protocolo de revisão sistemática e meta-análise foi registrado no banco de dados PROSPERO (CRD42015017053), ANEXO D. Foram seguidas as recomendações da declaração PRISMA para a realização desta revisão sistemática (Moher et al.,[31] 2010). A metodologia detalhada deste experimento está descrita no ARTIGO 4 (pág. 87).

3.3.1 Fontes de informação e estratégia de busca

O vocabulário controlado e palavras-chave livres na estratégia de busca foram definidos com base na seguinte pergunta PECO:

1. População (P): adultos.
2. Exposição (E): hábito de fumar.
3. Comparação (C): não fumantes.
4. Resultado (O): frequência de micronúcleos.
5. Estudos (S): estudos transversais.

Para identificar os estudos a serem incluídos nesta revisão, foi feita uma busca nas bases de dados eletrônicas do MEDLINE via PubMeb, Scopus, Web of Science, Literatura Latino-Americana em Ciências da Saúde (LILACS), Biblioteca Brasileira de Odontologia (BBO) e Biblioteca Cochrane. Também foi realizada uma busca na Literatura Cinzenta e nos Registros de Estudos Clínicos.

3.3.2 Critério de elegibilidade

Foram incluídos ensaios clínicos transversais que compararam a frequência de MN em fumantes e não fumantes em pacientes adultos. A frequência de MN foi o principal resultado do estudo. Foram incluídos apenas estudos clínicos em seres humanos.

3.3.3 Seleção dos estudos e processo de coleta de dados

Inicialmente, os artigos foram selecionados pelo título e resumos. O texto completo dos artigos foi obtido quando o título e resumo tinham informações suficientes para tomar uma decisão clara. Posteriormente, dois revisores classificaram aqueles que preencheram os critérios de inclusão. Detalhes sobre o estudo, os métodos, os participantes e os resultados foram extraídos utilizando formas de extração personalizadas.

3.3.4 Risco de viés individual dos estudos

A qualidade interna dos estudos incluídos foi avaliada por dois revisores independentes usando a escala EPHPP (Projeto Prática de Saúde Pública Eficaz) (Moher et al.,[31] 2010), ANEXO E, modificada.

O instrumento de avaliação de qualidade utilizado contém os seguintes componentes: 1) viés de seleção, 2) desenho do estudo, 3) identificação e tratamento de fatores de confusão,

4) cegamento de avaliadores e de participantes, 5) confiabilidade e validade dos métodos de coleta de dados e 6) desistências e abandonos. Os componentes são classificados como fortes, moderados ou fracos de acordo com o dicionário normatizado (http://www.ephpp.ca/PDF/QADictionary_dec 2009.pdf, ANEXO F) (Moher et al.,[31] 2010).

3.3.5 Meta-análise

A meta-análise foi realizada em estudos classificados como fortes e moderados, de acordo com a classificação final dos componentes de avaliação de qualidade.

4 ARTIGOS

4.1 One-year follow-up of at-home bleaching in smokers before and after dental prophylaxis: a randomized clinical trial

4.2 Effects of at-home bleaching in smokers: thirty-month follow-up

4.3 Determination of nicotine content in teeth submitted to prophylaxis and in-office bleaching by gas-chromatography

4.4 Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review and meta-analysis

ARTIGO 1

TÍTULO: ONE-YEAR FOLLOW-UP OF AT-HOME BLEACHING IN SMOKERS BEFORE AND AFTER DENTAL PROPHYLAXIS: A RANDOMIZED CLINICAL TRIAL

STATUS: PUBLICADO

REVISTA: JOURNAL OF DENTISTRY

One-year follow-up of at-home bleaching in smokers before and after dental prophylaxis: a randomized clinical trial

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ABSTRACT

Objective: This clinical study evaluated the color longevity after one-year of at-home bleaching with 10% carbamide peroxide (CP) in smokers and nonsmokers.

Methods: Sixty patients, 30 smokers and 30 non-smokers were subjected to bleaching with 10% CP during three hours daily for three weeks. The color was measured at baseline and one week, one month and one year after the completion of dental bleaching using the spectrophotometer Vita Easyshade (DE*), shade guide Vita classical organized by value and Vita Bleachedguide 3D-MASTER (DSGU). In the one-year recall, the color was assessed before and after dental prophylaxis with Robinson brush and prophylaxis paste. Data from color evaluation were analyzed by two-way repeated measures ANOVA and Tukey's test for the contrast of means ($a = 0.05$).

Results: Twenty-seven smokers and 28 non-smokers attended the one-year recall. For both study groups, only the main factor assessment time was statistically significant for DSGU (Vita classical) and DE* ($p < 0.001$). Effective whitening was observed for both groups at baseline, which was stable at one-month and one year after dental prophylaxis. A slight darkening was observed after one year when the color was measured without prophylaxis. For the Vita Bleachedguide 3D-MASTER, color rebound was observed irrespectively of dental prophylaxis.

Conclusion: The bleaching with 10% CP remained stable in both groups as long as extrinsic stains from diet and cigarette smoke were removed by professional dental prophylaxis. Clinical trials registry: NCT02017873.

Clinical relevance: The results of this study indicate that the bleaching is effective in smokers even after one-year, but dental prophylaxis may be necessary to remove extrinsic stains caused by diet and smoking.

Key-words: randomized clinical trial; color longevity; at-home bleaching; smokers; dental prophylaxis

1. Introduction

Currently, people give much value to the body and aesthetics. A large number of people wish not only to have a perfect body, but also a perfect smile [1]. In this context, smokers are likely good candidates for cosmetic dental procedures since the prevalence of self-assessed tooth discoloration in smokers is almost twice that reported by non-smokers [2]. They represent a significant portion of the population, since there are around 1.2 billion smokers in the world [3].

Unfortunately, clinical trials of bleaching agents usually exclude smokers from their clinical trials [4–13], which prevent us from assessing the feasibility of this cosmetic procedure in such patients. An earlier publication of de Geus et al. [14] demonstrated that effective whitening is achieved regardless of whether the patient is a smoker. It was reported that the magnitude of color change after at-home whitening is equivalent between smokers and non-smokers at one week [14]; however this equivalence was not seen one month after bleaching, with smokers having slightly darker teeth than non-smokers. This situation may be even more evident after some months as cigarette smoke deposits a dark extrinsic stain on the dental surface [2,15]. However, to the extent of our knowledge, no clinical study has evaluated the longevity of at-home bleaching in smokers.

Apart from that, we should be able to diagnose if the color rebound results from the deposition of dyes or smoke on the dental surface or from the reversal of the oxidizing action of the bleaching agent or dentin deposition over time. Therefore, the evaluation of the “real” whitening outcome in long-term recalls would require color assessment before and after removal of extrinsic staining by mechanical cleaning and dental prophylaxis [16].

Although there are numerous studies of at-home dental bleaching, only a few of them evaluate the color stability over time [7,8,17–24]. None of these studies have attempted to appraise the bleaching longevity after dental prophylaxis. Therefore, the aim of this controlled

clinical trial was to compare the one-year color change of at-home bleaching in smokers and non-smokers before and after dental prophylaxis. The following null hypotheses will be tested in this study: (1) no difference in color change of teeth will be observed between the immediate and one-year results for both study groups; (2) the color change before and after dental prophylaxis will be the same for both study groups.

2. Methods

The State University of Ponta Grossa (protocol 669.914/2014) and the Ethic Committee approved this equivalence clinical trial. This study is the one-year follow-up of an earlier study [14] registered at the clinicaltrials.gov under the identification number of NCT02017873. This earlier study was conducted in the Chile and Brazil centers [14], but the follow-up was only performed in the Brazilians participants.

2.1. Bleaching procedure

We asked the participants who met the inclusion criteria about their daily smoking habits. Those who did not smoke were part of the group of non-smokers, and those who smoked at least 10 cigarettes per day belonged to the group of smokers. We included 30 participants in each group.

We made alginate impressions of each participant's maxillary and mandibular arch and poured the impressions with dental stone. We did not apply block-out material to the labial surfaces of the teeth [25]. We used a 1-millimeter-thick soft vinyl material provided by the manufacturer (Whiteness, FGM Dental Products) to fabricate the custom-fitted tray to hold the bleaching gel. We trimmed the bleaching tray one mm beyond the marginal gingiva and delivered the tray and the 10% CP gel (Whiteness Perfect, FGM Dental Products) to each participant with oral instructions for use. We instructed all participants to wear the tray with the bleaching agent for 3 h daily for 3 weeks.

We instructed the participants to remove the tray after the daily bleaching period, wash it with water, and brush their teeth as usual. We also provided verbal instructions about oral hygiene, encouraging participants to brush their teeth regularly with fluoridated toothpastes without whitening components.

2.2. Sample size

This study is the one-year follow-up of an earlier study [14]. We based the sample size calculation on the color change measured with the spectrophotometer, the primary outcome of this study. Sixty participants were required to exclude a difference of means of 2.5 units of DE* at one week and one year (equivalence limit) with a power of 80% and a of 5%. With these calculations, we took into consideration a standard deviation of 3.3 in the DE*. The equivalence limit we chose was lower than the threshold of 3.0 measured with the spectrophotometer, above which color differences become clinically perceptible [26–28].

2.3. Shade evaluation

We evaluated the color of teeth using objective and subjective methods. For both devices, we checked the color in the middle third of the labial surface of the anterior central incisor according to the American Dental Association guidelines [29]. For the objective shade evaluation, we used a digital spectrophotometer (VITA Easyshade, VITA Zahnfabrik) because its reliability more than 96% [30]. For this purpose, we took an impression of the maxillary arch with dense silicone paste (Coltoflax and Perfil Cub, Vigodent), and we created a window on the labial surface of the silicone guide by using a metal device with a diameter of 6 mm. The purpose of this procedure was to standardize the area for color evaluation in all recall periods with the spectrophotometer.

We determined the color using the parameters of the digital spectrophotometer on which were indicated values: L*, a*, and b*, where L* represents luminosity (the value from

0 (black) to 100 (white)), and a* and b* represent color along the red–green axis and color along the yellow–blue axis, respectively. We calculated the difference between baseline and each recall period (DE*), by using the following formula [31]: DE* = [(DL*)2 + (Da*)2 + (Db*)2]1/2.

For the subjective evaluation, we used the Vita Bleachedguide 3D-MASTER (VITA Zahnfabrik), which is originally oriented from lightest to darkest color and the VITA classical shade guide (VITA Zahnfabrik). For the latter, we arranged the 16 tabs of the shade guide from lightest to darkest as follows: B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4. Although this scale is not linear in the truest sense, we treated the changes as continuous, with a linear ranking as has been used in several clinical trials on dental bleaching [9,10,32].

We calculated the color changes from the beginning of the active phase through the individual recall times by the change in shade guide units (DSGU) that occurred toward the lighter end of the value-oriented list of shade tabs. In the case of operator disagreement about color matching, we reached a consensus before dismissing the patient.

Two calibrated evaluators with a previous agreement of at least 85% determined by means of weighted k statistics recorded the shade of the maxillary right central incisor at baseline and one week, one month and one year after the end of the bleaching protocol. At one year, the evaluation was performed before and after dental prophylaxis with a Robinson brush and prophylaxis paste (Herjos, Vigodent Coltene SA Indústria e Comércio, Rio de Janeiro, Brazil). After dental prophylaxis, the treated teeth were rehydrated in the patient's mouth for 15 min before color assessment.

2.4. Statistical analysis

We performed all of the analyses using software (Statistica for Windows, StatSoft

Inc., Tulsa, OK, USA) and a 5% significance level. Statistical analyses were performed using per-protocol analysis (only for the available data) and the intention-to-treat approach, where the last observation was carried forward for the missing data. The color change in DSGU and in DE* was submitted to a two-way repeated measures ANOVA (Group vs. assessment period) and Tukey's test for pairwise comparisons.

3. Results

At baseline, we screened 305 patients to obtain 60 participants from the center in Brazil who met the eligibility criteria (Fig.1). The mean age and baseline color of the participants were similar between the groups. Most of the participants were men (Table 1). The smoking habit did not change among the majority of participants from the smoking group during the course of the year. Only three of them stopped smoking.

All participants included in this controlled clinical trial finished the bleaching protocol and attended the one-week and one-month recall visits (Fig. 1); however five participants did not attend the one-year recall (n = 3 in the smokers group and n = 2 in the nonsmokers group, Fig. 1). The reason for not attending the recall was that the participants lacked time to return to the university for a new color assessment.

3.1. Per-protocol vs. intention-to-treat analysis

All statistical analyses were performed with data imputation for missing outcomes (intention-to-treat) and without data imputation (per-protocol). The same overall conclusions were reached (data not shown) in all of the analyses. To avoid data repetition we opted to describe only the results obtained in the intention-to-treat analysis.

3.2. Shade guide data

For the Vita classical shade guide, the two-way repeated ANOVA revealed that the cross-product interaction group vs. assessment time ($p = 0.153$) and the main factor group (p

= 0.345) was not significant. Only the main factor assessment time was statistically significant (Table 2; $p < 0.001$). The lack of difference between the groups can also be seen by the effect size (mean difference) and the 95% confidence interval (Table 2).

A significant average color change (DSGU) of approximately 5.6 shade guide units was observed after bleaching for both groups, which was stable one month after the procedure (Table 2). At one year, color change was statistically similar to the immediate result only when the color was measured after dental prophylaxis. Without dental prophylaxis, the color change at one year was statistically different from the immediate result (one week postbleaching).

For Vita Bleachedguide 3D-MASTER, the two-way repeated measures ANOVA revealed that the cross-product interaction group vs. assessment time ($p = 0.80$) and the main factor group ($p = 0.05$) was not significant (Table 3). Only the main factor assessment time was statistically significant (Table 3; $p < 0.001$). The lack of difference between smokers and nonsmokers can alsobe seen by the effect size (mean difference) and the 95% confidence interval (Table 3).

According to this shade guide, a significant color rebound was observed over time for both groups (Table 3), and this color rebound was not affected by dental prophylaxis.

3.3. Spectrophotometer data

The two-way repeated measures ANOVA revealed that the cross-product interaction group vs. assessment time ($p = 0.158$) and the main factor group ($p = 0.311$) was not significant. Only the main factor assessment time was statistically significant (Table 4; $p < 0.001$). The lack of statistical difference between the groups can also be seen by the mean difference and the 95% confidence interval (Table 4).

Compared to the DSGU obtained with the Vita classical to spectrophotometer data, a

similar trend was observed. A significant color change was observed for both groups, which represented an average DE* of 10.8. This color change was statistically similar to that observed after one month and one year when color was measured after dental prophylaxis. Without dental prophylaxis, the color change at one year was statistically different from the immediate result (one week post-bleaching).

4. Discussion

Color matching and measurement in dentistry is performed using visual and/or instrumental methods. The Vita classical shade guide (VITA Zahnfabrik), when arranged from the lightest to the darkest tab, is the most frequent method used for visual evaluation of tooth whitening [9,10,32], thus this shade guide was chosen for color evaluation in the present study.

However, more recently, studies from the group of Paravina [33–35] developed a new shade guide for color assessment in bleaching studies. Vitapan 3D-Master (VITA Zahnfabrik) was found to have broader color range, better color distribution, and smaller coverage error as compared to other shade guides [36,37]. Despite these advantages, this scale is not yet routinely used for color evaluation in dentistry, so using it would prevent us from making comparisons with previous literature studies. In our opinion, this scale should be incorporated in future clinical trials to create a body of evidence regarding of whether it is superior or not to the traditional value-oriented shade guide Vita classical. In the present study the results of Vita Bleachedguide 3D-MASTER were not consistent with the results of the spectrophotometer and the Vita classical. The reason for such a difference among the shade guides and the spectrophotometer is not clear to the authors and should be a focus of future investigations.

There is a general acceptance that the consumption of staining beverages and foods is frequently associated to tooth discoloration [38,39]. This premise is based on the findings of

in vitro studies that reported that smoking, coffee, tea, and wine can lead to tooth discoloration [16,40–43] and therefore affect the longevity of tooth bleaching [16,43]. This is the reason of why dentists have been prescribing a white diet and precluding smokers from bleaching, to guarantee that the immediacy and longevity of the bleaching effect is not reduced as a result of diet [44] or smoking habits.

Fortunately, based on two out of the three tools for color evaluation, this was not confirmed in this clinical study and in others [10,14,45]. An earlier study [14], reported that neither smoking habits nor coffee consumption jeopardized the whitening produced by at-home bleaching [10]. This means that in a one month short-term follow-up, the deposition of cigarette smoke and dyes from coffee, wine, and other colorful foods and drinks does not produce significant color change, and the bleaching outcome is not affected. This was recently confirmed in a questionnaire-based study [44] in which the ingestion of different substances during bleaching was not found to be associated with a lower degree of whitening. Altogether, these findings suggest that the dentin substrate on which carbamide peroxide exerts its oxidizing action is probably similar irrespective of the smoking and dietary habits of the patient during the bleaching [10,14,45].

In regard to the longevity of at-home bleaching, the literature findings report controversial findings. While color rebound was observed after one year [7,46], two years [21,22] or longer follow-up recalls [19,20], other authors reported stable color in periods ranging from one to two years [8,17,18,22–24].

In the present investigation, we observed color stability (color assessed after dental prophylaxis) and color rebound (color assessed without dental prophylaxis) at the one-year recall, depending on the previous dental prophylaxis. Although diet and the smoking habit were not shown to affect the immediate outcome of bleaching [10,14], it is likely that this is the reason for the color rebound observed in the short-term follow-up of one year when dental

prophylaxis was not performed. Teeth exposed to coloring agents from diet indeed have greater potential to stain [47]. Similarly, smokers' teeth tend to develop tobacco stains over time [2], which may vary from yellow to black stains, and the severity is highly dependent on the length and frequency of the smoking habit.

Unfortunately, the majority of the clinical studies evaluating the longevity of at-home bleaching did not report the patients' dietary habits during and after tooth bleaching treatment, which prevents us from further comparisons. Only a few studies have attempted to associate the effect of dietary habits with the longevity of at-home bleaching [18,21,46], and they did not reach conclusive findings, which emphasizes the need for future studies.

It is worth mentioning however, that although significant differences were detected between the immediate results and the one-year follow-up without dental prophylaxis, the differences in the DSGU (less than 1 shade guide unit) and DE* (approximately 2 DE* units) were probably not within the visually perceptible range.

Visual thresholds for color differences are applied to correlate the instrumental color values with the clinical evaluation. According to Ghinea et al. [48], the threshold for acceptability was reported to be 3.5 color difference (DE*) units, and that for perceptibility was 1.8 DE* units based on the spectrophotometer readings.

In longer follow-ups, color rebound might be associated with other factors. As teeth get older, there is a continuous deposition of secondary dentin by the pulp [49]. As the dentin thickness increases, the teeth appear yellower. Unfortunately, the length of time that it takes to change one Vita shade tab due to deposition of secondary dentin is unknown, and it is probable that it takes longer than the one-year period of the current study.

Although it is not desirable that extrinsic staining may affect the overall perception of whiter teeth, such coloration may be easily removed by professional dental prophylaxis. This

also means that evaluation of the color of the dental structure in clinical trials and also in dental offices should be done after professional dental prophylaxis to prevent extrinsic staining from masking the whitening outcome produced by the bleaching procedure.

It is worth mentioning that though no significant difference was detected between smokers and nonsmokers, the data in Tables 2 and 4 highlight that the effect of dental prophylaxis was more evident in smokers than non-smokers. Had we recruited more patients or evaluated this study sample in longer follow-ups, this difference might have become statistically significant. It is likely that this difference becomes more evident and even significant after some years, with smokers eventually having darker teeth than do nonsmokers. This should encourage further clinical trials on this issue with longer follow-up periods.

5. Conclusion

At-home dental bleaching with 10% carbamide peroxide remained stable in both groups at one year as long as extrinsic stains caused by diet and smoking were removed by dental prophylaxis.

Acknowledgments

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References

[1] A. Devigus, The perfect smile, Int. J. Esthet. Dent. 9 (2014) 465.
[2] M.N. Alkhatib, R.D. Holt, R. Bedi, Smoking and tooth discolouration: findings from a national cross-sectional study, BMC Public Health 5 (2005) 27.
[3] V.T. DeVita Jr., T.S. Lawrence, S.A. Rosenberg, Cancer Principles & Practice of Oncology: Tobacco, 9th ed., Lippincott Williams & Wilkins, PA, Philadelphia, 2011.
[4] L.C. de Almeida, C.A. Costa, H. Riehl, P.H. dos Santos, R.H. Sundfeld, A.L. Briso, Occurrence of sensitivity during at-home and in-office tooth bleaching therapies with or without use of light sources, Acta Odontol. Latinoam. 25 (2012) 3–8.

[5] S.S. Meireles, S.S. Heckmann, F.L. Leida, S. dos Santos Ida, A. Della Bona, F.F. Demarco, Efficacy and safety of 10% and 16% carbamide peroxide tooth- whitening gels: a randomized clinical trial, Oper. Dent. 33 (2008) 606–612.
[6] F. Krause, S. Jepsen, A. Braun, Subjective intensities of pain and contentment with treatment outcomes during tray bleaching of vital teeth employing different carbamide peroxide concentrations, Quintessence Int. 39 (2008) 203–209.
[7] S.R. Grobler, R. Hayward, S. Wiese, M.H. Moola, W.K.T.J. van, Spectrophotometric assessment of the effectiveness of Opalescence PF 10%: a 14-month clinical study, J. Dent. 38 (2010) 113–117.
[8] M. Turkun, E.U. Celik, A. Aladag, N. Gokay, One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique, J. Esthet. Restor. Dent. 22 (2010) 139–146.
[9] E. Jadad, J. Montoya, G. Arana, L.A. Gordillo, R.M. Palo, A.D. Loguercio, Spectrophotometric evaluation of color alterations with a new dental bleaching product in patients wearing orthodontic appliances, Am. J. Orthod. Dentofacial Orthop. 140 (2011) e43–e47.
[10] M. Rezende, A.D. Loguercio, A. Reis, S. Kossatz, Clinical effects of exposure to coffee during at-home vital bleaching, Oper. Dent. 38 (2013) E229–E236.
[11] V. Alonso de la Pena, M. Lopez Raton, Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels, Oper. Dent. 39 (2014) 136–143.
[12] J.K. Bernardon, N. Sartori, A. Ballarin, J. Perdigão, G.C. Lopes, L.N. Baratieri, Clinical performance of vital bleaching techniques, Oper. Dent. 35 (2010) 3–10.
[13] E. Bonafe, C.L. Bacovis, S. Iensen, A.D. Loguercio, A. Reis, S. Kossatz, Tooth sensitivity and efficacy of in-office bleaching in restored teeth, J. Dent. 41 (2013) 363–369.
[14] J.L. de Geus, C. Bersezio, J. Urrutia, T. Yamada, E. Fernandez, A.D. Loguercio, et al., Effectiveness of and tooth sensitivity with at-home bleaching in smokers: a multicenter clinical trial, J. Am. Dent. Assoc. 146 (2015) 233–240.
[15] C. Bertoldo, D. Miranda, E. Souza-Júnior, F. Aguiar, D.A.N.L. Lima, R. Ferreira, et al., Surface hardness and color change of dental enamel exposed to cigarette smoke, Int. J. Dent. Clin. 3 (2011) 1–4.
[16] J.Z. Bazzi, M.J. Bindo, R.N. Rached, R.F. Mazur, S. Vieira, E.M. de Souza, The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel, J. Am. Dent. Assoc. 143 (2012) e1–e7.
[17] E.J.J. Swift Jr., K.N. May Jr., A.D. Wilder Jr., H.O. Heymann, S.C. Bayne, Two-year clinical evaluation of tooth whitening using an at-home bleaching system, J. Esthet. Dent. 11 (1999) 36–42.
[18] L.Y. Tay, C. Kose, D.R. Herrera, A. Reis, A.D. Loguercio, Long-term efficacy of inoffice and at-home bleaching: a 2-year double-blind randomized clinical trial, Am. J. Dent. 25 (2012) 199–204.
[19] R.H.J. Leonard, r, C. Bentley, J.C. Eagle, G.E. Garland, M.C. Knight, C. Phillips, Nightguard vital bleaching: a long-term study on efficacy, shade retention. side effects, and patients' perceptions, J. Esthet. Restor. Dent. 13 (2001) 357–369.
[20] A.V. Ritter, R.H.J. Leonard, r, G. St, A.J. eorges, D.J. Caplan, V.B. Haywood, Safety and stability of nightguard vital bleaching: 9 to 12 years post-treatment, J. Esthet. Restor. Dent. 14 (2002) 275–285.
[21] S.S. Meireles, I.S. Santos, A.D. Bona, F.F. Demarco, A double-blind randomized clinical trial of two carbamide peroxide tooth bleaching agents: 2-year follow-up, J. Dent. 38 (2010) 956–963.
[22] S. Tsubura, Clinical evaluation of three months' nightguard vital bleaching on tetracycline-stained teeth using Polanight 10% carbamide gel: 2-year follow-up study, Odontology 98 (2010) 134–138.

[23] T.M. Auschill, T. Schneider-Del Savio, E. Hellwig, N.B. Arweiler, Randomized clinical trial of the efficacy, tolerability, and long-term color stability of two bleaching techniques: 18-month follow-up, Quintessence Int. 43 (2012) 683–694.
[24] R.F. Mondelli, J.F. Azevedo, A.C. Francisconi, C.M. Almeida, S.K. Ishikiriama, Comparative clinical study of the effectiveness of different dental bleaching methods—two year follow-up, J. Appl. Oral Sci. 20 (2012) 435–443.
[25] B.A. Matis, Y.S. Hamdan, M.A. Cochran, G.J. Eckert, A clinical evaluation of a bleaching agent used with and without reservoirs, Oper. Dent. 27 (2002) 5–11.
[26] I.E. Ruyter, K. Nilner, B. Moller, Color stability of dental composite resin materials for crown and bridge veneers, Dent. Mater. 3 (1987) 246–251.
[27] W.M. Johnston, E.C. Kao, Assessment of appearance match by visual observation and clinical colorimetry, J. Dent. Res. 68 (1989) 819–822.
[28] C.M. Um, I.E. Ruyter, Staining of resin-based veneering materials with coffee and tea, Quintessence Int. 22 (1991) 377–386.
[29] American Dental Association. ADA Acceptance Program Guidelines Dentistdispensed home-use tooth bleaching products April, 2006. Retrieved online 20 May 2015 from: http://www.ada.org/ada/seal/.
[30] S. Kim-Pusateri, J.D. Brewer, E.L. Davis, A.G. Wee, Reliability and accuracy of four dental shade-matching devices, J. Prosthet. Dent. 101 (2009) 193–199.
[31] Commission Internationale de l'Eclairage. Recommendations on Uniform Color Spaces, Color Difference Equations, Psychometric Color Terms, Supplement 2 to CIE Publication 15 (E2-31.1) 1971 (TC-1.3) 1978 Paris, France: Bureau Central de la CIE.
[32] S.S. Meireles, S.S. Heckmann, I.S. Santos, A. Della Bona, F.F. Demarco, A double blind randomized clinical trial of at-home tooth bleaching using two carbamide peroxide concentrations: 6-month follow-up, J. Dent. 36 (2008) 878–884.
[33] C. da, J.B. osta, R. McPharlin, R.D. Paravina, J.L. Ferracane, Comparison of at home and in-office tooth whitening using a novel shade guide, Oper. Dent. 35 (2010) 381–388.
[34] R.D. Paravina, W.M. Johnston, J.M. Powers, New shade guide for evaluation of tooth whitening–colorimetric study, J. Esthet. Restor. Dent.19 (2007) 276–283.
[35] R.D. Paravina, New shade guide for tooth whitening monitoring: visual assessment, J. Prosthet. Dent. 99 (2008) 178–184.
[36] R.D. Paravina, J.M. Powers, R.M. Fay, Color comparison of two shade guides, Int. J. Prosthodont. 15 (2002) 73–78.
[37] M. Analoui, E. Papkosta, M. Cochran, B. Matis, Designing visually optimal shade guides, J. Prosthet. Dent. 92 (2004) 371–376.
[38] Association Dentaire Française (ADF). Medical devices commission: tooth bleaching treatments—a review. 2007. Retrieved online May 27, 2015 from: http://www.adf.asso.fr/pdf/DossiersADF_EN/TOOTH-BLEACHING.pdf.
[39] A. Joiner, The bleaching of teeth: a review of the literature, J. Dent. 34 (2006) 412–419.
[40] A. Watts, M. Addy, Tooth discolouration and staining: a review of the literature, Br. Dent. J. 190 (2001) 309–316.
[41] T. Attin, A. Manolakis, W. Buchalla, C. Hannig, Influence of tea on intrinsic colour of previously bleached enamel, J. Oral Rehabil. 30 (2003) 488–494.
[42] M. Ley, T. Wagner, M. Bizhang, The effect of different fluoridation methods on the red wine staining potential on intensively bleached enamel in vitro, Am. J. Dent. 19 (2006) 80–84.
[43] M.L. Attia, F.H. Aguiar, P. Mathias, G.M. Ambrosano, C.M. Fontes, P.C. Liporoni, The effect of coffee solution on tooth color during home bleaching applications, Am. J. Dent. 22 (2009) 175–179.

[44] B. Matis, G. Wang, J. Matis, N. Cook, G. Eckert, White diets is it necessary during tooth whitening? Oper. Dent. 40 (2015) 235–240.
[45] A.A. Mori, F.F. Lima, A.R. Benetti, R.S. Terada, M. Fujimaki, R.C. Pascotto, Susceptibility to coffee staining during enamel remineralization following the in-office bleaching technique: an in situ assessment, J. Esthet. Restor. Dent. (2015) , doi:http://dx.doi.org/10.1111/jerd.12134.
[46] S.S. Meireles, S. dos Santos Ida, A. Della Bona, F.F. Demarco, A double-blind randomized controlled clinical trial of 10 percent versus 16 percent carbamide peroxide tooth-bleaching agents: one-year follow-up, J. Am. Dent. Assoc. 140 (2009) 1109–1117.
[47] T.B. Téo, M.K. Takahashi, C.C. Gonzaga, M.d.G.K. Lopes, Avaliaéço, após clareamento, da alteraéço de cor de dentes bovinos imersos em soluéóes com elevado potencial de pigmentaéço, Revista Sul-Brasileira de Odontologia 7 (2010) 401–405.
[48] R. Ghinea, M.M. Perez, L.J. Herrera, M.J. Rivas, A. Yebra, R.D. Paravina, Color difference thresholds in dental ceramics, J. Dent. 38 (2010) e57–e64.
[49] A. Nanci, Ten Cate's Oral Histology: Development, Structure, and Function, eighth ed., Elsevier, 2013.

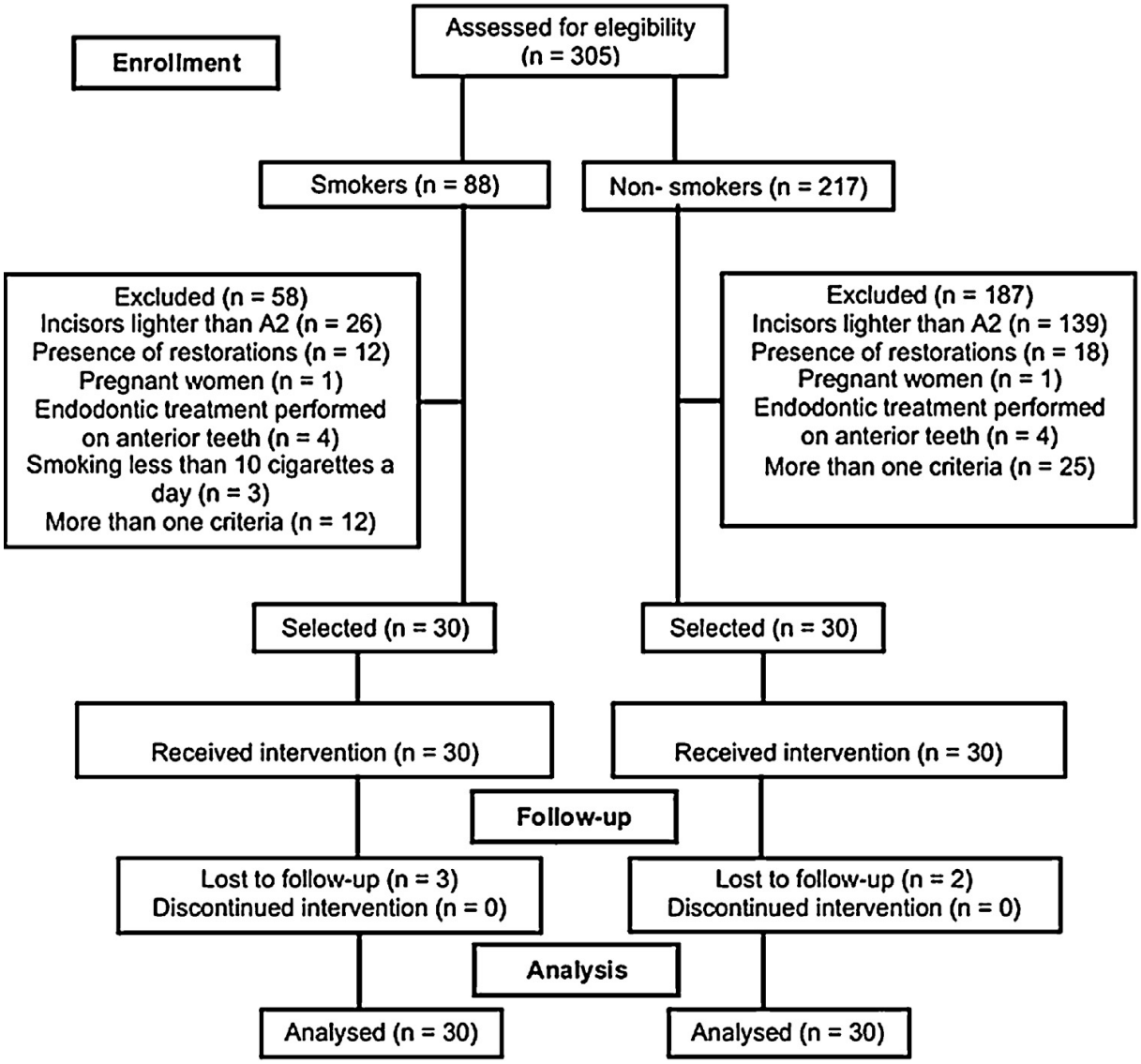

Figure 1. Flow diagram of the clinical trial, including detailed information regarding the excluded participants.

Table 1. Demographic characteristics of the participants.

Characteristics	Groups	
	Smokers	**Non-smokers**
Baseline color (SGU*; mean ± SD**)	7.8 ± 1.1	8.2 ± 1.3
Baseline L* (mean ± SD)	83 ± 19.9	82.6 ± 9.1
Baseline a* (mean ± SD)	0.4 ± 0.6	-0.5 ± 0.4
Baseline b* (mean ± SD)	26.8 ± 5.5	23.4 ± 1.6
Age (years; mean ± SD)	26.3 ± 6.5	24.1 ± 6.8
Sex (male; %)	63.3	53.3
Cigarettes/day (mean ± SD)	13.2 ± 4.0	--
Average smoking years (mean ± SD)	8.0 ± 5.9	--

** SGU = Shade Guide Unit; **SD = standard deviation.*

Table 2. Means and standard deviations of color change in shade guide units (ΔSGU) obtained with the value-oriented shade guide Vita classical at the different assessment points along with the effect size (mean difference) and the 95% confidence interval (CI).

Assessment time	Groups		Main factor Time*	Mean difference (95% CI)
	Smokers	**Non-smokers**		
Baseline *vs.* 1 wk	5.4 ± 2.0	5.8 ± 2.0	5.6 ± 2.3 a	- 0.4 (-1.4 to 0.6)
Baseline *vs.* 1 mth	5.2 ± 2.1	5.7 ± 2.1	5.4 ± 2.3 ab	-0.5 (-1.6 to 0.6)
Baseline *vs.* 1 yr before prophy	4.9 ± 2.1	5.6 ± 2.1	5.3 ± 2.4 b	-0.7 (-1.8 to 0.4)
Baseline *vs.* 1 yr after prophy	5.2 ± 2.2	5.6 ± 2.2	5.4 ± 2.4 ab	-0.4 (1.6 to 0.7)

** Groups identified with the same letter are statistically similar*

Table 3. Means and standard deviations of color change in shade guide units (ΔSGU) using the Vita Bleachedguide 3D-MASTER at the different assessment points along with the effect size (mean difference) and the 95% confidence interval (CI).

Assessment time	Groups		Main factor	Mean difference
	Smokers	Non-smokers	Time*	(95% CI)
Baseline *vs.* 1 wk	4.4 ± 1.0	5.0 ± 1.4	4.7 ± 1.2 a	- 0.6 (-1.2 to 0.03)
Baseline *vs.* 1 mth	4.1 ± 1.1	4.7 ± 1.4	4.4 ± 1.3 b	-0.6 (-1.2 to -0.05)
Baseline *vs.*1 yr before prophy	3.5 ± 1.2	4.2 ± 1.6	3.8 ± 1.4 c	-0.7 (-1.4 to 0.03)
Baseline *vs.* 1 yr after prophy	3.5 ± 1.2	4.2 ± 1.6	3.9 ± 1.4 c	-0.7 (-1.4 to 0.03)

** Groups identified with the same letter are statistically similar.*

Table 4. Means and standard deviations of color change in shade guide units (ΔE*) at the different assessment points along with the effect size (mean difference) and the 95% confidence interval.

Assessment time	Groups		Main factor	Mean difference
	Smokers	Non-smokers	Time*	(95% CI)
Baseline *vs.* 1 wk	10.5 ± 3.9	11.1 ± 3.3	10.8 ± 3.6 ab	-0.6 (-2.5 to 1.3)
Baseline *vs.* 1 mth	8.8 ± 3.9	10.7 ± 4.0	9.7 ± 4.0 a	-1.9 (-3.9 to 0.1)
Baseline *vs.*1 yr before prophy	8.2 ± 4.8	9.2 ± 3.5	8.7 ± 4.2 c	-1.0 (3.2 to 1.2)
Baseline *vs.* 1 yr after prophy	9.9 ± 4.6	10.3 ± 3.7	10.1 ± 4.2 b	-0.4 (2.6 to 1.8)

** Groups identified with the same letter are statistically similar.*

ARTIGO 2

TÍTULO: EFFECTS OF AT-HOME BLEACHING IN SMOKERS: THIRTY-MONTH FOLLOW-UP

STATUS: SUBMETIDO

REVISTA: OPERATIVE DENTISTRY

Effects of at-home bleaching in smokers: thirty-month follow-up

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Clinical Relevance

The results of this study indicate that the dental bleaching is effective in smokers even after 30 months, but dental prophylaxis may be necessary to remove extrinsic stains caused by diet and smoking.

SUMMARY

Objective: This clinical study evaluated the color longevity after thirty months of at-home bleaching with 10 % carbamide peroxide (CP) in smokers and non-smokers.

Methods: Sixty patients, 30 smokers (S) and 30 non-smokers (NS) were subjected to bleaching with 10% CP (Whiteness Perfect - FGM) for three hours daily for three weeks. The color was measured at baseline, one month and 30 months after the completion of dental bleaching, using the shade guide Vita classical organized by value (ΔSGU) and the shade guide Vita Bleachedguide 3D-MASTER. In the 30 months recall, the color was assessed before and after dental prophylaxis. Data from color evaluation was analyzed by two-way repeated measures ANOVA and Tukey's test for the contrast of means ($\alpha = 0.05$).

Results: Twenty-one smokers and 22 non-smokers attended the 30 months recall. For both shade guides, only the main factor assessment time was statistically significant ($p < 0.001$). Effective bleaching was observed in both groups at the baseline, which was stable at one-month. However, color rebound was observed after 30 months for both groups of participants when color was measured before and after dental prophylaxis.

Conclusion: Thirty months after at-home bleaching with 10% carbamide peroxide gel, dental darkening was detected in both groups, which cannot be solely attributed to stains caused by extrinsic staining from the daily food, drinks and smoke (in smokers).

INTRODUCTION

Currently dental bleaching is one of the most requested treatments by patients, due to the fact that white and well-aligned teeth are considered important factors for the concept of a beautiful smile.[1] At-home dental bleaching using 10% carbamide peroxide gel with custom-trays is the most widely used bleaching technique for tooth discoloration treatments.[2]

Although many studies of at-home bleaching have been conducted, most of them exclude smokers from their clinical trials,[3-7] which prevent us from assessing the effect of this cosmetic procedure on such patients. Contrary to this widespread concept, an earlier publication of de Geus et al.[8] demonstrated that effective whitening is achievable regardless of whether or not the patient is smoker.

However smokers' teeth tend to develop tobacco stains over time.[9, 10] Considering that these stains may vary from yellow to black and the severity is highly dependent on the length and frequency of the smoking habit, concerns about the longevity of such treatment were raised. The whitening outcome in smokers was shown to remain stable one year after the bleaching treatment as long as teeth were submitted to dental prophylaxis before color evaluation.[11] These results suggests that color rebound after bleaching results from the deposition of pigments or cigarette smoke on the dental surface in such a short one-year follow-up period.

Although there are numerous studies that evaluated the longevity of at-home bleaching, even for periods as long as 12 years,[12-16] none of them have attempted to appraise bleaching longevity after dental prophylaxis, mainly in smokers patients.

Therefore, the aim of this controlled clinical trial was to compare the 30-month color change of at-home bleaching with 10% carbamide peroxide in smokers and non-smokers before and after dental prophylaxis. The null hypotheses tested is that no significant difference will be detected between smokers and non-smokers after 30 months and no color rebound will be detected in both groups of participants before and after dental prophylaxis.

METHODS AND MATERIALS

The State University of Ponta Grossa and the Ethic Committee approved this equivalence clinical trial (protocol number 16211/ 2014). This study is the thirty-month follow-up of an earlier study[8] registered at clinicaltrials.gov under the identification number NCT02017873. This earlier study was conducted in Chilean and Brazilian centers,[8] but the follow-up was only performed on the Brazilians participants.

Inclusion and exclusion criteria

We evaluated participants in a dental chair, after dental prophylaxis with pumice and water to check whether they met the study's eligibility criteria. Participants included in this clinical trial were aged between 18 and 54 years and had good general and oral health. Each participant had at least 1 central incisor of shade A2 or darker as assessed by means of comparison with a value-oriented shade guide (VITA classical, VITA Zahnfabrik, Bad Säckingen, Germany). Color A2 is the 5th color in the light to dark value VITA classical shade guide scale, so that there are still 5 shades to allow measurement of color changes with this scale. This minimal color shade was already employed in many other clinical trials.[4-7]

We did not include participants who had undergone previous dental bleaching procedures during orthodontic treatment or those who were pregnant, lactating or with bruxism habits. In addition, we excluded participants with non-carious cervical lesions and buccal restorations in anterior teeth as well as those having veneers or full crowns, dental fluorosis, gingival recession, spontaneous tooth pain, internal tooth discoloration and endodontically treated anterior teeth. Patients with bruxism habits were excluded as they usually have a high prevalence of dentin sensitivity.[17]

Study groups

We asked the participants who met the inclusion criteria about their daily smoking habits. Those who did not smoke were part of the non-smoker group, and those who smoked at least 10 cigarettes per day belonged to the group of smokers. We included 30 participants in each group.

Bleaching procedure

We made alginate impressions of each participant's maxillary and mandibular arch, pouring the impressions with dental stone. We did not apply block-out material to the labial surfaces of the teeth.[18] We used a 1-millimeter–thick soft vinyl material provided by the manufacturer (Whiteness, FGM, Joinville, SC, Brazil) to fabricate the custom-fitted tray to hold the bleaching gel. We trimmed the bleaching tray one mm beyond the marginal gingiva and delivered the tray and the 10% CP gel (Whiteness Perfect, FGM, Joinville, SC, Brazil) to each participant with oral instructions for use. We instructed all participants to wear the tray with the bleaching agent for 3 hours daily for 3 weeks.

We instructed the participants to remove the tray after the daily bleaching period, wash it with water, and brush their teeth as usual. We also provided verbal instructions about oral hygiene, encouraging participants to brush their teeth regularly with fluoridated toothpastes without whitening components.

Color evaluation

We checked the color in the middle one-third area of the labial surface in the anterior central incisor according to the American Dental Association guidelines.[19] We used the Vita Bleachedguide 3D-MASTER (VITA Zahnfabrik), which is originally oriented from lightest to darkest color and the VITA classical shade guide (VITA Zahnfabrik). The 16 classical shade guide tabs (VITA classical, VITA Zahnfabrik) were arranged from whitest to darkest as follows: B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4. Although this scale is not linear, we treated the changes as continuous, with linear ranking was used in several clinical trials on dental bleaching.[4, 6]

We calculated the color changes from the beginning of the active phase through the individual recall times by the change in shade guide units (**Δ**SGU) that occurred toward the lighter end of the value-oriented list of shade tabs. In cases where operators disagree about color matching, we reached a consensus before dismissing the patient.

Two calibrated evaluators, with a previous agreement of at least 85% determined by means of weighted k statistics, recorded the shade of the maxillary right central incisor at the baseline, one

week, one month, 12 and 30 months after finishing the bleaching protocol. At 12 and 30 months, the evaluation was performed before and after dental prophylaxis with a rotating brush and prophylaxis paste (Herjos, Vigodent Coltene SA Indústria e Comércio, Rio de Janeiro, Brazil). After dental prophylaxis, teeth were rehydrated in the patient's mouth for 15 min before color assessment. This care was taken due to the teeth becoming lighter as they were dehydrated [20] which could have affected the reliability of the data collected.

Satisfaction assessment

In the 30-month recall, the participants were asked to answer some closed-ended questions about their satisfaction level concerning the bleaching outcome, their perception of color change, and their perception of color rebound after 30 months.

Statistical analysis

We performed all the analyses using the statistical software Statistica for Windows (StatSoft Inc, Tulsa, OK, USA) with a 5% significance level. Two statistical analyses were performed using the per-protocol (only for the available data) and the intention-to-treat approaches. In the latter, the last observation was carried forward for the missing data. The color change in **Δ**SGU from both shade guide units was submitted to two-way repeated measures ANOVA (group vs. assessment period) and Tukey's test for pairwise comparisons. Due to the exploratory nature of the satisfaction assessment data, we did not submit these data to statistical analysis, only descriptive analyses were performed.

RESULTS

At the baseline, we screened 305 patients to obtain 60 participants from the Brazilian center who met the eligibility criteria (Figure 1). Most of non-smokers evaluated were excluded because they have incisors lighter than A2. Although this is also the main reason for exclusion of smokers, a much smaller number of these participants had to be evaluated for the selection. The average age and baseline color of the participants were similar between the groups. Most of the participants were men (Table 1). The 12-month data was published earlier.[11] At the 30-month recall, smoking habits did not change among the majority of participants from the smoking group. Only five of them stopped

smoking while four reduced the number of cigarettes smoked per day (less than ten cigarettes a day).

All participants included in this controlled clinical trial finished the bleaching protocol and attended the one-week and one-month recall visits (Figure 1), however 17 patients did not attend the 30-month recall. The reasons for not attending the recall were due to the change of housing location and l participant's lacking time availability to return to the university, for a new color assessment.

Per-protocol *vs.* Intention-to-treat analysis

All statistical analyses were performed with data imputation for missing outcomes (intention-to-treat) and without data imputation (per-protocol). In all analyses, the same overall conclusions were reached (data not shown). To avoid data repetition we opted to describe only the results and statistics obtained in the per-protocol analysis due to the fact that a high percentage of patients (17 out of 60 [28%]) could not be evaluated in the 30-month recall. The distribution of missing data was homogeneous among groups (n = 9 in the smokers group and n = 8 in the non-smokers group).

Shade evaluation

For both Vita classical shade guide and Vita Bleachedguide 3D-MASTER, the two-way repeated ANOVA revealed that the cross-product interaction group *vs*. assessment time ($p = 0.079$ and $p = 0.378$, respectively) and the main factor group ($p = 0.517$ and $p = 0.051$, respectively) was not significant. Only the main factor assessment time was statistically significant (Tables 2 and 3; $p < 0.001$). The lack of difference between the groups (smokers vs. non-smokers) can also be seen with the 95% CI interval of the effect size (mean difference) (Tables 2 and 3) that does include zero.

A significant average color change (ΔSGU) of approximately 5 shade guide units in the Vita classical guide (Table 2) and 4 shade guide units in the Vita Bleachedguide 3D-MASTER (Table 3) was observed one month after bleaching for both groups. At 30 months, a slight but significant color rebound could be detected regardless of whether color was measured before or after dental prophylaxis (Tables 2 and 3; $p < 0.001$).

Satisfaction assessment

Participants that attended the shade evaluation at 30 months after bleaching treatment were

questioned about their satisfaction level (Table 4). The majority of the smokers reported that they still observed moderate bleaching, while participants in the non-smokers group reported there was still significant bleaching. Most participants from both groups felt happy with the bleaching result and would repeat this procedure. After 30 months of the bleaching procedure, 70% of participants reported that their teeth darkened slightly.

DISCUSSION

As a part of daily life many people smoke, eat dark-colored food, drink coffee, tea, red wine and other colored drinks. Some investigators have reported that colored beverages and foods can induce tooth discoloration.[21, 22] This fact along with slight demineralization that acidic bleaching gels produce on dental surfaces[23] led dentists and product manufacturers' to request their patients to avoid smoking, drinking and eating colored beverages and foods during the active bleaching treatment phase.

However, these dentists' recommendation seems to be indorsed by bleaching myths, in relation to efficacy and safety, rather than evidence-based findings.[24] It was reported in a recent publication that at-home bleaching did not induce DNA damage to gingival tissue during the bleaching period in smokers and non-smokers.[25] The genotoxicity potencial of smoking detected by the mean number of micronuclei in exfoliated cells was not increased by at-home bleaching procedure.[25]

Considering the effectiveness, the results of the present study highlight that effective bleaching is achievable in smokers even without requesting them to stop smoking during the active phase of the bleaching treatment. In a similar trend, an earlier study reported that exposure to coffee four times a day also did not jeopardize the bleaching efficacy when compared to patients that followed a white diet.[4] White diet was a term introduced by Prof. Matis in a recent publication[24] that refers to a diet free of colored drinks and foods. This was also confirmed in a recent published study.[24] Altogether these three studies provide contrary evidence to this widespread myth that patients should keep a white diet and/or quit smoking while having their teeth bleached. The self-assessment of the participants is also in agreement with the results of the shade guide units; most of the participants from

both groups reported to be happy with the whitening degree and would repeat dental bleaching if necessary.

On the other hand, we cannot deny the fact that smokers are theoretically more prone to have stain deposition on their dental surfaces than non-smokers over time. Consequently, concerns about durability and longevity of the bleaching protocol in such groups of patients are critical. Tobacco has much nicotine[26] and though it is an inherently colorless substance, it turns yellow when put in contact with oxygen. Nicotine penetrates the nooks and crannies[27] of teeth leading to teeth stains. Apart from nicotine, tobacco smoke contains carbon monoxide, thiocyanate, herbicide, fungicide and pesticide residues, tars, sugar, and cocoa,[28] which cause dental discoloration due to their dark hue and ability to adhere to dental surfaces.[9]

However, our results demonstrated that color rebound was equal in both groups of participants. We expected that teeth of smokers would be darker than those of non-smokers, which was not detected by the findings of the present investigation. Perhaps 30-months is still a short-term follow-up for nicotine and tar to penetrate in the tooth and change its color intrinsically. Another factor to be considered is that in the 12-month assessment, dental prophylaxis was performed,[18] so the extrinsic pigments observed in the 30-month recall were the result of an 18-month accumulation. Furthermore, some participants stopped or decreased smoking during this 30-month follow-up. Perhaps the evaluation of such sample in longer follow-ups might allow us to detect if indeed differences in the longevity of the bleaching outcomes in smokers comparatively to non-smokers may become evident in longer follow-ups.

Professional mechanical cleaning, such as dental prophylaxis and enamel polishing are effective means to produce partial or complete stain removal.[29] Indeed, this was observed in the present investigation and in the one-year follow-up of this study. By removing the extrinsic stains presented in the dental surface of the smoker group (produced by diet + cigarette smoke) and in the non-smokers group (produced by diet), teeth became significantly whiter. This was probably one of the reasons that reduced the patient's overall perception of whiter teeth after 30 months of follow-up.

On the other hand, the present study demonstrated that stain deposition is not the single factor

responsible for the color rebound observed in the present investigation. Contrary to what was observed in the one-year follow-up,[11] the ΔSGU baseline *vs*. 30-month after prophylaxis was not statistically similar to the whitening degree obtained one month post bleaching. Meaning that other factors apart from superficial dental staining might be associated with such slight but significant color rebound.

As teeth get older, there is a continuous enamel wear and deposition of secondary dentin by the pulp.[30,] As the dentin thickness increases and enamel thickness decreases,[31] teeth become increasingly yellow irrespectively of the dietary conditions or smoking habits. Interestingly, most of the patients reported that they felt their teeth were darker at the 30-month recall than immediately after bleaching. In the 30 months recall most of participants of both groups reported that their teeth darkened slightly.

The participant's perception is important for clinicians, since a positive correlation was found between participants' self-assessment of their tooth shade and that of the clinician.[32] One of the most important factors in determining satisfaction with self-appearance is the tooth color.[33] In the present study most participants, both smokers and non-smokers feel happy with the bleaching treatment result, which was previously shown in a study to assess patient satisfaction with the whitening treatment performed.[34] Some participants reported that the bleaching treatment provided a slight color change, noticed or not by other people. It has been shown that the patient's expectations regarding the outcome of the bleaching treatment are higher than those of dentists,[35] which could lead to a divergence between them. Meaning that clinical trials should include more patient-centered outcomes rather than evaluator-centered outcomes, since the patient's satisfaction or treatment success perception is more important than the care provider's perception. It is worth pointing out, however, that the data provided by the questionnaire included in this trial is just exploratory, as we have not used a validated instrument for such goal.

The literature findings report controversial findings in regard to the longevity of at-home bleaching. Some authors reported stable color in periods ranging from one to two years.[12, 13, 16] Other authors reported color rebound after one year[36, 37]and two years[13, 38] and also after longer follow-up recalls,[39, 40] as demonstrated in this study. Indeed, the longevity of such bleaching procedure is yet to

be determined. In spite of this, 70% of patients reported that they observed a slight color change in their teeth.

Additionally, the majority of clinical trials that evaluate the longevity of at-home bleaching did not report the patients' dietary habits during and after dental bleaching treatment. Only few studies have attempted to associate the effect of dietary habits with the longevity of at-home bleaching[12, 37,38] although they did not reach conclusive findings, which emphasizes the need for future studies.

CONCLUSION

After a follow-up of 30 months we detected a significant color rebound in smokers and non-smokers with 10% carbamide peroxide, which cannot be attributed to extrinsic stains only, as even after dental prophylaxis, teeth appeared slightly darker than the immediate whitening result.

REFERENCES

1. Samorodnitzky-Naveh GR, Geiger SB & Levin L (2007) Patients' satisfaction with dental esthetics *Journal of the American Dental Association***138(6)** 805-808.
2. Machado LS, Anchieta RB, Dos Santos PH, Briso AL, Tovar N, Janal MN, Coelho PG & Sundfeld RH (2016) Clinical Comparison of At-Home and In-Office Dental Bleaching Procedures: A Randomized Trial of a Split-Mouth Design The *International Journal of Periodontics and Restorative Dentistry***36(2)** 251-260.
3. de Almeida LC, Costa CA, Riehl H, dos Santos PH, Sundfeld RH & Briso AL (2012) Occurrence of sensitivity during at-home and in-office tooth bleaching therapies with or without use of light sources *Acta Odontológica Latinoamericana***25(1)** 3-8.
4. Rezende M, Loguercio AD, Reis A & Kossatz S (2013) Clinical effects of exposure to coffee during at-home vital bleaching *Operative Dentistry***38(6)** E229-236.
5. Alonso de la Pena V & Lopez Raton M (2014) Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels *Operative Dentistry***39(2)** 136-143.
6. Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC & Baratieri LN (2010) Clinical performance of vital bleaching techniques *Operative Dentistry***35(1)** 3-10.
7. Bonafe E, Bacovis CL, Iensen S, Loguercio AD, Reis A & Kossatz S (2013) Tooth sensitivity and efficacy of in-office bleaching in restored teeth *Journal of Dentistry***41(4)** 363-369.
8. de Geus JL, Bersezio C, Urrutia J, Yamada T, Fernandez E, Loguercio AD, Reis A & Kossatz S (2015) Effectiveness of and tooth sensitivity with at-home bleaching in smokers: A multicenter clinical trial *Journal of the American Dental Association***146(4)** 233-240.
9. Bertoldo C, Miranda D, Souza-Júnior E, Aguiar F, DANLLima, Ferreira R, Claes I & Lovadino J (2011) Surface hardness and color change of dental enamel exposed to cigarette smoke *International Journal of Dental Clinics***3(4)** 1-4.

10. Alkhatib MN, Holt RD & Bedi R (2005) Smoking and tooth discolouration: findings from a national cross-sectional study *BMC Public Health***5** 27.
11. de Geus JL, de Lara MB, Hanzen TA, Fernandez E, Loguercio AD, Kossatz S & Reis A (2015) One-year follow-up of at-home bleaching in smokers before and after dental prophylaxis *Journal of Dentistry***43(11)** 1346-1351.
12. Tay LY, Kose C, Herrera DR, Reis A & Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: A 2-year double-blind randomized clinical trial *American Journal of Dentistry***25(4)** 199-204.
13. Tsubura S (2010) Clinical evaluation of three months' nightguard vital bleaching on tetracycline-stained teeth using Polanight 10% carbamide gel: 2-year follow-up study *Odontology***98(2)** 134-138.
14. Turkun M, Celik EU, Aladag A & Gokay N (2010) One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique *Journal of Esthetic and Restorative Dentistry***22(2)** 139-146.
15. Auschill TM, Schneider-Del Savio T, Hellwig E & Arweiler NB (2012) Randomized clinical trial of the efficacy, tolerability, and long-term color stability of two bleaching techniques: 18-month follow-up *Quintessence International***43(8)** 683-694.
16. Mondelli RF, Azevedo JF, Francisconi AC, Almeida CM & Ishikiriama SK (2012) Comparative clinical study of the effectiveness of different dental bleaching methods - two year follow-up *Journal of Applied Oral Science***20(4)** 435-443.
17. Scaramucci T, de Almeida Anfe TE, da Silva Ferreira S, Frias AC & Sobral MA (2014) Investigation of the prevalence, clinical features, and risk factors of dentin hypersensitivity in a selected Brazilian population *Clinical Oral Investigations***18(2)** 651-657
18. Matis BA, Hamdan YS, Cochran MA & Eckert GJ (2002) A clinical evaluation of a bleaching agent used with and without reservoirs *Operative Dentistry***27(1)** 5-11.
19. American Dental Association (2006) ADA Acceptance Program Guidelines Dentist-dispensed home-use tooth bleaching products. Retrieved online March 12, 2016 from: http://www.ada.org/ada/seal/.
20. Stevenson B (2009) Current methods of shade matching in dentistry: a review of the supporting literature *Dental Update***36(5)** 270-272, 274-276.
21. Attin T, Manolakis A, Buchalla W & Hannig C (2003) Influence of tea on intrinsic colour of previously bleached enamel *Journal of Oral Rehabilitation* **30(5)** 488-494.
22. Ley M, Wagner T & Bizhang M (2006) The effect of different fluoridation methods on the red wine staining potential on intensively bleached enamel in vitro *American Journal of Dentistry***19(2)** 80-84.
23. Attin T, Schmidlin PR, Wegehaupt F & Wiegand A (2009) Influence of study design on the impact of bleaching agents on dental enamel microhardness: a review *Dental Materials***25(2)** 143-157.
24. Matis B, Wang G, Matis J, Cook N & Eckert G (2015) White Diet: Is It Necessary During Tooth Whitening? *Operative Dentistry***40(3)** 235-240.
25. de Geus JL, Rezende M, Margraf LS, Bortoluzzi MC, Fernandez E, Loguercio AD, Reis A & Kossatz S (2015) Evaluation of genotoxicity and efficacy of at-home bleaching in smokers: a single-blind controlled clinical trial *Operative Dentistry***40(2)** E47-55.
26. Neergaard J, Singh P, Job J & Montgomery S (2007) Waterpipe smoking and nicotine exposure: a review of the current evidence *Nicotine & Tobacco Research***9(10)** 987-994.
27. Kassis C & Zogheib T (2015) Extrinsic tooth discoloration, an updated review *Dental Tribune***6(B)** 8B - 11B.
28. Wasilewski Mde S, Takahashi MK, Kirsten GA & de Souza EM (2010) Effect of cigarette smoke and whiskey on the color stability of dental composites *American Journal of Dentistry***23(1)** 4-8.

29. Bazzi JZ, Bindo MJ, Rached RN, Mazur RF, Vieira S & de Souza EM (2012) The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel *Journal of the American Dental Association* **143(5**) e1-7.
30. Nanci A (2013) Ten Cate's oral histology: development, structure, and function Eighth edition, Elsevier.
31.Morse DR (1991) Age-related changes of the dental pulp complex and their relationship to systemic aging *Oral Surgery, Oral Medicine and Oral Pathology***72(6)** 721-745.
32. Samorodnitzky-Naveh GR, Grossman Y, Bachner YG & Levin L (2010) Patients' self-perception of tooth shade in relation to professionally objective evaluation *Quintessence International***41(5)** e80-83.
33. Tin-Oo MM, Saddki N & Hassan N (2011) Factors influencing patient satisfaction with dental appearance and treatments they desire to improve aesthetics *BMC Oral Health***11** 6.
34. Martin J, Rivas V, Vildosola P, Moncada L, Oliveira Junior OB, Saad JR, Fernandez E & Moncada G (2016) Personality Style in Patients Looking for Tooth Bleaching and Its Correlation with Treatment Satisfaction *Brazilian Dental Journal* **27(1)** 60-65.
35. Carlsson GE, Wagner IV, Odman P, Ekstrand K, MacEntee M, Marinello C, Nanami T, Ow RK, Sato H, Speer C, Strub JR & Watanabe T (1998) An international comparative multicenter study of assessment of dental appearance using computer-aided image manipulation *The International Journal of Prosthodontics***11(3)** 246-254.
36. Grobler SR, Hayward R, Wiese S, Moola MH & van WKTJ (2010) Spectrophotometric assessment of the effectiveness of Opalescence PF 10%: a 14-month clinical study *Journal of Dentistry***38(2)** 113-117.
37. Meireles SS, dos Santos Ida S, Della Bona A & Demarco FF (2009) A double-blind randomized controlled clinical trial of 10 percent versus 16 percent carbamide peroxide tooth-bleaching agents: one-year follow-up *Journal of the American Dental Association***140(9)** 1109-1117.
38. Meireles SS, Santos IS, Bona AD & Demarco FF (2010) A double-blind randomized clinical trial of two carbamide peroxide tooth bleaching agents: 2-year follow-up *Journal of Dentistry***38(12)** 956-963.
39. Ritter AV, Leonard RH, Jr., St Georges AJ, Caplan DJ & Haywood VB (2002) Safety and stability of nightguard vital bleaching: 9 to 12 years post-treatment *Journal of Esthetic and Restorative Dentistry***14(5)** 275-285.
40. Leonard RH, Jr., Bentley C, Eagle JC, Garland GE, Knight MC & Phillips C (2001) Nightguard vital bleaching: a long-term study on efficacy, shade retention. side effects, and patients' perceptions *Journal of Esthetic and Restorative Dentistry***13(6)** 357-369.

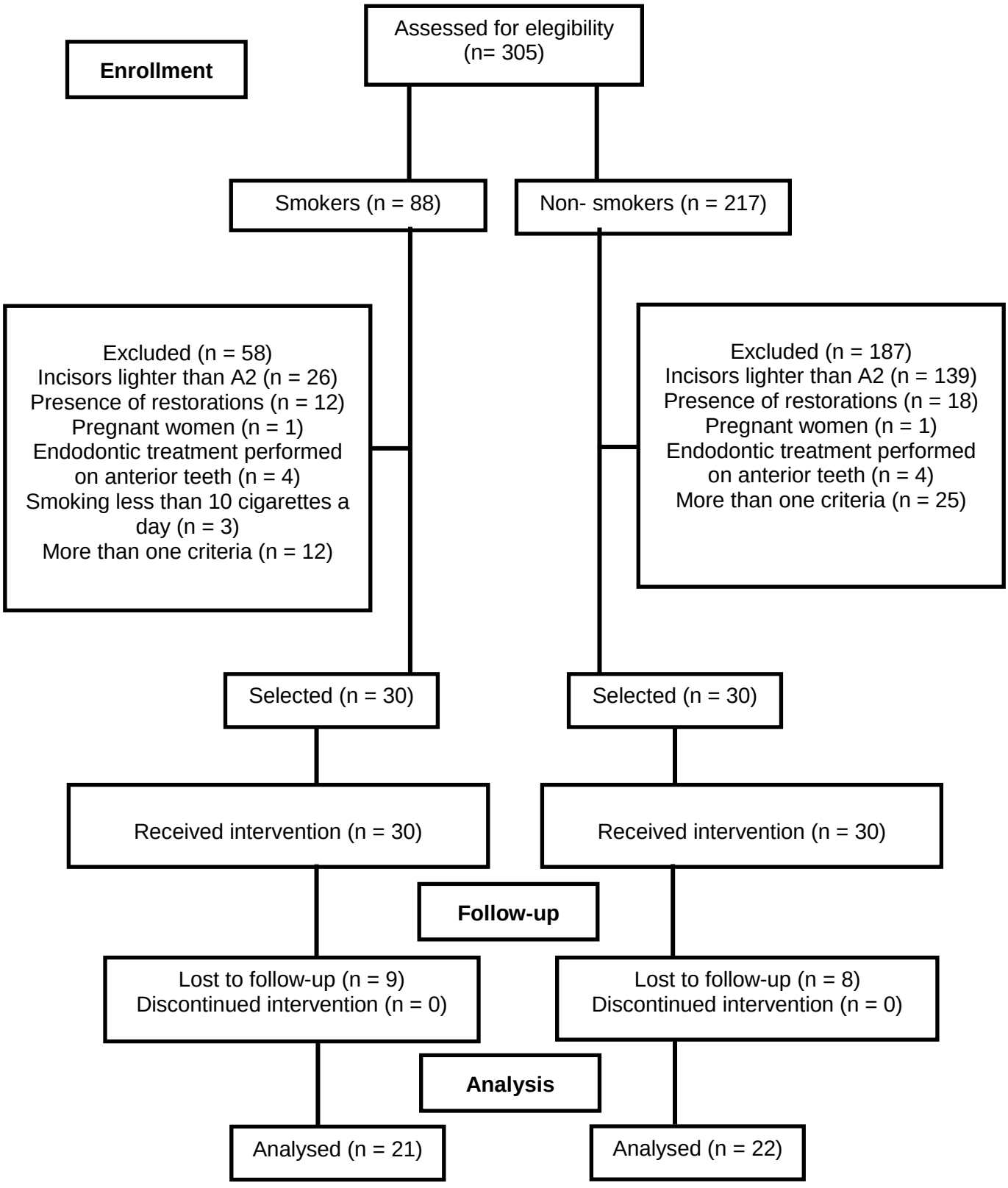

Figure 1. Flow diagram of the clinical trial, including detailed information regarding the excluded participants. *An intention-to-treat analysis, where unit imputation was used for missing information, was also performed and the overall conclusions were the same as the per protocol analysis.

Table 1. Demographic characteristics of the participants included in this randomized clinical trial.

Characteristics	Groups	
	Smokers	**Non-smokers**
Baseline color (SGU classical; mean ± SD*)	6.8 ± 2.3	7.3 ± 2.5
Baseline color (SGU Bleachedguide 3D-MASTER; mean ± SD)	7.8 ± 1.1	8.2 ± 1.3
Age (years; mean ± SD)	26.3 ± 6.5	24.1 ± 6.8
Sex (male; %)	63.3	53.3
Cigarettes/day (mean ± SD)	13.2 ± 4.0	--
Cigarettes/day at 30 months (mean ± SD)	11.8 ± 5.1	--
Average smoking years (mean ± SD)	8.0 ± 5.9	--

**SD = standard deviation.*

Table 2. Color change in shade guide units (ΔSGU) obtained with the value-oriented shade guide Vita Classical at the different assessment points along with the effect size (mean difference) and the 95% confidence interval (CI).

Assessment time	Medians (interquartile range)		Means ± standard deviations			
	Smokers	Non-smokers	Smokers	Non-smokers	Main factor Time (*)	Mean difference (95% CI)
Baseline *vs*. 1 mth	4 (3.75 – 6.25)	5.5 (4 – 8)	5.2 ± 2.1	5.7 ± 2.3	5.5 ± 2.2[a]	-0.5 (-1.9 – -0.9)
Baseline *vs*. 30 mths before prophy	4 (3 – 6)	4.5 (3 – 7)	4.7 ± 2.2	5.1 ± 2.2	5.0 ± 2.2[c]	-0.4 (1.8 – 1.0)
Baseline *vs*. 30 mths after prophy	4 (3.5 – 6.25)	4.5 (3 – 7)	5 ± 2.2	5.2 ± 2.3	5.2 ± 2.3[b]	-0.2 (-1.6 – 1.2)

** Groups identified with the same letter are statistically similar.*

Table 3. Color change in shade guide units (ΔSGU) using the Bleachedguide 3D-MASTER shade guide at the different assessment points along with the effect size (mean difference) and the 95% confidence interval (CI).

Assessment time	Medians (interquartile range)		Means ± standard deviations			
	Smokers	Non-smokers	Smokers	Non-smokers	Main factor Time*	Mean difference (95% CI)
Baseline *vs*. 1 mth	4 (3 – 4.25)	4.5 (4 – 5)	4.1 ± 1.1	4.7 ± 1.4	4.4 ± 1.3[a]	-0.6 (-1.4 – 0.2)
Baseline *vs*. 30 mths before prophy	3 (2 – 3.25)	3.5 (3 – 4)	2.6 ± 1.1	3.3 ± 1.2	3.0 ± 1.2[c]	-0.7 (-1.4 – 0.0)
Baseline *vs*. 30 mths after prophy	3 (2.75 – 4)	4 (3 – 4)	3.0 ± 1.1	3.4 ± 1.2	3.2 ± 1.2[b]	-0.4 (-1.1 – 0.3)

** Groups identified with the same letter are statistically similar.*

Table 4. Degree of participants' satisfaction 30 months after bleaching.

QUESTIONS	Smokers	Non-smokers	Statistical significance*
1 – After the bleaching treatment, you observed that			
a) there was no color change in teeth	0	0	n.s.
b) there was mild whitening, not noticed by others	1	0	n.s.
c) there was mild bleaching, noticed by others	2	5	n.s.
d) there was moderate whitening	10	5	n.s.
e) there was a significant whitening	8	12	n.s.
2 – What is your level of satisfaction with the performed bleaching treatment?			
a) Very happy	9	6	n.s.
b) Happy	9	8	n.s.
c) Satisfied	3	8	n.s.
d) Indifferent	0	0	n.s.
e) Dissatisfied	0	0	n.s.
3 – Would you repeat the bleaching treatment in case your teeth get darker?			
a) Yes, because I liked the result	16	16	n.s.
b) Yes, because I would like my teeth become lighter than it is	5	6	n.s.
c) No, I'm satisfied	0	0	n.s.
d) No, because I experienced pain	0	0	n.s.
4–Does your teeth look darker now (30 months after bleaching)?			
a) No	0	1	n.s.
b) Little	14	15	n.s.
c) Reasonable	6	5	n.s.
d) Too much	0	1	n.s.
e) I don't know	1	0	n.s.

** Chi-square test, $\alpha = 0.05$.*

ARTIGO 3

TÍTULO: DETERMINATION OF NICOTINE CONTENT IN TEETH SUBMITTED TO PROPHYLAXIS AND IN-OFFICE BLEACHING BY GAS-CHROMATOGRAPHY

STATUS: SUBMETIDO

REVISTA: CLINICAL ORAL INVESTIGATIONS

Determination of nicotine content in teeth submitted to prophylaxis and in-office bleaching by gas-chromatography

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Abstract

Objectives To evaluate the dental color exposed to acute cigarette smoke treatment and quantify the amount of nicotine in samples exposed to cigarette smoke, after dental prophylaxis and after in-office bleaching.

Materials and Methods Sixty-nine healthy human molars were subjected to cigarette smoke in a cigarette machine. The teeth were divided into three groups: positive control, prophylaxis, and bleaching. Forty cycles of smoke exposition with duration of 15 min each were performed using 10 cigarettes (positive control). Dental prophylaxis was performed with a rotating brush and prophylaxis paste; in-office bleaching was performed with 35% hydrogen peroxide, in two sessions of three 15-min applications, with a one week interval between sessions. The color was evaluated at the baseline, after exposure to cigarette smoke, after dental prophylaxis, and after in-office bleaching. Teeth from each group were powered and analyzed by gas chromatography-mass spectrometry in order to measure the amount of nicotine present in each group. Data from quantification of nicotine and color change were analyzed by one-way ANOVA and Tukey's test ($\alpha = 0.05$).

Data For subjective and objective color evaluation, a perceptible dental darkening occurred in teeth after exposure to cigarette smoke. Dental prophylaxis was able to recover the original color of teeth however, only after bleaching teeth became whiter than at the baseline ($p < 0.001$). The amount of nicotine was significantly different and higher in positive control group (3.3 ± 1.3 μg/g of tooth), followed by the prophylaxis group (2.1 ± 1.4 μg/g) and the bleaching group (0.8 ± 0.3 μg/g) ($p < 0.001$).

Conclusions Cigarette smoke penetrates into the dental structure. Dental prophylaxis and bleaching with 35% hydrogen peroxide can partially remove the nicotine from tobacco smoke. However, when in-office bleaching was applied, a more significant nicotine removal was achieved.

Clinical Significance Nicotine deposit on the external dental surface, and penetration within the dental structure. Although dental prophylaxis decreased the quantity of nicotine in teeth exposed to cigarette

smoke, in-office bleaching decreased it even further.

Key words Tobacco Products • Gas Chromatography-Mass Spectrometry • Nicotine • Tooth Bleaching Agents.

Introduction

Since 1980, large reductions in the estimated prevalence of daily smoking were observed at the global scale both for men and women, but due to population growth, the number of smokers has increased significantly [1].

When burning, cigarette components such as tar, sugar, nicotine, and cocoa are transferred to smoke due to heating [2]. Nicotine is the most specific component of a cigarette, which is responsible for tobacco dependency. It is present in a relatively large amount (1-2 mg per cigarette). It is absorbed and measured in active smokers as liabilities [3, 4].

Cigarette smoke by products was already observed in composite resins and dental structures [5]. Nicotine deposit on a dental surface might penetrate enamel grooves leading to dental yellowing. Although nicotine is a colorless substance, it turns yellow when exposed to oxygen [6].

Whether or not this dental yellowing in smokers is caused by external staining or by the deposits of cigarette smoke by products within the dental structure is yet to be evaluated. If this yellowing was caused by the deposits of dark hue cigarette smoke on the dental surfaces of teeth [7], mechanical and professional cleaning could be appropriate means of recovering natural dental color [8] by decreasing the amount of nicotine and other pigments on the dental surface. Recently, de Geus et al. (2015) [9] showed that effective and similar whitening was achieved in smokers; however 1-month post-bleaching smokers showed slightly darker teeth [10], probably due to the deposition of cigarette smoke stains on the dental surfaces. After one-year, de Geus et al. (2015) [11] observed the same trend: smokers having slightly darker teeth than nonsmokers, but this could be reverted by performing dental prophylaxis.

However, one cannot rule out the fact that this yellowing could also be the result of nicotine deposit within the dental structure. In this case only oxidizing methods, such as dental bleaching could change the molecular structure of these colorful molecules and recover natural dental color.

Therefore, the aim of this *in vitro* study was to evaluate the color of teeth exposed to acute

cigarette smoke treatment and quantify the amount of nicotine in these samples after dental prophylaxis and in-office bleaching.

Materials and methods

The State University of Ponta Grossa (protocol 11265/2015) and the Ethics Committee from the same University (protocol number 1.065.444) approved this *in vitro* study. Sixty-nine sound human molars were obtained from the Bank of extracted teeth from the same university.

Chemicals

The cigarettes (Marlboro Red) were purchased from Philip Morris Brazil Ind. e Com. Ltda. (Santa Cruz do Sul, RS, Brazil). Water was purified using a Millipore Milli-Q system (Millipore) and it was used for all the experiments. Methylene chloride ($CHCl_2$) was GC grade (JT Baker) and ammonium hydroxide (NH_4OH) was analytical grade (Fisher Scientfic). The standards of nicotine (> 97% purity) and diphenylamine (> 99% purity, IS) were purchased from Sigma–Aldrich (St. Louis, Missouri, EUA).

Teeth samples and cigarette smoke exposure

The sound human molars were placed in a smoke machine to allow for impregnation of cigarette smoke into the dental structures. Briefly, the home-made machine (Figure 1) aspires and conducts smoke from the cigarette through glass cannulas aiming to allow it to circulate and deposit the chemical products on the dental surface. Forty cycles (15 min/each cycle) with 10 cigarettes were performed, totaling 400 cigarettes. This cycle resembles a 1-year smoking habit, considering: 1) an average of 15 cigarettes a day; 2) a 4-second contact time of cigarette smoke with the teeth per inhalation; 3) 11 inhalations per cigarette.

Staining removal procedure

After cigarette smoke exposure, teeth were divided in three groups (n = 23 each). In specimens from the positive control group, no attempt was made to remove the staining produced by the cigarette smoke. In specimens from the prophylaxis group, teeth were submitted to dental treatment with a rotating brush and prophylaxis paste (Herjos, Vigodent Coltene, Rio de Janeiro, RJ, Brazil). On each dental

surface, the paste was scrubbed with the rotating brush for 60 s using intermittent motion; after that, the specimens were rinsed abundantly with water (± 100 mL) for 30 s and kept in water at 37°C. On the specimens from the bleach group, the teeth were fixed in styrofoam and in-office bleaching was performed using 35% hydrogen peroxide gel (Whiteness HP MAXX – FGM Dental Products, Joinville, SC, Brazil) following the manufacturer's instructions. The bleaching agent was applied on all surfaces and refreshed every 15 min during the in-office process for three times. Two bleaching sessions, with a one-week interval between them, were performed. In the meantime, teeth were always kept immersed in water at 37°C.

Shade evaluation

The color of the teeth was evaluated using an objective and subjective method. For the objective shade evaluation, we used a digital spectrophotometer (VITA Easyshade, VITA Zahnfabrik, Bad Säckingen, Germany). Before color evaluation, an impression of the teeth was taken with dense silicone paste (Coltoflax and Perfil Cub, Vigodent, Rio de Janeiro, RJ, Brazil), a window was created on the buccal surface of the silicone guide by using a metal device with a radius of 6 mm, to standardize the area for color evaluation at the different assessment points.

The color was determined using the parameters of the digital spectrophotometer on which the following values were indicated: L*, a*, and b*, where L* represents luminosity (the value from 0 [black] to 100 [white]), and a* and b* represent color along the red-green axis and color along the yellow-blue axis, respectively. The difference between the baseline and each recall period (ΔE*) was calculated by using the following formula (CIE 1978)[12]: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

For the subjective evaluation, two shade guides were used. The 16 tabs from the VITA classical shade guide (VITA Zahnfabrik) were arranged from whitest to darkest as follows: B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4. The Vita Bleachedguide 3D-MASTER was also used, developed for bleaching studies [9, 13-16], in which the color tabs are already organized from whitest to darkest. The color in the middle one-third area of the labial surface of the teeth was checked. Color

changes were calculated from the beginning of the active phase through the individual recall times by the change in shade guide units (ΔSGU) that occurred toward the lighter end of the value-oriented list of shade tabs.

The color was measured before exposure to cigarette smoke (baseline), after exposure to cigarette smoke, and after each treatment for stain removal (dental prophylaxis and in-office bleaching).

Sample Analysis

After color measurements, teeth from each group were broken in pieces, 5 g was pulverized by a ball mill machine (SPEX SamplePrep 8000M Mixer/Mill, Metuchen, NJ, USA) for 60 min at 1725 rpm. This procedure was repeated 4 times producing four samples for each study group (Figure 2). The study groups were processed using the same procedure described below for the calibration curve standards extraction: 15 mg of sample was added to 1 mL NH_4OH 2%, vortex for 20 s, incubated in a sonication bath at 80°C for 30 min and then 1 mL of $CHCl_2$ was added to the solution and the mixture was homogenized by a vortex for 1 min and centrifuged for 15 min at 9000 rpm. The organic layer was transferred to a vial prior to gas chromatography-mass spectrometry [17].

Gas Chromatography-Mass Spectrometry analysis (GC-MS)

The analysis was performed using Agilent 7898B gas chromatography (Santa Clara, CA, USA) coupled with Agilent 5977 with a single quadrupole mass specific detector. The Agilent J&W DB-23MS fused silica capillary column [(50%-Cyanopropyl) - methylpolysiloxane, 30 m in length x 0.25 mm i.d., 0.25 µm film thickness] was used with helium as the gas carrier at a flow rate of 1.0 mL/min. The oven temperature program started at 100 °C, then the column was sequentially heated at a rate of 20 °C/min to 250 °C and was held for 10 min. Both the injector temperature and the transfer line temperature were programmed at 250 °C. The split less injection mode was used with 2 µL injection. The EI spectrum was recorded at 70 eV from m/z 50 to 500 in both scan and selected ion monitoring (SIM) modes. The ions of m/z 162 and 169 were selected to monitor nicotine and IS, respectively. The MS source temperature was set to 230 °C, the single quadrupole temperature was 150 °C. The instrument was controlled by the

Agilent Enhanced MDS Productivity ChemStation software (version E.02.02). Wiley and NIST library (version 2.0 g) was used to assist substance identification. Further identification was based on the relative retention index compared with literature and the reference standards.

Calibration curve and quality control preparation

Nicotine stock solutions (Figure 3) were prepared in MeOH (1000, 500, 200, 100, 50 and 10 ng/mL, volumetric flask). From stock solutions, the calibration curve in matrix was prepared. For this, we added in an eppendorf 50 µL of IS solution (1 µg/mL in MeOH), 20 mg of matrix (blank powder tooth), 100 µL of nicotine stock solutions to reach final concentrations of 100, 50, 20, 10, 5 and 1 ng/mL) and 1mL NH_4OH 2%. This solution was vortexed for 20 s and in the sequence incubated in a sonic bath at 80°C for 30 min.

After that, we added 1 mL of $CHCl_2$ and the mixture was homogenized by a vortex for 1 min and centrifuged for 15 min at 9000 rpm. The $CHCl_2$ layer (bottom) was transferred to a vial and injected into the GC-MS. The quality controls (80, 30 and 15 ng/mL) were prepared in the same way. The calibration curve and quality control standards were prepared in triplicate and the calibration curve was drawn by plotting the peak area ratios between nicotine and IS [18].

Recovery, intermediate precision and accuracy

The recovery was evaluated comparing 3 quality control solutions prepared as described above (extracted solutions) and 3 standard solutions (50 µL of IS solution (1 µg/mL in MeOH), 100 µL of nicotine stock solutions (80, 30 and 15 ng/mL), and 850 µL of $CHCl_2$ to get the perceptual recovery. The intermediate precision (intra and inter day) was obtained by evaluating the quality control samples in three non-consecutives days. The accuracy was determined by back calculation of the quality controls measures (calculated concentrations).

Limits of detection and quantification

The limit of detection (LOD) was determined by taking the signal-to-noise ratio of the three as

criterion, the limit of quantification (LOQ) was the lowest concentration evaluated with coefficient of variation (RSD%) smaller than 5% and accuracy below 20% [19].

Statistical analysis

All analyses were performed using a software (Statistica for Windows, Stat Soft Inc.) and a 5% significance level. The quantification of nicotine and color change in shade guide units and in ΔE was submitted to a one-way ANOVA and Tukey's test for pairwise comparisons. In case the data did not show normal distribution, non-parametric statistics (Kruskall-Wallis and Dunn's test) were performed instead.

Results

Color change

The mean shade guide units obtained with both color guides are shown in Table 1. Non-parametric statistics revealed a significant difference among the groups (Table 1; $p < 0.001$) for both scales. Color at the baseline got darker after exposition to cigarette smoke. After dental prophylaxis, color was statistically similar to the baseline. The lightest color was obtained after dental bleaching (Table 1; $p < 0.001$).

The ΔE mean values can be seen in Table 2. One-way ANOVA revealed a significant difference among groups (Table 2; $p < 0.001$). After dental prophylaxis, we could observe a reduction of the ΔE values compared to the ΔE obtained at the baseline vs. after cigarette exposition. The difference was even more expressive and statistically different when we observe the baseline vs. after dental bleaching values (Table 2; $p < 0.001$).

Nicotine determination on teeth

Initially, chromatographic (temperature, injection amount and ion source energy) and extraction conditions ($NaOH/NH_4OH$, hexane/$CHCl_2$ and different times of sonic procedure) were determined to get the best compromise for nicotine determination. A liquid-liquid procedure using an alkaline solution (NH_4OH) and $CHCl_2$ (to improve the nicotine extraction from the matrix) was optimized and a 7.5 min

chromatographic run was developed and allowed to observe the nicotine peak at 3.8 min showing a fragment at m/z 162 and the peak of internal standard at 6.6 min (m/z 169) (Figure 4).

The chromatogram was well resolved and peaks from the substances were confirmed, injecting the standard of both solutions (nicotine and IS) in the same conditions. The nicotine quantification in teeth samples was obtained after method validation was conducted according to the Brazilian government validation rules [18, 19]. The linearity was confirmed with the adequate regression equation $y = 0.0024x + 0.0065$, and good correlation coefficient (r^2) of 0.9987. Table 3 presents the obtained values of precision and accuracy parameters.

The limits of detection (LOD = 0.3 ng/mL) and quantification (LOQ =1 ng/mL) of nicotine were adequate for the goal of this study, getting RSD% for the LOQ and for the intra and inter day study lower than 20%. This criterion is considered satisfactory considering that the analysis was executed in a complex matrix (teeth). The validation of the method was considered adequate for the quantitative purpose and the method was applied to analyze the teeth samples from the different groups.

The sample from cigarette non-exposed teeth samples did not reveal the presence of nicotine (data not shown), highlighting that there was no contamination in process. The means and standard deviations of nicotine in the different groups are shown in Table 4. One-way ANOVA revealed the significant differences among groups (Table 4; $p < 0.001$). For the positive control group one can observe the presence of nicotine in the dental structure of teeth after exposure to cigarette smoke in high amount. Dental prophylaxis was able to remove approximately 36% of the nicotine presented on the dental structure; but an expressive reduction (approximately 75%) could only be removed after dental bleaching.

Discussion

Several methods have been proposed to investigate the amount of nicotine within the dental structure. Deciduous teeth have been proposed as a matrix for measuring the cumulative exposure to environmental tobacco smoke during the whole childhood [20] and the gas chromatography–mass spectrometry (GC–MS) technique can be used for the determination of this biomarker in teeth [17].

Chromatographic methods to measure nicotine in different biological matrix are very useful and described in the literature [21-23]. However direct methods that allow the determination of this substance in these matrixes normally requires relatively expensive analytical instruments [23]. The chromatographic method developed in this study, using the GC-MS equipment, presented an excellent resolution for nicotine and IS without additional peaks in the short analysis time (7.5 min of run and initial conditions were restored in 10 min).

For this chromatographic analysis, only a two-step sample preparation method without sample dry step in the end of the extraction was developed. Marchei et al. (2008) [21] also published a method to measure nicotine in teeth using chromatography method (LC-MS) but for this goal these authors developed a very long and with several steps for sample pre-treatment. Additionally, Miyazawa et al. (2011) [22] developed a sensitive and useful method for the determination of nicotine using the liquid chromatography. The authors described that the method presented adequate resolution of nicotine and the sample preparation was very simple, achieving the purpose of the investigation.

Using the GC-MS technique, Shin et al. (2002) [24] proposed the determination of nicotine and cotinine in human urine, plasma, and saliva and Pascual et al. (2003) [17] the measurement of these substances in children teeth samples. These last authors described that a validated GC-MS was simple and reliable to determine the nicotine amount in the samples. In the present study we also reached the same conclusions using a chromatographic method to determine the amount of nicotine in different samples of teeth with the advantage to develop a fast and reliable pre-treatment method to extract the nicotine from the teeth samples.

It is known that cigarette smoke, coffee, and wine and as well as other colorful dyes are macromolecular compound chains that may adhere to the dental surfaces over time [25]. Nicotine is the most specific component of cigarettes and its deposition on dental surfaces as well as penetration in teeth might lead to dental yellowing [6]. Teeth exposed to coloring agents from diet indeed have greater potential to stain [26]. Similarly, smokers' teeth tend to develop tobacco stains over time [27], which may

vary from yellow to black stains, and the severity is highly dependent on the length and frequency of the smoking habit.

In the present study we could observe that teeth color got darker after exposition to cigarette smoke, which is consistent with the findings of some *in vitro* studies [7, 8]. By using the GC-MS methodology, we could observe the presence of nicotine in samples that were exposed to cigarette smoke (positive control).

However, after dental prophylaxis, we could observe a reduction mean SGU (teeth getting whiter) compared to that of teeth exposed to cigarette smoke. This was even more expressive in the bleaching groups. The first conclusion that we can reach from these results is that cigarette smoke really makes a deposit on the external surface of teeth, as dental prophylaxis, which can only clean teeth superficially, was capable to reduce nicotine.

Nevertheless, as this cleaning procedure was not capable to eliminate all nicotine presented in the teeth specimens, one can suggest that nicotine can penetrate the dental structure and become entrapped within enamel and/or dentin. Indeed, a significant amount of nicotine (around 64%) was still detected in teeth sample after dental prophylaxis.

Although dental prophylaxis could remove only 36% of the nicotine presented on the external dental surface of the teeth, this procedure was already enough to allow the teeth to recover their original color, measured with a shade guide in the shade guide units. This means that the nicotine presented in the internal structure of the teeth was not in amount enough to alter the color of the teeth. The cigarette cycle exposure employed in this study resembles a 1-year smoking habit, which is perhaps a short-term exposition to significantly alter the color of the dental structure by the penetration of the cigarette smoke. Perhaps, using the same methodology but using longer exposure times to cigarette smoke, one could detect significant color changes in teeth even after dental prophylaxis. More research on this topic should be conducted.

In a recent one-year clinical study of at-home bleaching in smokers [11], the authors observed that

color rebound was observed in smokers when color at one-year was measured before dental prophylaxis (without removal of cigarette smoke stains), but not after dental prophylaxis. In both situations, in the clinical study [11] and in this *in vitro* study, the deposition of dark hue cigarette smoke on dental surface was responsible for such yellowing.

When we observe the baseline *vs.* after dental bleaching values, one can see that dental bleaching with 35% hydrogen peroxide was effective to whiten teeth as already reported in several clinical studies [28-35] and also effective in removing nicotine significantly. In-office dental bleaching removed approximately 75% of the nicotine presented on and within the dental structure of the dental specimens. Therefore, dental bleaching may be considered an effective way to significantly reduce the amount of nicotine that becomes entrapped within the dental structure produced by smoking habits. Further *in vitro* and *in vivo* studies should be conducted to investigate the hypothesis herein raised.

Conclusions

The chromatographic method allowed the evaluation of nicotine in dental specimens after cigarette smoke exposure. Cigarette smoke deposit on the external dental surface and also on the internal dental structure. Dental prophylaxis and in-office bleaching with 35% hydrogen peroxide can reduce the amount of nicotine deposited on the external surface of teeth as well as the nicotine that penetrates it by cigarette smoke. However, in-office bleaching showed a more significant removal of nicotine.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee.

Informed consent

For this type of study, formal consent is not required.

References

1. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, Wollum A, Sanman E, Wulf S, Lopez AD, Murray CJ and Gakidou E (2014) Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. Jama 311:183-192. doi: 10.1001/jama.2013.284692
2. Wasilewski Mde S, Takahashi MK, Kirsten GA and de Souza EM (2010) Effect of cigarette smoke and whiskey on the color stability of dental composites. American journal of dentistry 23:4-8.
3. Benowitz NL and Jacob P, 3rd (1993) Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clinical pharmacology and therapeutics 53:316-323.
4. Robinson DE, Balter NJ and Schwartz SL (1992) A physiologically based pharmacokinetic model for nicotine and cotinine in man. Journal of pharmacokinetics and biopharmaceutics 20:591-609.
5. Takeuchi CY, Correa-Afonso AM, Pedrazzi H, Dinelli W and Palma-Dibb RG (2011) Deposition of lead and cadmium released by cigarette smoke in dental structures and resin composite. Microscopy research and technique 74:287-291. doi: 10.1002/jemt.20903
6. Kassis C ZT, Mehanna C. (2015) Extrinsic tooth discoloration, an updated review. . Dental Tribune 6B:5B-8B.
7. Bertoldo C, Miranda D, Souza-Júnior E, Aguiar F, DANLLima, Ferreira R, Claes I and Lovadino J (2011) Surface hardness and color change of dental enamel exposed to cigarette smoke. International Journal of Dental Clinics 3:1-4.
8. Bazzi JZ, Bindo MJ, Rached RN, Mazur RF, Vieira S and de Souza EM (2012) The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel. Journal of the American Dental Association (1939) 143:e1-7.
9. de Geus JL, Rezende M, Margraf LS, Bortoluzzi MC, Fernandez E, Loguercio AD, Reis A and Kossatz S (2015) Evaluation of genotoxicity and efficacy of at-home bleaching in smokers: a

single-blind controlled clinical trial. Operative dentistry 40:E47-55. doi: 10.2341/14-121-c
10. de Geus JL, Bersezio C, Urrutia J, Yamada T, Fernandez E, Loguercio AD, Reis A and Kossatz S (2015) Effectiveness of and tooth sensitivity with at-home bleaching in smokers: A multicenter clinical trial. Journal of the American Dental Association (1939) 146:233-240. doi: 10.1016/j.adaj.2014.12.014
11. de Geus JL, de Lara MB, Hanzen TA, Fernandez E, Loguercio AD, Kossatz S and Reis A (2015) One-year follow-up of at-home bleaching in smokers before and after dental prophylaxis. Journal of dentistry 43:1346-1351. doi: 10.1016/j.jdent.2015.08.009
12. Commission Internationale de l'Eclairage (1978). Recommendations on Uniform Color Spaces, Color Difference Equations, Psychometric Color Terms. Supplement 2 to CIE Publication 15 (E2-31.1)1971 (TC-1.3)1978. Paris, France: Bureau Central de la CIE.
13. Rezende M, Bonafe E, Vochikovski L, Farago PV, Loguercio AD, Reis A and Kossatz S (2016) Pre- and postoperative dexamethasone does not reduce bleaching-induced tooth sensitivity: A randomized, triple-masked clinical trial. Journal of the American Dental Association (1939) 147:41-49. doi: 10.1016/j.adaj.2015.07.003
14. Kwon SR, Wang J, Oyoyo U and Li Y (2013) Evaluation of bleaching efficacy and erosion potential of four different over-the-counter bleaching products. American journal of dentistry 26:356-360.
15. Ontiveros JC, Eldiwany MS and Paravina R (2012) Clinical effectiveness and sensitivity with overnight use of 22% carbamide peroxide gel. Journal of dentistry 40 Suppl 2:e17-24. doi: 10.1016/j.jdent.2012.08.009
16. Da Costa J, Lubisich E, Ferracane J and Hilton T (2011) Comparison of efficacy of an in-office whitening system used with and without a whitening priming agent. Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al] 23:97-104. doi: 10.1111/j.1708-8240.2010.00400.x
17. Pascual JA, Diaz D, Segura J, Garcia-Algar O, Vall O, Zuccaro P, Pacifici R and Pichini S (2003) A simple and reliable method for the determination of nicotine and cotinine in teeth by gas chromatography/mass spectrometry. Rapid communications in mass spectrometry : RCM 17:2853-2855. doi: 10.1002/rcm.1279
18. Brasil (2003) Agência Nacional de Vigilância Sanitária. Resolução nº 899/2003. Diário Oficial da União.
19. Brasil (2012) Agência Nacional de Vigilância Sanitária. Resolução nº 27/2012. Diário Oficial da União.
20. Garcia-Algar O, Vall O, Segura J, Pascual J, Diaz D, Mutnoz L, Zuccaro P, Pacifici R and Pichini S (2003) Nicotine Concentrations in Deciduous Teeth and Cumulative Exposure to Tobacco Smoke During Childhood. JAMA - Journal of the American Medical Association 290:196 - 197.
21. Marchei E, Joya X, Garcia-Algar O, Vall O, Pacifici R and Pichini S (2008) Ultrasensitive detection of nicotine and cotinine in teeth by high-performance liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry : RCM 22:2609-2612. doi: 10.1002/rcm.3636
22. Miyazawa M, Kawauchi Y, Okuno Y and Oda Y (2011) The novel assay method for nicotine metabolism to cotinine using high performance liquid chromatography. Chemical & pharmaceutical bulletin 59:295-297.
23. Piller M, Gilch G, Scherer G and Scherer M (2014) Simple, fast and sensitive LC-MS/MS analysis for the simultaneous quantification of nicotine and 10 of its major metabolites. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 951-952:7-15.

doi: 10.1016/j.jchromb.2014.01.025
24. Shin HS, Kim JG, Shin YJ and Jee SH (2002) Sensitive and simple method for the determination of nicotine and cotinine in human urine, plasma and saliva by gas chromatography-mass spectrometry. Journal of chromatography B, Analytical technologies in the biomedical and life sciences 769:177-183.
25. Nicholson JW (1996) Biologic considerations In: Schwartz RS, Summitt JB, Robbins JW, Santos J (eds) Fundamentals of Operative Dentistry: A Contemporary Approach Quintessence Publishing Co Inc. Chicago 3:1-25.
26. Téo TB, Takahashi MK, Gonzaga CC and Lopes MdGK (2010) Avaliação, após clareamento, da alteração de cor de dentes bovinos imersos em soluções com elevado potencial de pigmentação. Revista Sul-Brasileira de Odontologia 7:401-405.
27. Alkhatib MN, Holt RD and Bedi R (2005) Smoking and tooth discolouration: findings from a national cross-sectional study. BMC public health 5:27. doi: 10.1186/1471-2458-5-27
28. Bernardon JK, Sartori N, Ballarin A, Perdigao J, Lopes GC and Baratieri LN (2010) Clinical performance of vital bleaching techniques. Operative dentistry 35:3-10. doi: 10.2341/09-008cr
29. Basting RT, Amaral FLB, França FMG and Flório FM (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents. Operative dentistry 37:464-473. doi: 10.2341/11-337-C
30. Tay LY, Kose C, Herrera DR, Reis A and Loguercio AD (2012) Long-term efficacy of in-office and at-home bleaching: A 2-year double-blind randomized clinical trial. American journal of dentistry 25:199-204.
31. Zhao K, Zong L, Zhang Q and Att W (2013) Clinical comparison between two bleaching techniques: a 180-day follow-up study. Quintessence international (Berlin, Germany : 1985) 44:601-607. doi: 10.3290/j.qi.a29702
32. de Moraes IQ, Silva LN, Porto IC, de Lima Neto CF, Dos Santos NB and Fragoso LS (2015) Effect of in-office bleaching with 35% hydrogen peroxide with and without addition of calcium on the enamel surface. Microscopy research and technique 78:975-981. doi: 10.1002/jemt.22561
33. Lo Giudice R, Pantaleo G, Lizio A, Romeo U, Castiello G, Spagnuolo G and Giudice GL (2016) Clinical and Spectrophotometric Evaluation of LED and Laser Activated Teeth Bleaching. The open dentistry journal 10:242-250. doi: 10.2174/1874210601610010242
34. Mena-Serrano AP, Garcia E, Luque-Martinez I, Grande R, Loguercio AD and Reis A (2016) A Single-Blind Randomized Trial About the Effect of Hydrogen Peroxide Concentration on Light-Activated Bleaching. Operative dentistry. doi: 10.2341/15-077-c
35. Rezende M, Ferri L, Kossatz S, Loguercio AD and Reis A (2016) Combined Bleaching Technique Using Low and High Hydrogen Peroxide In-Office Bleaching Gel. Operative dentistry 41:388-396. doi: 10.2341/15-266-c

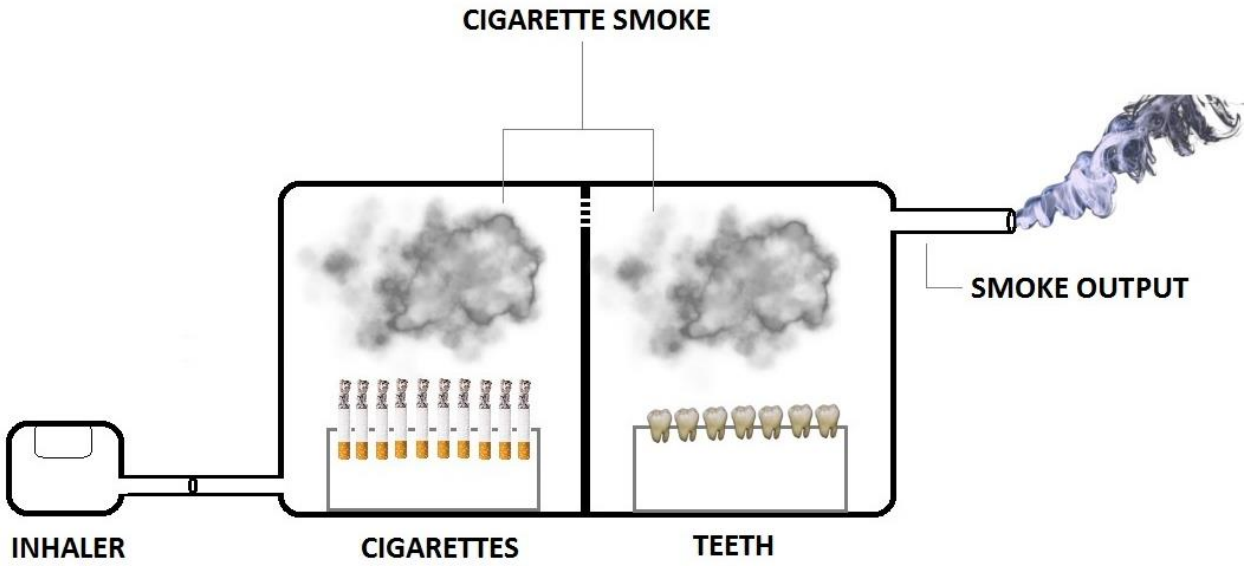

Figure 1. Home-made cigarette smoke machine.

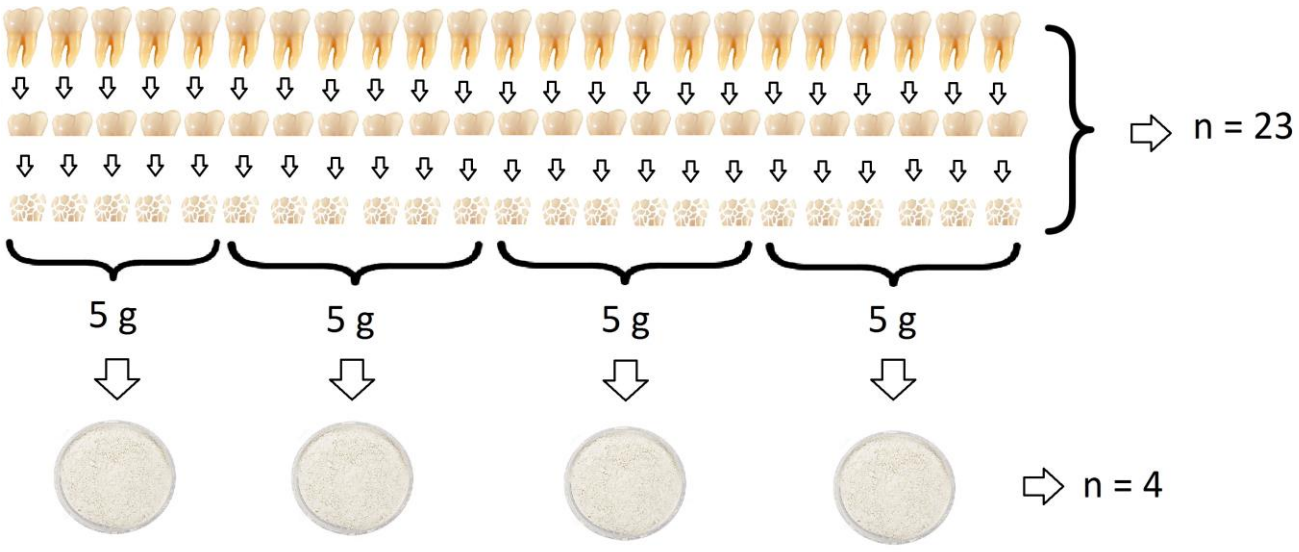

Figure 2 - Schematic drawing of the milling process of the teeth.

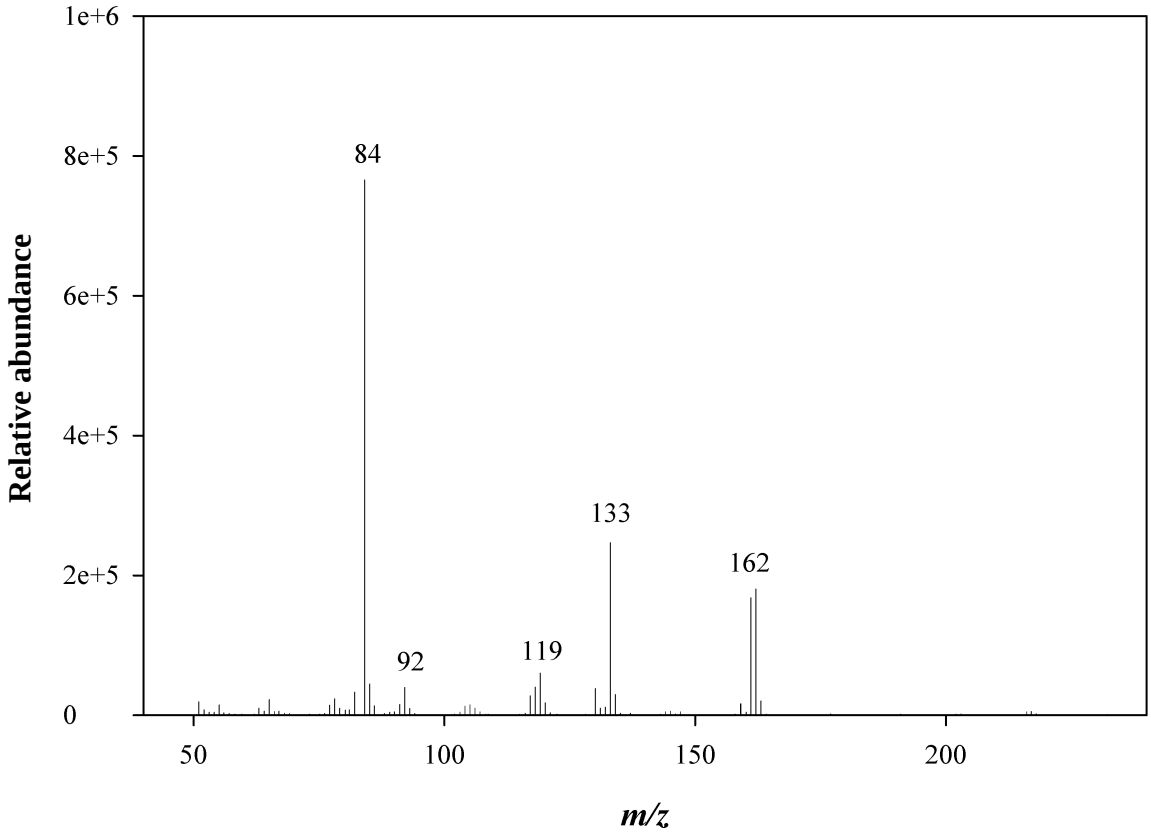

Figure 3. Mass spectrum and chemical ionization of nicotine.

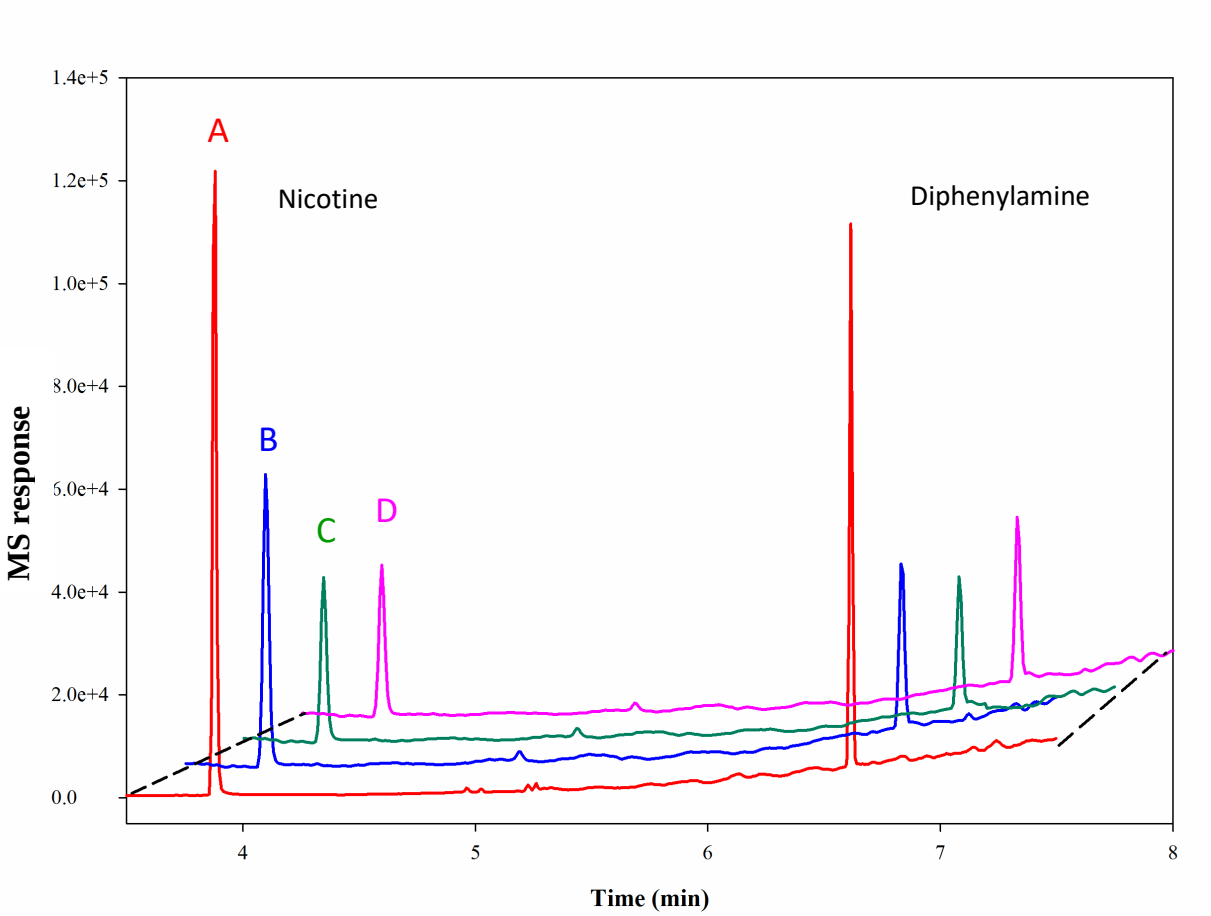

Figure 4. Chromatogram of samples. (A) Standard, (B) Positive group tooth sample group, (C) Prophylaxis tooth sample group, (D) Bleaching tooth sample group; Nicotine Rt = 3.8 min and diphenylamine Rt = 6.6 min.

Table 1 – Means and standard deviations (SD) of color in shade guide units (SGU) with Vita classical shade guide and Vita Bleachedguide 3D-MASTER for the different groups (*)

Groups	Means ± SD		Medians (interquartile range)	
	Vita Classical	Bleachguide	Vita Classical	Bleachguide
Baseline	5.9 ± 3.3[a]	7.5 ± 2.4 [A]	5 (3 – 9)	8 (5.5 – 10)
After cigarette smoke	9.1 ± 2.6[b]	9.1 ± 1.5 [B]	9 (8.5 – 11)	9 (8 – 10.5)
After dental prophylaxis	5.0 ± 3.2 [a]	6.4 ± 2.3 [A]	5 (2 – 8.5)	7 (4 – 9)
After dental bleaching	1.0 ± 0.2 [c]	2.5 ± 1.1 [C]	1 (1 – 1)	2 (2 – 3)

(*) Comparisons are valid within each column. Kruskall-Wallis test and Dunn's test ($p < 0.001$). Distinct letters indicate means that are statistically different.

Table 2 – Means and standard deviations of color change in shade guide units (ΔE) of the different groups (*)

Groups	ΔE
Baseline *vs.* after cigarette exposition	20.8 ± 6.4[A]
Baseline *vs*. after dental prophylaxis	17.5 ± 6.2[B]
Baseline *vs*. after dental bleaching	11.6 ± 5.2[C]

(*) One-way analysis of variance and Tukey's test ($p < 0.001$). Distinct letters indicate means that are statistically different.

Table 3. Precision (n=3; RDT%) and accuracy (n=9; %) evaluation for nicotine (*)

NICOTINE	*1st day*		*2nd day*		*3rd day*	
Parameters	*Precision*	*Accuracy*	*Precision*	*Accuracy*	*Precision*	*Accuracy*
80 ng/mL	4.1	101.2	1.1	93.9	3.2	83.6
30 ng/mL	10.0	93.2	6.3	91.1	1.0	100.4
15 ng/mL	3.5	102.8	3.7	109.3	5.0	118.5

(*) Analysis in triplicate

Table 4 – Means and standard deviations of nicotine in teeth (*)

Groups	Nicotine (µg/g of tooth)
After cigarette smoke exposure	3.3 ± 1.3[a]
After dental prophylaxis	2.1 ± 1.4[b]
After dental bleaching	0.8 ± 0.3[c]

(*) One-way analysis of variance and Tukey's test ($p < 0.001$). Distinct letters indicate means that are statistically different.

ARTIGO 4

TÍTULO: DOES SMOKING HABIT INCREASE THE MICRONUCLEI FREQUENCY IN THE ORAL MUCOSA OF ADULTS COMPARED TO NON-SMOKERS? A SYSTEMATIC REVIEW AND META-ANALYSIS

STATUS: SUBMETIDO

REVISTA: CLINICAL ORAL INVESTIGATIONS

Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review and meta-analysis

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Abstract

Objectives A systematic review of clinical studies to evaluate the micronuclei frequency in the oral mucosa of smokers and non-smokers in adult patients was performed.

Materials and Methods A comprehensive search was carried out on MEDLINE via PubMeb, Scopus, Web of Science, LILACS, BBO and Cochrane Library and SIGLE without restrictions. Dissertations and thesis were searched using the ProQuest Dissertations and Periodicos Capes Thesis Databases. We included only cross-sectional clinical trials that compared the frequency of micronuclei in the oral mucosa of smokers and non-smokers in adult patients.

Data After title and abstract screening, 35 studies remained. Eighteen studies were further excluded, whereas 17 studies remained for qualitative analysis and 16 for the meta-analysis of the primary and secondary outcomes. The standardized mean difference of the frequency of micronuclei between groups was 1.64, with a 95% confidence interval of 1.09 to 2.20 ($p < 0.001$). Based on these studies, a significant statistical difference between the groups could be identified. Data were heterogeneous (chi-square test, $p < 0.00001$; $I^2 = 92\%$), which means that all studies included in the analysis did not share a common effect size.

Conclusions Despite the high variation in the methodology of the assessed studies, this study showed a higher frequency of micronuclei in exfoliated cells of smokers compared to non-smokers.

Clinical significance The use of tobacco is associated with cytotoxic and genotoxic effects because a higher frequency of micronuclei in exfoliated cells of smokers was observed.

Key words Systematic review • Meta-analysis • Micronucleus tests • Tobacco use

Introduction

In 2011 there were around 1.2 billion smokers in the world [1]. The number of smokers has increased steadily worldwide, and there are preliminary indications that global prevalence among men increased in recent years [2]. Cigarette smoke contains over 4000 chemical compounds. More than 60 of these have been identified as cancer causing [3]. The smoking habit causes more than 1 million cancer deaths per year [1].

DNA damage in cells from the oral mucosa of smokers usually sign the genotoxicity potential of the smoking habit [4, 5]. This can be indirectly observed by the increase in the frequency of micronuclei in exfoliated epithelial cells (MN). During the division of the cells from the basal layer of the mucosa, the damage to the DNA molecule leads to the formation of micronuclei. This generally occurs days or weeks after the contact with the carcinogenic agent [6, 7]. Chromosome fragments or whole chromosomes that are not included in the main cores during nuclear division produce MNs and they reflect chromosomal damage providing an early marker of carcinogenesis [8]. The evaluation of the frequency of MN is a viable method for the detection of the risk of cancer in humans, because the majority of tumors possess epithelial origin [8]. An increased frequency of MN in exfoliated cells of the oral mucosa has traditionally served as an index to assess the genotoxicity of exposure to various carcinogens [9, 10].

This explains why the majority of recent studies have shown that the frequency of micronuclei is significantly higher in smokers than in non-smokers [5, 11-13]. However, the literature in this issue is not consensual, as there are some studies that did not found difference in micronuclei frequency of smokers and non-smokers [14, 15]. In face of the controversial results, the aim of this systematic review of the literature was to answer the following focused question: Does smoking habit increase the micronuclei frequency in the oral mucosa of adults compared to non-smokers?

Materials and methods

Protocol and registration

This study protocol were registered at the PROSPERO database (CRD42015017053), and we followed the recommendations of the PRISMA statement for the report of this systematic review [16].

Information sources and search strategy

The controlled vocabulary (mesh terms) and free keyword in the search strategy was defined based on the following PECO question:

1. Population (P): adults.
2. Exposure (E): smoking habit.
3. Comparison (C): non-smokers.
4. The outcome (O): frequency of micronuclei.
5. Study design (S): cross-sectional studies.

To identify trials to be included for this review, we searched on the electronic databases MEDLINE via PubMeb, Scopus, Web of Science, Latin American and Caribbean Health Sciences Literature database (LILACS), Brazilian Library in Dentistry (BBO) and Cochrane Library. No restrictions to publication date or languages were performed.

Other sources were also used to identify more articles. We explored the grey literature using the database System for Information on Grey literature in Europe (SIGLE), and dissertations and thesis using the ProQuest Dissertations and Thesis Fulltext database, as well as the Periodicos CAPES Thesis database.

To locate unpublished and ongoing trials related to the review question, we searched the following clinical trials registry: Current Controlled Trials (www.controlled-trials.com), International Clinical trials registry platform (http://apps.who.int/trialsearch/), the ClinicalTrials.gov (www.clinicaltrials.gov), Rebec (www.rebec.gov.br), and EU Clinical Trials Register (https://www.clinicaltrialsregister.eu).

Eligibility criteria

We included cross-sectional clinical trials that compared the frequency of micronuclei in smokers and non-smokers in adult patients of any age group. The frequency of micronuclei was the primary outcome of the study. We included only clinical studies in humans.

Non-controlled clinical trials, editorial letters, pilot studies, historical reviews, *in vitro* studies, descriptive studies, such as case reports and case series were excluded. Additionally, clinical studies were excluded if 1) studies compared frequency of micronuclei in smokers and passive smokers; 2) studies evaluated DNA damage in patients who have smokeless tobacco habits; 3) studies that used "Papanicolau" technique as staining method as this method is not specific for DNA.

Study selection and data collection process

Initially, the articles were selected by title and abstracts. Articles that appear in more than one database were considered only once. Full-text articles were obtained when the title and abstract had insufficient information to make a clear decision. Subsequently, two reviewers classified those that met the inclusion criteria. Details about the study, methods, participants and outcomes were extracted using customized extraction forms.

Risk of bias in individual studies

The internal quality of included studies was assessed using the EPHPP Modified scale (Effective Public Health Practice Project) [17] by two independent reviewers. This quality assessment tool evaluates the design and quality of randomized clinical trials and observational studies and also facilitates incorporation of quality assessments in the interpretation of the meta-analysis results, albeit not used as criteria for inclusion or exclusion of articles.

The quality assessment instrument used contains the following components: 1) selection bias, 2) study design, 3) identification and treatment of confounders, 4) blinding of outcome assessors and of participants, 5) reliability and validity of data collection methods, and 6) withdraws and dropouts. The components are rated strong, moderate, or weak according to a standardized dictionary (http://www.ephpp.ca/PDF/QADictionary_dec 2009.pdf) [17].

The overall rating for the study is determined by assessing the six component ratings. In the original instrument, [17] those with no weak ratings and at least four strong ratings should be considered strong. Those with less than four strong ratings and one weak rating are considered moderate. Finally, those with two or more weak ratings are considered weak. Strong and moderate studies were included in the review [17].

As on our review we only included cross-sectional studies, the item study design was not computed for the overall rating of the study. As blinding of participants was never possible and was not likely to affect the outcomes, only evaluator blinding was rated.

We listed three important confounders that should have been taken into consideration in the study: alcoholism habit, time of smoking and number of cigarettes a day. If the article

controlled two or three of these items, this study item was considered strong, if the study controlled only one of these items, this study item was considered moderate, and if the article did not control any of these confounders, this study item was considered weak.

During data selection and quality assessment, any disagreements between the reviewers were solved through discussion, and if needed, by consulting a third reviewer.

Summary measures and synthesis of the results

When authors evaluated the micronuclei frequency in different regions in the oral cavity or when more than one group of smokers was added, we combined these data to make a single entry in the meta-analysis.

All analyses were conducted using RevMan 5.3 (Review Manager, The Cochrane Collaboration, Copenhagen, Denmark). Data from eligible studies were continuous (frequency of micronuclei). The random-effects models were employed for the continuous data.

Results

Study selection

After database screening and removal of duplicates, 1338 studies were identified (Figure 1). After title and abstract screening, 35 studies remained and this number was reduced to 17 after careful examination of the abstracts.

Characteristics of included articles

The characteristics of the 17 studies selected are listed in Table 2. The mean of cigarettes a day smoked by the participants in the trials varied from 4 to 34 [11, 14, 15,18-24].

Five out of the 17 studies did not report this data [6, 13,25-27]. Two studies reported the mean of packs a year [28, 29].

The mean time of smoking ranged from 0.5 to 18 years [11, 13-15, 18-20,23-28]. Four out of the 17 studies did not report this information [6, 21, 22,29].

The smallest sample size per group was 10 [14] and the highest was 83 in the smokers group [11] . The mean age of participants ranged from 25 to 51 [11, 13-15, 19-24, 26, 27], and five out of 17 studies did not report this information [6, 18, 25, 28, 29]. Most of participants of the studies were male [11, 13-15, 22, 24, 26, 28], with exception of four studies [19-21, 29]. Five out of 17 studies did not report the sex of participants [6, 18, 23, 25,27].

All studies (n = 17) smeared the mucosa of the cheek [6, 11, 13-15,18-29]. Some of these studies also used the tongue for collecting exfoliated cells [15, 23, 24], the lower lip [23, 26] and the palate [23, 24].

The number of cells counting per participant ranged from 100 to 2000. Six out from 17 studies performed 1000 cells counting per participant [18, 19, 21, 27-29], four studies performed 2000 cells counting per participant [11, 15, 20, 25] and the remaining seven studies performed different cells counting per participant [6, 13, 14, 22-24, 26].

Ten out of 17 studies just evaluated micronucleated cells [6, 13, 21, 23-29]. Three studies evaluated binucleated cells [11, 14, 20], four evaluated karyolysis, karyorrhexis and pycnosis [11, 14, 15, 20]. One analyzed apoptosis [19], nuclear bud [20], aberrant cells [18] and spindle disturbances [22].

According to the staining method used for coloring the cytologic smears, the Feulgen stain was the most used method, applied in twelve out of the 17 studies [6, 11, 13-15, 18, 20-

23, 26, 29], followed by Giemsa stain, used in five studies [14, 19, 25, 27, 28]. Two studies used Acridine orange [24], one study used papanicolau [28] and another one used various staining techniques [14].

Assessment of the risk of bias

The quality assessment of selected studies is presented in Table 3. In summary, from the 17 studies, thirteen were considered moderate [11, 14, 15, 18, 20-28] and four were considered strong [6, 13, 19, 29], according to the quality assessment components of the Effective Public Health Practice Project (EPHPP). Therefore all the studies met the best requirement features for inclusion in the meta-analysis of the frequency of micronuclei.

Meta-analysis

All meta-analysis was performed on studies classified as strong and moderate on the final rating of quality assessment components.

Frequency of micronuclei

This analysis was based on 16 studies [6, 11, 13-15, 18-28]. One study [29] was not included in this meta-analysis as it did not provide means and standard deviations for this outcome. This study showed the distribution of 0-5 positive wells for micronuclei separately among smokers and non-smokers, therefore, we could not extract the mean and standard deviation data.

The standardized mean difference of the frequency of micronuclei between groups was 1.64, with a 95% confidence interval of 1.09 to 2.20 ($p < 0.001$). Based on these studies, a significant statistical difference between the groups could be identified (Figure 2). Data were heterogeneous (chi-square test, $p < 0.00001$; $I^2 = 92\%$; Figure 2), which means that all studies included in the analysis did not share a common effect size.

Frequency of other DNA damage

The other genotoxic changes were binucleated cells, karyolysis, karyorrhexis and pyknosis. These genotoxic changes were evaluated by three studies [11, 15, 20] and the effect estimate and heterogeneity data produced after running the meta-analyses can be seen in Table 4. In all cases, significant differences were observed between groups, with more significant DNA damage in the smokers group. Heterogeneity was also significant in almost all cases, except for binucleated cells and karyorrhexis.

Sensitivity analysis

In order to identify some predictor factors that could be responsible for the high heterogeneity observed in the frequency of micronuclei we evaluated the impact of the staining method (Feulgen or Giemsa), the local of smear (cheek or tongue), the quality of studies (strong or moderate), the mean number of cigarettes a day (less or more than 15), the smoking time (less or more than 10 years), and the type of smoking (cigarette or beedi) on the effect estimate as well as in the heterogeneity. In none of the cases, we observed significant reduction of the heterogeneity, which remained significant in all situations (data not shown).

Discussion

The study of DNA damage in exfoliated cells collected from the oral cavity holds great promise as a minimally invasive method for monitoring exposure to genotoxic agents [30]. Oral exfoliative cytology has been largely used for screening of nuclear abnormalities, such as micronucleus formation, karyolysis, karyorrhexis, pyknosis, binucleated cells, broken egg nucleus, anucleation, and so on [30]. Its faithfulness to detect malignant changes are around 95%, which gives sufficient credibility to use it as a screening test in populations at high risk of oral cancer development [31].

An increased frequency of chromosome breaks has been recently demonstrated to be an initial event in carcinogenesis, suggesting that these alterations may play a significant role

in assessing oncogenic risk [32, 33]. Among biomarkers that can be used for this purpose, the measurement of MN appears to be one of the most suitable. An increased frequency of MN in exfoliated cells from oral mucosa has served traditionally as an index for evaluating the genotoxicity of exposure to various carcinogens [9, 10]. MN originates from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division. They reflect chromosome damage and may thus provide a marker of early-stage carcinogenesis.

Several studies have been conducted to determine the association between tobacco smoking and induction of micronuclei in oral cells [11-14, 18-20, 34, 35]. The carcinogenic effect of smoking habit is determined largely by the mutagenicity of compounds present in cigarette smoke. Cigarettes contain a mixture of several carcinogens including polycyclic aromatic hydrocarbons (PAHs), specific N-nitrosamines, aromatic amines, aldehydes and other carcinogens [36].

The results of this study point out for the fact that greater genotoxicity in cells of oral cavity occurs in the smokers comparing to non-smokers. Taking into consideration the mutagenic effects of tobacco-specific nitrosamines [37, 38], one can consider that these compounds may product an important function in the induction of MN in oral mucosa cells of smokers. Tobacco has many genotoxic substances, so cytogenetic damage can be considered a good biological marker for assessing the effect of exposure to tobacco [37].

The literature shows a variety of staining methods used in micronucleus assessment studies. Most studies used specific-DNA stains, such as Feulgen staining [6, 11, 13-15, 18, 20-23, 26, 29]. However, we still observed a high fraction of the studies, around 30%, that used non-specific stains, such as Giemsa [14, 19, 25, 27, 28], reason why we included the two staining methods in this study. However, it has been shown that the results of the

micronucleus test in exfoliated oral mucosa cells of tobacco users and non-users of tobacco depended strongly on the staining method [28].

Another common variation in the micronucleus evaluation method is the site of smear. Most studies collect the smear from the cheek [6, 11, 13-15, 18-25, 27-29], however collection from other sites, such as the tongue [15, 23, 24], palate [23, 24] and lower lip [23, 26], have also been performed. Buccal cells have been shown to have limited DNA repair capacity concerning to peripheral blood lymphocytes, and therefore may more precisely reflect genomic instability events in epithelial tissues [39].

This highlights that differences may occur depending on the site where the smear was collected for micronuclei frequency evaluation. Despite claiming that the distribution patterns of micronucleated cells from different oral sites could be constructed and compared with distribution patterns of micronucleated cells in patients with oral cancer [24], it has been shown that high frequencies of micronucleated cells occur in places where the tobacco comes in close contact with the oral mucosa, and precancerous lesions and carcinomas develop [23].

As already reported in the above two paragraphs, these two factors (type of staining method and local of smear collection) can affect the frequency of micronuclei evaluation. However, on a sensitivity analysis, we could not reduce the heterogeneity of the meta-analyses by controlling these factors. This means that other factors may play an important role on the high heterogeneity of the results. Perhaps population-related factors or even a better standardization of technique could reduce this heterogeneity. Large, multicentre, longitudinal studies involving a standard method are needed to confirm the findings of the present study.

Conclusions

Despite the high heterogeneity in the methodology of the assessed studies, this meta-analysis showed a higher frequency of micronuclei in exfoliated cells of the oral cavity of smokers compared to non-smokers.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

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Ethical approval

Since the study was a systematic review with meta-analysis, no ethical approval was required.

Informed consent

For this type of study, formal consent is not required.

References

1. Devita VT Jr, Lawrence TS, & Rosberg AS (2011) Cancer Principles & Practice of Oncology: Tobacco 9th ed Lippincott Williams & Wilkins, Philadelphia 150-156.
2. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, Wollum A, Sanman E, Wulf S, Lopez AD, Murray CJ and Gakidou E (2014) Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. Jama 311:183-192. doi:10.1001/jama.2013.284692.
3. International Agency for Research on Cancer (1986) Cumulative Index to IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. IARC Monogr Eval Carcinog Risk Chem Hum 39:379-403.
4. Belien JA, Copper MP, Braakhuis BJ, Snow GB and Baak JP (1995) Standardization of counting micronuclei: definition of a protocol to measure genotoxic damage in human exfoliated cells. Carcinogenesis 16:2395-2400.
5. Sivasankari NP KS, Reddy KS, Rao KR, Kumar SJM (2012) Micronucleus assay - screening tool in the diagnosis of oral carcinoma in tobacco users. International Journal of Pharma and Bio Sciences 3:646-651.
6. Stich HF and Rosin MP (1983) Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. Int J Cancer 31:305-308.
7. Stich HF, Rosin MP and Vallejera MO (1984) Reduction with vitamin A and beta-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betal nut and tobacco chewers. Lancet 1:1204-1206.
8. Cairns J (1975) Mutation selection and the natural history of cancer. Nature 255:197-200.

9. Bhattathiri VN, Bindu L, Remani P, Chandralekha B, Davis CA and Nair MK (1996) Serial cytological assay of micronucleus induction: a new tool to predict human cancer radiosensitivity. Radiother Oncol 41:139-142.
10. Basu A, Ghosh P, Das JK, Banerjee A, Ray K and Giri AK (2004) Micronuclei as biomarkers of carcinogen exposure in populations exposed to arsenic through drinking water in West Bengal, India: a comparative study in three cell types. Cancer Epidemiol Biomarkers Prev 13:820-827.
11. Nersesyan A, Muradyan R, Kundi M and Knasmueller S (2011) Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. Mutagenesis 26:295-301. doi: 10.1093/mutage/geq092
12. Bansal H, Sandhu VS, Bhandari R and Sharma D (2012) Evaluation of micronuclei in tobacco users: A study in Punjabi population. Contemp Clin Dent 3:184-187. doi: 10.4103/0976-237x.96825
13. Naderi NJ, Farhadi S and Sarshar S (2012) Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian J Pathol Microbiol 55:433-438. doi: 10.4103/0377-4929.107774
14. Nersesyan A, Kundi M, Atefie K, Schulte-Hermann R and Knasmuller S (2006) Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiol Biomarkers Prev 15:1835-1840. doi: 10.1158/1055-9965.epi-06-0248
15. Angelieri F, Goncalves Moleirinho TdC, Carlin V, Fujiyama Oshima CT and Ribeiro DA (2010) Biomonitoring of oral epithelial cells in smokers and non-smokers submitted to panoramic X-ray: comparison between buccal mucosa and lateral border of the tongue. Clin Oral Investig 14:669-674. doi: 10.1007/s00784-009-0345-6
16. Moher D, Liberati A, Tetzlaff J and Altman DG PRISMA group (2010) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg 8:336-41. doi: 10.1016/j.ijsu.2010.02.007
17. Thomas BH, Ciliska D, Dobbins M and Micucci S (2004) A process for systematically reviewing the literature: providing the research evidence for public health nursing interventions. Worldviews Evid Based Nurs 1:176-184. doi: 10.1111/j.1524-475X.2004.04006.x
18. Chandirasekar R, Kumar BL, Sasikala K, Jayakumar R, Suresh K, Venkatesan R, Jacob R, Krishnapriya EK, Kavitha H and Ganesh GK (2014) Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. Mutat Res Genet Toxicol Environ Mutagen 767:21-27. doi: 10.1016/j.mrgentox.2014.04.007
19. Haveric A, Haveric S and Ibrulj S (2010) Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers. Toxicol Mech Methods 20:260-266. doi: 10.3109/15376516.2010.482962
20. Kausar A, Giri S, Mazumdar M, Giri A, Roy P and Dhar P (2009) Micronucleus and other nuclear abnormalities among betel quid chewers with or without sadagura, a unique smokeless tobacco preparation, in a population from North-East India. Mutat Res Genet Toxicol Environ Mutagen 677:72-75. doi: 10.1016/j.mrgentox.2009.05.007
21. Konopacka M (2003) Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells. Neoplasma 50:380-382.
22. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R and Levis AG (1987) The micronucleus assay in exfoliated cells of the human buccal mucosa. Mutagenesis 2:11-17.
23. Stich HF, Parida BB and Brunnemann KD (1992) Localized formation of micronuclei in the oral-mucosa and tobacco-specific nitrosamines in the saliva of reverse smokers, khaini-tobacco chewers and gudakhu users. Int J Cancer 50:172-176. doi: 10.1002/ijc.2910500203

24. Suhas S GK, Gayatridevi,Ramesh C. (2004) Application of the micronucleus test to exfoliated epithelial cells from the oral cavity of beedi smokers, a high-risk group for oral cancer. Mutat Res Genet Toxicol Environ Mutagen 561:15-21. doi: 10.1016/j.mrgentox.2004.03.001
25. Mr P, Guruprasad Y, Jose M, Saxena K, K D and Prabhu V (2014) Comparative Study of Genotoxicity in Different Tobacco Related Habits using Micronucleus Assay in Exfoliated Buccal Epithelial Cells. J J Clin Diagn Res 8:ZC21-ZC24. doi: 10.7860/jcdr/2014/8733.4357
26. Ozkul Y, Donmez H, Erenmemisoglu A, Demirtas H and Imamoglu N (1997) Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis 12:285-287.
27. Sellappa S, Balakrishnan M, Raman S and Palanisamy S (2009) Induction of micronuclei in buccal mucosa on chewing a mixture of betel leaf, areca nut and tobacco. J Oral Sci 51:289-292.
28. Palaskar S and Jindal C (2010) Evaluation of micronuclei using Papanicolaou and may Grunwald Giemsa stain in individuals with different tobacco habits -A comparative study. JCDR 4:3607-3613.
29. Piyathilake CJ, Macaluso M, Hine RJ, Vinter DW, Richards EW and Krumdieck CL (1995) Cigarette-smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial-cells of the buccal mucosal. Cancer Epidemiol Biomarkers Prev 4:751-758.
30. Harshvardhan S. Jois ADK, Mohan Kumar K.P. (2010) Micronucleus as Potential Biomarker of Oral Carcinogenesis. IJDA 2:197-202.
31. Chaudhry AP, Schmutz JA, Jr. and Hanks CT (1967) Comparison of cytologic and histologic findings in induced carcinoma. J Dent Res 46:253-256.
32. Hagmar L, Bonassi S, Stromberg U, Brogger A, Knudsen LE, Norppa H and Reuterwall C (1998) Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res 58:4117-4121.
33. Bonassi S, Hagmar L, Stromberg U, Montagud AH, Tinnerberg H, Forni A, Heikkila P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE and Norppa H (2000) Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res 60:1619-1625.
34. Braga FL, Meneguzzi RD, Paiva RL and Rados PV Avaliação citopatólogica da mucosa bucal de fumantes e não-fumantes Rev odonto ciênc 19:157-163.
35. Motgi AA, Chavan MS, Diwan NN, Chowdhery A, Channe PP and Shete MV (2014) Assessment of cytogenic damage in the form of micronuclei in oral epithelial cells in patients using smokeless and smoked form of tobacco and non-tobacco users and its relevance for oral cancer. J Cancer Res Ther 10:165-170. doi: 10.4103/0973-1482.131454
36. Hoffmann D, Hoffmann I and El-Bayoumy K (2001) The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. Chem Research Toxicol 14:767-790.
37. International Agency for Research on Cancer (1985) Tobacco habits other than smoking; betel-quid and areca-nut chewing; and some related nitrosamines. IARC Working Group. Lyon, 23-30 October 1984. IARC Monogr Eval Carcinog Risk Chem Hum 37:1-268.
38. International Agency for Research on Cancer (2007) Smokeless tobacco and some tobacco-specific N-nitrosamines. IARC Monogr Eval Carcinog Risks Hum 89:1-592.
39. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S and Fenech M (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: the HUMN project perspective on current status and knowledge gaps. Mutat Res 659:93-108. doi: 10.1016/j.mrrev.2008.03.007

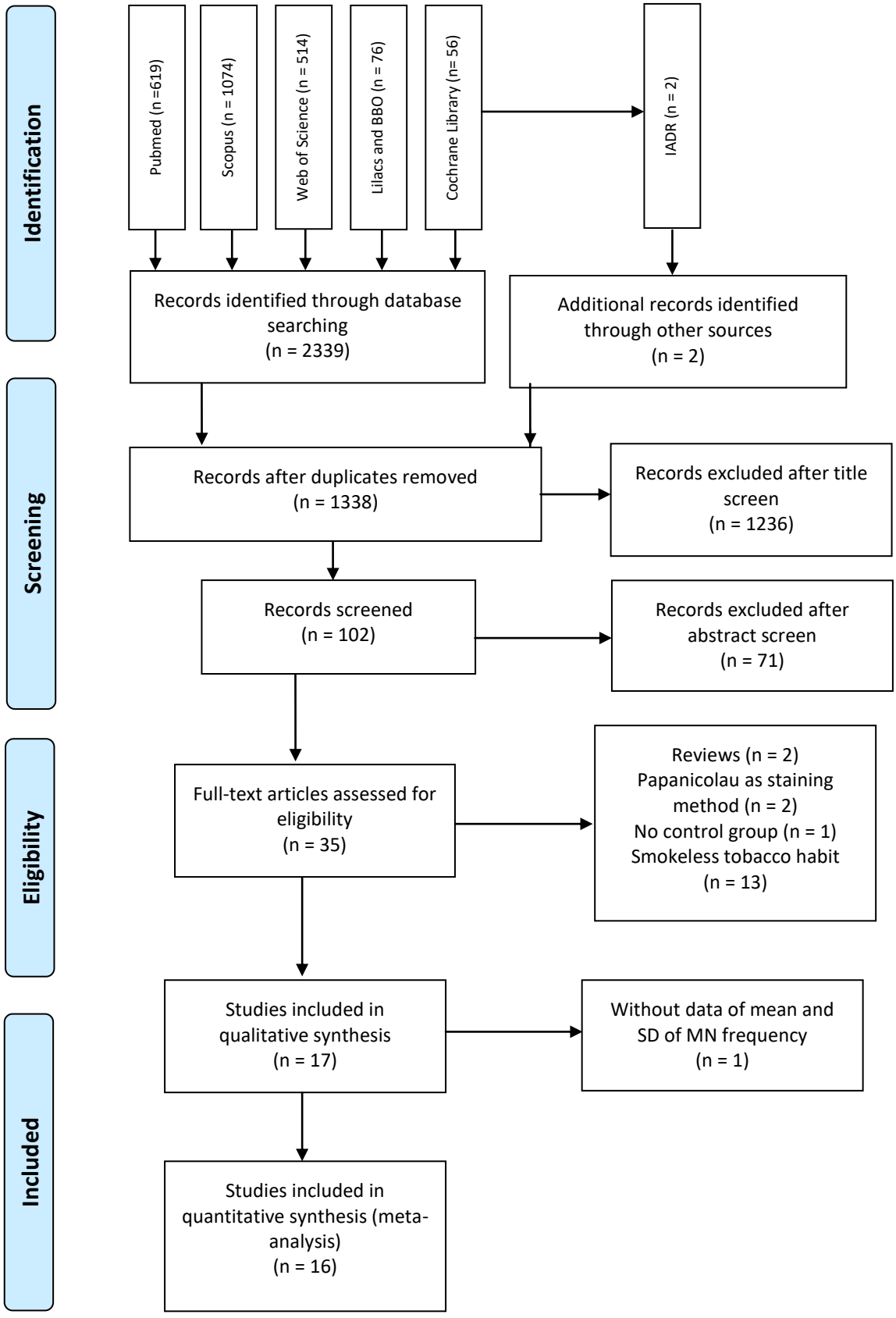

Fig. 1 Flow diagram of literature search and selection criteria

Table 1 Electronic database and search strategy.

Pubmed (23/December/2014)		
1#((((((((((((((((((((((((Cheek[MeSH Terms]) OR Mouth mucosa[MeSH Terms]) OR Gingiva[MeSH Terms]) OR "jugal mucosa"[Title/Abstract]) OR "oral mucosa"[Title/Abstract]) OR "buccal mucosa"[Title/Abstract]) OR "buccal mucosa cell"[Title/Abstract]) OR "exfoliative cytology"[Title/Abstract]) OR "buccal cells"[Title/Abstract]) OR "exfoliated cells"[Title/Abstract]) OR "exfoliated oral cells"[Title/Abstract]) OR "exfoliated oral mucosa cell"[Title/Abstract]) OR "exfoliated cell"[Title/Abstract]) OR "exfoliated oral cell"[Title/Abstract]) OR "buccal mucosa cells"[Title/Abstract]) OR "buccal mucosal cell"[Title/Abstract]) OR "buccal cell"[Title/Abstract]) OR "buccal mucosal cells"[Title/Abstract]) OR "ephitelial cell"[Title/Abstract]) OR "gingival tissue"[Title/Abstract]) OR gum[Title/Abstract]) OR gums[Title/Abstract]))))	**#2** ((((((((((((((((((((Smoke[MeSH Terms]) OR Tobacco Products[MeSH Terms]) OR Smoking[MeSH Terms]) OR Tobacco[MeSH Terms]) OR cigarette[Title/Abstract]) OR cigarettes[Title/Abstract]) OR "cigarette smoke"[Title/Abstract]) OR "tobacco addictive"[Title/Abstract]) OR "cigarette smoking"[Title/Abstract]) OR "smokeless tobacco users"[Title/Abstract]) OR "smokeless tobacco user"[Title/Abstract]) OR "tobacco harm"[Title/Abstract]) OR cigar[Title/Abstract]) OR cigars[Title/Abstract]) OR smoke[Title/Abstract]) OR smoker[Title/Abstract]) OR smokers[Title/Abstract]) OR nicotine[Title/Abstract]) OR tar[Title/Abstract]))	**#3**(((((((Healthy Volunteers[MeSH Terms]) OR Tobacco, Smokeless[MeSH Terms]) OR "non smokers"[Title/Abstract]) OR "non smoker"[Title/Abstract]) OR smokeless[Title/Abstract]) OR "no smoker"[Title/Abstract]) OR "no smokers"[Title/Abstract]))
#1 AND #2 AND #3		
Scopus (23/December/2014)		
#1(TITLE-ABS-KEY (cheek) OR TITLE-ABS-KEY ("mouth mucosa") OR TITLE-ABS-KEY ("jugal mucosa") OR TITLE-ABS-KEY (gingiva) OR TITLE-ABS-KEY ("oral mucosa") OR TITLE-ABS-KEY ("buccal mucosa") OR TITLE-ABS-KEY ("buccal mucosa cell") OR TITLE-ABS-KEY ("exfoliativecitology") OR TITLE-ABS-KEY ("buccal cell") OR TITLE-ABS-KEY ("exfoliated cell") OR TITLE-ABS-KEY ("exfoliated oral cell") OR TITLE-ABS-KEY ("exfoliated oral mucosa cell") OR TITLE-ABS-KEY ("buccal mucosa cell") OR TITLE-ABS-KEY ("ephitelial cell") OR TITLE-ABS-KEY ("gingival tissue") OR TITLE-ABS-KEY (gum))	**#2**(TITLE-ABS-KEY (smoke) OR TITLE-ABS-KEY ("tobacco products") OR TITLE-ABS-KEY (smoking) OR TITLE-ABS-KEY (tobacco) OR TITLE-ABS-KEY (cigarette) OR TITLE-ABS-KEY ("cigarette smoke") OR TITLE-ABS-KEY ("tobacco addictive") OR TITLE-ABS-KEY ("cigarette smoking") OR TITLE-ABS-KEY ("smokeless tobacco user") OR TITLE-ABS-KEY ("tobacco harm") OR TITLE-ABS-KEY (cigar) OR TITLE-ABS-KEY (smoker) OR TITLE-ABS-KEY (nicotine) OR TITLE-ABS-KEY (tar))	**#3** (TITLE-ABS-KEY ("healthy volunteer") OR TITLE-ABS-KEY ("tobacco smokeless") OR TITLE-ABS-KEY ("non smoker") OR TITLE-ABS-KEY ("no smoker") OR TITLE-ABS-KEY (smokeless))
#1 AND #2 AND #3		
Web of Science (27/December/2014)		
#1 Topic: (cheek) OR Topic: ("mouth mucosa") OR Topic: (gingiva) OR Topic: ("oral mucosa") OR Topic: ("buccal mucosa") OR Topic: ("buccal mucosa cell") OR Topic: ("exfoliativecitology") OR Topic: ("buccal cell*") OR Topic: ("exfoliated cell*") OR Topic: ("exfoliated oral cell*") OR Topic: ("exfoliated oral mucosa cell*") OR Topic: ("buccal mucosa cell*") OR Topic: ("buccal mucosal cell*") OR Topic: ("ephitelial cell") OR Topic: ("gingival tissue") OR Topic: (gum*)	**#2** Topic: (smoke) OR Topic: ("tobacco products") OR Topic: (smoking) OR Topic: (tobacco) OR Topic: (cigarette*) OR Topic: ("cigarette smok*") OR Topic: ("tobacco addictive") OR Topic: ("smokeless tobacco user*") OR Topic: ("tobacco harm") OR Topic: (smoker*) OR Topic: (cigar*) OR Topic: (nicotine) OR Topic: (tar)	**3#**Topic: ("healthy volunteers") OR Topic: ("no* smoker*") OR Topic: (smokeless)
#1 AND #2 AND #3		
Lilacs and BBO (27/December/2014)		
#1(MH: cheeck OR "mouth mucosa" OR gingiva OR gengiva OR goma OR "jugal mucosa" OR	**#2**(MH: smoke OR MH: "tobacco products" OR MH: smoking OR MH:	**#3**(MH: "healthy volunteers" OR

"mucosa jugal" OR "oral mucosa" OR "mucosa oral" OR "buccal mucosa" OR "mucosa bucal" OR "buccal mucosa cell" OR "células da mucosa bucal" OR "exfoliative citology" OR "citologia esfoliativa" OR "buccal cell" OR "célulabucal" OR "buccal cells" OR "células bucais" OR "exfoliated cell" OR "célula exfoliada" OR "exfoliated cells" OR "célulasexfoliadas" OR "exfoliated oral cell" OR "exfoliated oral cells" OR "células orais exfoliadas" OR "exfoliated oral mucosa cell" OR "exfoliated oral mucosa cells" OR "células exfoliadas da mucosa oral" OR "buccal mucosa cell" OR "buccal mucosa cells" OR "buccal mucosal cell" OR "buccal mucosal cells" OR "ephitelial cell" OR "célula epitelial" OR "gingival tissue" OR "tecido gengival" OR "tejido gengival" OR gum OR gums)	tobacco OR cigarette OR cigarro OR cigarrillo OR cigarettes OR cigarros OR cigarrillos OR "cigarette smoke" OR "fumaça de cigarro" OR fumo OR "tobacco addictive" OR "cigarette smoking" OR "fumar cigarro" OR "smokeless tobacco user" OR "smokeless tobacco users" OR "usuários de tabaco" OR "consumidores de tabaco" OR "tobacco harm" OR "dano do tabaco" OR "daño del tabaco" OR smoker OR fumante OR fumador OR smokers OR fumantes OR fumadores OR cigar OR cigars OR nicotine OR nicotina OR tar OR alcatrão OR alquitrán)	"non smoker" OR "não fumante" OR "no fumador" OR "non smokers" OR "no fumadores" OR "não fumantes" OR smokeless OR "no smoker" OR "não-fumante" OR "no smokers" OR "não-fumantes")
#1 AND #2 AND #3		

Cochrane Library (15/December/2014)

#1 MeSH descriptor: [Cheek] explode all trees #2 MeSH descriptor: [Mouth Mucosa] explode all trees #3 MeSH descriptor: [Gingiva] explode all trees #4 "jugalmocosa":ti,ab,kw or "oral mucosa":ti,ab,kw or "buccal mucosa":ti,ab,kw or "buccal mucosa cell":ti,ab,kw or "buccal mucosa cels":ti,ab,kw (Word variations have been searched) #5 "buccal cell":ti,ab,kw or "buccal cells":ti,ab,kw or "exfoliated cell":ti,ab,kw or "exfoliated cells":ti,ab,kw or "exfoliated oral cell":ti,ab,kw (Word variations have been searched) #6 "exfoliated oral cells":ti,ab,kw or "exfoliated oral mucosa cells":ti,ab,kw or "buccal mucosal cell":ti,ab,kw or "buccal mucosal cells":ti,ab,kw or "ephitelial cell":ti,ab,kw (Word variations have been searched) #7 "gingival tissue":ti,ab,kw or gum:ti,ab,kw or gums:ti,ab,kw (Word variations have been searched) #8 #1 or #2 or #3 or #4 or #5 or #6 or #7	#9 MeSH descriptor: [Tobacco Products] explode all trees #10 MeSH descriptor: [Tobacco] explode all trees #11 smoke:ti,ab,kw or smoking:ti,ab,kw or cigarette:ti,ab,kw or cigarettes:ti,ab,kw or "cigarette smoke":ti,ab,kw (Word variations have been searched) #12 "tobacco addictive":ti,ab,kw or "cigarette smoking":ti,ab,kw or "smokeless tobacco user":ti,ab,kw or "smokeless tobacco users":ti,ab,kw or "tobacco harm":ti,ab,kw (Word variations have been searched) #13 smoker:ti,ab,kw or smokers:ti,ab,kw or cigar:ti,ab,kw or cigars:ti,ab,kw or nicotine:ti,ab,kw (Word variations have been searched) #14 tar:ti,ab,kw (Word variations have been searched) #15 #9 or #10 or #11 or #12 or #13 or #14	#16 MeSH descriptor: [Healthy Volunteers] explode all trees #17 "non smoker":ti,ab,kw or "non smokers":ti,ab,kw or "no smoker":ti,ab,kw or "no smokers":ti,ab,kw or smokeless:ti,ab,kw (Word variations have been searched) #18 #16 or #17
#8 AND #15 AND #18		

Table 2 Summary of the studies selected for this systematic review.

Study ID	Mean cigarettes/ day	Smoking time (yrs) /range	Number of participants/ group	Subjects' age mean±SD [range] (yrs)	% of subjects [Male]	Local of smear	Cell counting/ participant	Other genotoxic alterations evaluated?	Stain method used
Angelieri 2010 [15]	≥ 20	≥10	S – 15 NS – 17	S – 37.7 ± 6.5 NS – 39.6 ± 5.4	S – 60 NS – 65	Cheek and lateral border of the tongue	2000	Pyknosis, karyolysis, karyorrhesis	Feulgen
Chandirasekar 2014 [18]	10	12.5 ± 6.3	S – 58 NS – 57	n.r.	n.r.	Cheek	1000	Aberrant cells	Feulgen
Haveric 2010 [19]	16.8 ± 7.5	8.3 ± 3.6	S – 43 NS – 44	S – 26.0 ± 3.5 NS – 25.3 ± 3.30	S – 47 NS – 39	Cheek	1000	Apoptosis	Giemsa
Kausar 2009 [20]	≥ 4	≥ 0.5	S – 15 NS – 30	S – 37.0 ± 7.6 NS – 39.7 ± 5.6	S – 0 NS – 50	Cheek	2000	Binucleated, karyolysis, karyorrhesis, pkcnosis and nuclear bud	Feulgen
Konopacka 2003 [21]	≥ 10	n.r.	S – 50 NS – 70	36.8 ± 12.4	S – 50 NS – 50	Cheek	1000	No	Feulgen
Mr 2014 [25]	n.r.	≥ 5	S – 45 NS – 45	n.r.	n.r.	Cheek	2000	No	Giemsa
Naderi 2012 [13]	n.r.	3 - 52	S – 40 NS – 23	S – 39.0 ± 20.5 NS – 37.8 ± 17.3	S – 100 NS - 100	Cheek	500	No	Feulgen
Nersesyan 2006 [14]	34 ± 11.9	12.2 ± 4.0	S – 20 NS – 10	S – 36.1 ± 8.1 NS – 30.3 ± 8.7	S – 80 NS – 60	Cheek	1500	Broken eggs, binucleated, condensed chromatin, karyolisis, karyorrhexis and pyknosis.	Giemsa, May-Grünwald-Giemsa, Feulgen, DAPI and acridine orange
Nersesyan 2011 [11]	31.8 ± 7.6	17.2± 4.5	S – 83 NS – 20	S – 47.0 ± 9.1 NS – 43.9 ± 10.9	S – 100 NS - 100	Cheek	2000	Broken eggs, binucleated, condensed chromatin, karyolisis, karyorrhexisand pyknosis.	Feulgen
Ozkul 1997 [26]	n.r.	17.6 ± 2.9	S – 14 NS - 15	S – 46.2 ± n.r. NS – 40.62 ± n.r.	S – 100 NS – 100	Lower lip mucosa	100	No	Feulgen
Palaskar 2010 [28]	≥ 80 packs/yr	≥ 5	S – 15 NS - 15	n.r.	S – 100 NS – 100	Cheek	1000	No	Papanicolau and Giemsa
Piyathilake 1995 [29]	20 packs/year	n.r.	S – 39 NS - 60	n.r.	S – 51 NS – 20	Cheek	1000	No	Feulgen
Sarto 1987 [22]	21.6 ± 9.0	n.r.	S – 25 NS – 25	S – 37.7 ± 11.1 NS – 37.7 ± 9.0	S – 60 NS – 60	Cheek	S – 2927 NS – 3187	Splindle disturbances	Feulgen
Sellappa 2009 [27]	n.r.	13.2 ± 5.7	S – 14 NS – 15	S – 34.5 ± 0.9 NS – 38.6 ± 0.5	n.r.	Cheek	1000	No	Giemsa
Stich 1983 [6]	n.r.	n.r.	S – 36 NS – 15	n.r.	n.r.	Cheek	500	No	Feulgen
Stich 1992 [23]	8.8 ± 4.9	11 - 36	S – 25 NS – 50	S – 51.4 ± 7.2 NS – n.r.	n.r.	Palate, tongue, cheek and lower lip	300	No	Feulgen
Suhas 2004 [24]	13 (5 – 60)	15 (2 – 45)	S – 25 NS – 25	S – 37.6 ± 11.6 NS – 30.5 ± 11.2	S – 100 NS – 100	Cheek, tongue and palate	500	No	Acridine Orange

ID – identification; SD – standard deviation; yrs – years; # – number; n.r. – Not reported; S – smokers; NS – non smokers.

Table 3 Quality assessment components and final rating of the studies.

STUDY ID	SELECTION BIAS	CONFOUNDERS	BLINDING	DATA COLLECTION METHODS	WITHDRAWS AND DROPOUTS	FINAL RATING
Angelieri 2010 [15]	WEAK	STRONG	MODERATE	STRONG	STRONG	MODERATE
Chandirasekar 2014 [18]	MODERATE	WEAK	MODERATE	STRONG	STRONG	MODERATE
Haveric 2010 [19]	MODERATE	STRONG	STRONG	STRONG	STRONG	STRONG
Kausar 2009 [20]	WEAK	STRONG	STRONG	STRONG	STRONG	MODERATE
Konopacka 2003 [21]	WEAK	MODERATE	MODERATE	STRONG	STRONG	MODERATE
Mr 2014 [25]	STRONG	WEAK	STRONG	STRONG	STRONG	MODERATE
Naderi 2012 [13]	MODERATE	STRONG	STRONG	STRONG	STRONG	STRONG
Nersesyan 2006 [14]	WEAK	STRONG	MODERATE	STRONG	STRONG	MODERATE
Nersesyan 2011 [11]	WEAK	STRONG	MODERATE	STRONG	STRONG	MODERATE
Ozkul 1997 [26]	WEAK	STRONG	STRONG	STRONG	STRONG	MODERATE
Palaskar 2010 [28]	WEAK	STRONG	STRONG	STRONG	STRONG	MODERATE
Piyathilake 1995 [29]	MODERATE	STRONG	STRONG	STRONG	STRONG	STRONG
Sarto 1987 [22]	WEAK	STRONG	STRONG	STRONG	STRONG	MODERATE
Sellappa 2009 [27]	WEAK	MODERATE	STRONG	STRONG	STRONG	MODERATE
Stich 1983 [6]	STRONG	MODERATE	MODERATE	STRONG	STRONG	STRONG
Stich 1992 [23]	WEAK	MODERATE	MODERATE	STRONG	STRONG	MODERATE
Suhas 2004 [24]	MODERATE	WEAK	MODERATE	STRONG	STRONG	MODERATE

Table 4 Data from secondary outcomes.

Outcome	Studies	Participants	Statistical Method	Effect Estimate	Heterogeneity	
					Chi-square	I^2
Binucleated cells	3	178	Std. Mean Difference (IV, Random, 95% CI)	1.28 [0.90, 1.66]	0.36	3%
Karyolisis	3	178	Std. Mean Difference (IV, Random, 95% CI)	1.12 [0.11, 2.14]	0.05	75%
Karyorrhexis	3	178	Std. Mean Difference (IV, Random, 95% CI)	1.22 [0.81, 1.62]	0.32	13%
Pycnosis	3	178	Std. Mean Difference (IV, Random, 95% CI)	1.52 [0.10, 2.95]	< 0.00001	92%

Study or Subgroup	Smokers Mean	SD	Total	Non-smokers Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Angelieri 2010	0.05	0.04	15	0.05	0.04	17	6.3%	0.00 [-0.69, 0.69]
Chandirasekar 2014	2.06	1.28	58	0.3	0.22	57	6.6%	1.90 [1.45, 2.34]
Haveric 2010	3.04	3	43	2	2.03	44	6.7%	0.40 [-0.02, 0.83]
Kausar 2009	0.46	0.38	15	0.07	0.05	30	6.2%	1.74 [1.01, 2.46]
Konopacka 2003	1.5	0.47	50	0.55	0.32	70	6.6%	2.42 [1.95, 2.90]
Mr 2014	3.11	0.4	45	0.5	0.8	45	6.2%	4.09 [3.35, 4.83]
Naderi 2012	14.1	2.79	40	2.26	2.17	23	5.7%	4.53 [3.56, 5.49]
Nersesyan 2006	1.79	1.85	20	0.63	0.6	10	6.1%	0.72 [-0.06, 1.51]
Nersesyan 2011	1.26	0.5	83	1	0.1	20	6.6%	0.57 [0.08, 1.06]
Ozkul 1997	1.99	1.12	14	0.84	0.85	15	6.1%	1.13 [0.34, 1.92]
Palaskar 2010	17.57	5.76	15	3.53	1.4	15	5.4%	3.26 [2.12, 4.40]
Sarto 1987	0.66	0.46	25	0.35	0.34	25	6.5%	0.75 [0.18, 1.33]
Sellappa 2009	2	1.11	14	0.81	0.65	15	6.0%	1.28 [0.47, 2.09]
Stich 1983	0.3	0.03	36	0.23	0.04	15	6.2%	2.08 [1.34, 2.81]
Stich 1992	1.3	1	25	0.5	0.2	50	6.5%	1.33 [0.80, 1.86]
Suhas 2004	2.72	1.1	25	2.04	0.79	25	6.5%	0.70 [0.13, 1.27]
Total (95% CI)			**523**			**476**	**100.0%**	**1.64 [1.09, 2.20]**

Heterogeneity: $Tau^2 = 1.15$; $Chi^2 = 189.89$, $df = 15$ ($P < 0.00001$); $I^2 = 92\%$

Test for overall effect: $Z = 5.82$ ($P < 0.00001$)

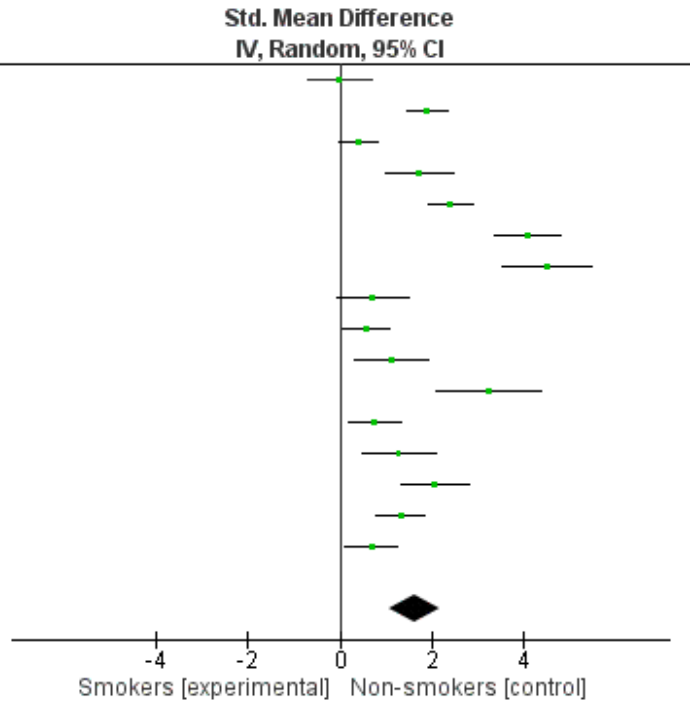

Fig. 2 Forest plot of the frequency of micronuclei for smokers vs non-smokers.

5 DISCUSSÃO

Há um consenso geral de que o consumo de bebidas e alimentos com corantes é frequentemente associado ao escurecimento dental (Joiner,[32] 2006). Esta premissa é baseada nos achados de estudos *in vitro* que relataram que o cigarro, café, chá e vinho podem levar ao escurecimento dos dentes (Bazzi et al.,[24] 2012; Watts e Addy,[33] 2001; Attin et al.,[34] 2003; Ley et al.,[35] 2006; Attia et al.,[36] 2009) e, por conseguinte, afetar a longevidade do clareamento dental (Bazzi et al.,[24] 2012; Attia et al.,[36] 2009). Esta é a razão pela qual dentistas têm prescrito uma dieta sem corantes e impedido fumantes de realizar o clareamento, para garantir que os efeitos imediatos e a longevidade do clareamento não sejam reduzidos pelos resultados da dieta (Matis et al.,[37] 2015) ou hábito de fumar.

Dentes expostos a agentes corantes na dieta realmente tem um maior potencial para manchar (Téo et al.,[28] 2010). Da mesma forma, os dentes dos fumantes tendem a desenvolver manchas de cigarro ao passar do tempo (Alkhatib et al.,[11] 2005), as quais podem variar de amarelas a marrons, e a gravidade é altamente dependente da duração e frequência do hábito de fumar. Embora não seja desejável que a coloração extrínseca possa afetar a percepção geral de dentes mais brancos, estas manchas podem ser facilmente removidas por profilaxia dentária profissional, como demonstrado no Estudo 1. Em estudos de longos acompanhamentos (Tay et al.,[38] 2012; Tsubura,[39] 2010; Turkun et al.[16] 2010; Auschill et al.,[40] 2012; Mondelli et al.,[41] 2012), a recidiva de cor pode estar associada a outros fatores, como o envelhecimento dos dentes, onde há uma deposição contínua de dentina secundária pela polpa (Nanci,[42] 2013). À medida que a espessura da dentina aumenta, os dentes aparecem mais amarelados, independentemente das condições dietéticas ou hábitos tabagistas. Uma alteração de cor significativa foi demonstrada tanto para fumantes como para não fumantes no Estudo 2.

No Estudo 3 pode-se observar que a cor dos dentes ficou mais escura após a exposição à fumaça do cigarro, o que é consistente com os resultados de alguns estudos *in vitro* (Bazzi et al.,[24] 2012; Bertoldo et al.,[22] 2011). Usando a metodologia CG-MS, foi possível observar a presença de nicotina em amostras que foram expostas à fumaça do cigarro (controle positivo). A profilaxia dental removeu apenas 36% da nicotina apresentada na superfície externa dos dentes e o clareamento em consultório removeu cerca de 75% da nicotina apresentada na superfície externa e interna dos dentes. Estes resultados sugerem que a nicotina penetra na estrutura dental e embora não tenha afetado negativamente a cor dos

dentes tanto nos experimentos clínicos como no laboratorial, isto possa ser atribuído ao pouco tempo de avaliação que foi de 12 a 30 meses.

A nicotina possui a capacidade de promover a progressão de tumores já estabelecidos, estando associada principalmente com a progressão do câncer de pulmão, de estômago e de colo (Grando,[43] 2014). Um aumento da frequência de quebra do cromossomo foi demonstrado como um evento inicial na carcinogênese, sugerindo que estas alterações podem desempenhar um papel significativo na avaliação do risco oncogênico (Hagmar et al.,[44] 1998; Bonassi et al.,[45] 2000). Entre os marcadores que podem ser utilizados para este propósito, a contagem de micronúcleos (MN) parece ser um dos mais adequados. Um aumento da frequência de MN em células esfoliadas do tecido gengival tem servido tradicionalmente como um índice para avaliar a genotoxicidade de exposição a vários agentes cancerígenos (Bhattathiri et al.[7] 1996, Basu et al.[8] 2004). MN se originam de fragmentos do cromossomo ou cromossomos inteiros que não estão incluídos nos principais núcleos filhos durante a divisão nuclear. Eles refletem danos cromossômicos e podem, assim, proporcionar um marcador inicial da carcinogênese.

Vários estudos têm sido realizados para determinar a associação entre tabagismo e indução de MN em células orais (Nersesyan et al.,[46] 2011; Bansal et al.,[47] 2012; Naderi et al.,[48] 2012; Nersesyan et al.,[9] 2006; Chandirasekar et al.,[49] 2014; Haveric et al.,[50] 2010; Kausar et al.,[51] 2009; Braga et al.,[52] 2004; Motgi et al.,[53] 2014). O efeito cancerígeno do hábito de fumar é determinado em grande parte pela mutagenicidade dos compostos presentes na fumaça do cigarro. Os cigarros contêm uma mistura de vários agentes cancerígenos incluindo os hidrocarbonetos aromáticos policíclicos (PAH), a N-nitrosaminas específicas, aminas aromáticas, aldeídos e outros agentes cancerígenos (Hoffmann et al.,[54] 2001).

Os resultados do Estudo 4 apontam para o fato de ocorrer uma maior genotoxicidade em células da cavidade oral nos fumantes comparando-os aos não fumantes. Levando em consideração os efeitos mutagênicos de nitrosaminas específicas do tabaco (IARC,[55] 2007), pode-se considerar que estes compostos podem ter uma função importante na indução de MN em células da mucosa bucal dos fumantes. Entretanto, um estudo recente publicado por nosso grupo de pesquisa, mostrou que não houve uma maior genotoxicidade em fumantes quando este foi submetido ao tratamento clareador caseiro (de Geus et al.,[56] 2015). Apesar de ter sido demonstrado que o PH pode causar alterações nos tecidos moles (Weitzman et al.,[57] 1986; Minoux e Serfaty,[58] 2008), o clareamento em consultório com PH não aumentou induziu danos ao DNA no tecido gengival e bucal durante o tratamento clareador (Rezende et al.,[59] 2016).

6 CONCLUSÃO

A cor obtida com o clareamento dental caseiro com peróxido de carbamida 10% manteve-se estável em ambos os grupos no Estudo 1, quando as manchas extrínsecas causadas pela dieta e tabagismo foram removidas por profilaxia dental.

No Estudo 2, após trinta meses de acompanhamento, detectou-se uma alteração de cor significativa em fumantes e não-fumantes, que não pode ser atribuída exclusivamente a manchas extrínsecas, pois mesmo após a profilaxia dental, os dentes aparentaram um pouco mais escuros do que o resultado imediato do clareamento.

O método de cromatografia do Estudo 3 permitiu a avaliação de nicotina em espécimes dentários após a exposição à fumaça do cigarro. Houve depósito de fumaça de cigarro na superfície dentária externa e também sobre a estrutura dental interna. A profilaxia e o clareamento em consultório com peróxido de hidrogênio 35% reduziram a quantidade de nicotina depositada sobre a superfície externa dos dentes, bem como a nicotina que penetrou pela fumaça do cigarro. No entanto, o clareamento em consultório mostrou uma remoção mais significativa da nicotina.

A meta-análise realizada no Estudo 4 mostrou uma maior frequência de micronúcleos em células esfoliadas da cavidade oral de fumantes em comparação com não fumantes.

***REFERÊNCIAS**

1- WHO. World Health Organization. Tobacco, 2016. Disponível em http://www.who.int/mediacentre/factsheets/fs339/en/. Acesso em agosto de 2016.

2- Devita Jr VT, Lawrence TS, Rosberg SA. Cancer Principles & Practice of Oncology: Tobacco, 9th edition, Lippincott Williams & Wilkins publisher, 2011, 150-156.

3- International Agency for Research on Cancer. Cumulative Index to IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. IARC Monogr Eval Carcinog Risk Chem Hum.1986;39:379-403.

4- Beliën JA, Copper MP, Braakhuis BJ, et al. Standardization of counting micronuclei: definition of a protocol to measure genotoxic damage in human exfoliated cells. Carcinogenesis. 1995 Oct;16(10):2395-2400.

5- Sivasankari NP, Kaur S, Reddy KS, et al. Micronucleus assay - screening tool in the diagnosis of oral carcinoma in tobacco users. Int J Pharm Bio Sci. 2012 Oct;3(4):646-651.

6- Cairns J. Mutation selection and the natural history of cancer. Nature. 1975 May;255(5505):197-200.

7- Bhattathiri VN, Bindu L, Remani P, et al. Serial cytological assay of micronucleus induction: a new tool to predict human cancer radiosensitivity. Radiother Oncol. 1996 Nov;41(2):139-142.

8- Basu A, Ghosh P, Das JK, et al. Micronuclei as biomarkers of carcinogen exposure in populations exposed to arsenic through drinking water in West Bengal, India: a comparative study in three cell types. Cancer Epidemiol Biomarkers Prev. 2004 May;13(5):820-827.

9- Nersesyan A, Kundi M, Atefie K, et al. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiol Biomarkers Prev. 2006 Oct;15(10):1835-1840.

10- Angelieri F, Goncalves Moleirinho TdC, Carlin V et al. Biomonitoring of oral epithelial cells in smokers and non-smokers submitted to panoramic X-ray: comparison between buccal mucosa and lateral border of the tongue. Clin Oral Investig. 2010 Dec;14(6):669-674.

11- Alkhatib MN, Holt RD, Bedi R. Smoking and tooth discolouration: findings from a national cross-sectional study. BMC Public Health 2005 Mar;5:27.

12- Krause F, Jepsen S, Braun A. Subjective intensities of pain and contentment with treatment outcomes during tray bleaching of vital teeth employing different carbamide peroxide concentrations. Quintessence Int. 2008 Mar;39(3):203-209.

13- Meireles SS, Heckmann SS, Santos IS, et al. A double blind randomized clinical trial of at-home tooth bleaching using two carbamide peroxide concentrations: 6-month follow-up. J Dent. 2008 Nov;36(11):878-884.

*De acordo com as normas do PPGO-UEPG.

14- Bernardon JK, Sartori N, Ballarin A, et al. Clinical performance of vital bleaching techniques. Oper Dent. 2010 Jan-Feb;35(1):3-10.

15- Grobler SR, Hayward R, Wiese S, et al. Spectrophotometric assessment of the effectiveness of Opalescence PF 10%: A 14-month clinical study. J Dent. 2010;38(2):113-117.

16- Türkün M, Celik EU, Aladağ A, et al. One-year clinical evaluation of the efficacy of a new daytime at-home bleaching technique. J Esthet Restor Dent. 2010 Apr;22(2):139-146.

17- De Almeida LCAG, Costa CAS, Riehl H, et al. Occurrence of sensitivity during at-home and in-office tooth bleaching therapies with or without use of light sources. Acta Odontol Latinoam. 2012;25(1):3-8.

18- Bonafé E, Bacovis CL, Iensen S, et al. Tooth sensitivity and efficacy of in-office bleaching in restored teeth. J Dent. 2013 Apr;41(4):363-369.

19- Rezende M, Loguercio AD, Reis A, et al. Clinical effects of exposure to coffee during at-home vital bleaching. Oper Dent. 2013 Nov-Dec;38(6):E229-36.

20- De la Peña VA, Ratón ML. Randomized clinical trial on the efficacy and safety of four professional at-home tooth whitening gels. Oper Dent. 2014 Mar-Apr;39(2):136-43.

21- De Geus JL, Bersezio C, Urrutia J, et al. Effectiveness of and tooth sensitivity with at-home bleaching in smokers: a multicenter clinical trial. J Am Dent Assoc. 2015 Apr;146(4):233-40.

22- Bertoldo CES, Miranda DA, Souza-Júnior EJ, et al. Surface hardness and color change of dental enamel exposed to cigarette smoke. Int J Dent Clin. 2011;3(4):1-4.

23- Wasilewski M de S, Takahashi MK, Kirsten GA, et al. Effect of cigarette smoke and whiskey on the color stability of dental composites. Am J Dent. 2010 Feb;23(1):4-8.

24- Bazzi JZ, Bindo MJF, Rached RN, et al. The effect of at-home bleaching and toothbrushing on removal of coffee and cigarette smoke stains and color stability of enamel. JADA. 2012 May;143:e1-e7

25- Benowitz NL, Jacob P, 3rd. Nicotine and cotinine elimination pharmacokinetics in smokers and nonsmokers. Clin Pharmacol Ther.1993 Mar;53(3):316-323.

26- Robinson DE, Balter NJ, Schwartz SL.A physiologically based pharmacokinetic model for nicotine and cotinine in man. Journal Pharmacokinet Biopharm. 1992 Dec;20(6):591-609.

27- Pascual JA, Diaz D, Segura J, et al.A simple and reliable method for the determination of nicotine and cotinine in teeth by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom. 2003;17(24):2853-2855.

*De acordo com as normas do PPGO-UEPG.

28- Téo TB, Takahash MK, Gonzaga CC, et al. Avalição, após clareamento, da alteração de cor de dentes bovinos imersos em soluções com elevado potencial de pigmentação, RSBO. 2010;7:401–405.

29- Armênio RV, Fitarelli F, Armênio MF, et al. The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial. J Am Dent Assoc. 2008 May;139(5):592-597.

30- Reis A, Dalanhol AP, Cunha TS, et al. Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. Oper Dent. 2011 Jan-Feb;36(1):12-17.

31- Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg.2010;8(5):336-341.

32- Joiner A. The bleaching of teeth: a review of the literature. J Dent. 2006 Aug;34(7):412–419.

33- Watts A, Addy M. Tooth discolouration and staining: a review of the literature. Br Dent. J. 2001 Mar;190(6):309–316.

34- Attin T, Manolakis A, Buchalla W, et al. Influence of tea on intrinsic colour of previously bleached enamel. J Oral Rehabil. 2003 May;30(5):488–494.

35- Ley M, Wagner T, Bizhang M. The effect of different fluoridation methods on the red wine staining potential on intensively bleached enamel in vitro. Am J Dent. 2006 Apr;19(2):80–84.

36- Attia ML, Aguiar FH, Mathias P, et al. The effect of coffee solution on tooth color during home bleaching applications. Am J Dent. 2009 Jun;22(3):175–179.

37- Matis B, Wang G, Matis J, et al. White diets is it necessary during tooth whitening? Oper Dent. 2015 May-Jun;40(3):235–240.

38- Tay LY, Kose C, Herrera DR et al. Long-term efficacy of in-office and at-home bleaching: A 2-year double-blind randomized clinical trial. Am J Dent. 2012 Aug;25(4):199-204.

39- Tsubura S. Clinical evaluation of three months' nightguard vital bleaching on tetracycline-stained teeth using Polanight 10% carbamide gel: 2-year follow-up study Odontology. 2010 Jul;98(2):134-138.

40- Auschill TM, Schneider-Del Savio T, Hellwig E, et al. Randomized clinical trial of the efficacy, tolerability, and long-term color stability of two bleaching techniques: 18-month follow-up. Quintessence Int. 2012 Sep;43(8):683-694.

41- Mondelli RF, Azevedo JF, Francisconi AC, et al. Comparative clinical study of the effectiveness of different dental bleaching methods - two year follow-up. J Appl Oral Sci. 2012 Jul-Aug;20(4):435-443.

*De acordo com as normas do PPGO-UEPG.

42- Nanci A. Ten Cate's Oral Histology: Development, Structure, and Function, eighth ed., Elsevier, 2013.

43- Grando SA. Connections of nicotine to cancer. Nat Rev Cancer. 2014 Jun;14(6):419-429.

44- Hagmar L, Bonassi S, Stromberg U, et al. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res. 1998 Sep;58(18):4117-4121.

45- Bonassi S, Hagmar L, Stromberg U, et al. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. European Study Group on Cytogenetic Biomarkers and Health. Cancer Res. 2000 Mar;60(6):1619-1625.

46- Nersesyan A, Muradyan R, Kundi M, et al. Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. Mutagenesis. 2011Mar;26(2):295-301.

47- Bansal H, Sandhu VS, Bhandari R, et al. Evaluation of micronuclei in tobacco users: A study in Punjabi population. Contemp Clin Dent. 2012Apr;3(2):184-187.

48- Naderi NJ, Farhadi S, Sarshar S. Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian J Pathol Microbiol. 2012 Oct-Dec;55(4):433-438.

49- Chandirasekar R, Kumar BL, Sasikala K, et al. Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. Mutat Res Genet Toxicol Environ Mutagen. 2014Jun;767:21-27.

50- Haveric A, Haveric S, Ibrulj S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers. Toxicol Mech Methods. 2010 Jun;20(5):260-266.

51- Kausar A, Giri S, Mazumdar M, et al. Micronucleus and other nuclear abnormalities among betel quid chewers with or without sadagura, a unique smokeless tobacco preparation, in a population from North-East India. Mutat Res Genet Toxicol Environ Mutagen. 2009 Jun-Jul;677(1-2):72-75.

52- Braga FL, Meneguzzi RD, Paiva RL, et al. Avaliação citopatólogica da mucosa bucal de fumantes e não-fumantes Rev Odonto Ciênc. 2004;19:157-163.

53- Motgi AA, Chavan MS, Diwan NN, et al. Assessment of cytogenic damage in the form of micronuclei in oral epithelial cells in patients using smokeless and smoked form of tobacco and non-tobacco users and its relevance for oral cancer. J Cancer Res Ther. 2014 Jan-Mar;10(1):165-170.

54- Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. Chem Research Toxicol. 2001 Jul;14(7):767-790.

55- International Agency for Research on Cancer. Smokeless tobacco and some tobacco-specific N-nitrosamines. IARC Monogr Eval Carcinog Risks Hum. 2007;89:1-592.

56- De Geus JL, Rezende M, Margraf LS, et al. Evaluation of genotoxicity and efficacy of at-home bleaching in smokers: a single-blind controlled clinical trial. Oper Dent. 2015 Mar-Apr;40(2):E47-55.

57- Weitzman SA, Weitberg AB, Stossel TP, et al. Effects of hydrogen peroxide on oral carcinogenesis in hamsters. J Periodontol. 1986 Nov;57(11):685-688.

58- Minoux M, Serfaty R. Vital tooth bleaching: Biologic adverse effects—A review Quintessence Int. 2008 Sep;39(8):645-659.

59- Rezende M, De Geus JL, Loguercio AD, et al. Clinical Evaluation of Genotoxicity of In-office Bleaching. Oper Dent. 2016 Nov/Dec;41(6):578-586.

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ANEXO A

UNIVERSIDADE ESTADUAL DE PONTA GROSSA - UEPG

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação clínica da sensibilidade, genotoxicidade e efetividade do clareamento dental caseiro em pacientes fumantes.

Pesquisador: Stella Kossatz Pereira

Área Temática:

Versão: 4

CAAE: 07114312.8.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 669.914

Data da Relatoria: 23/05/2014

Apresentação do Projeto:

Projeto de pesquisa que teoriza a avaliação clínica da sensibilidade, genotoxicidade e efetividade do clareamento dental caseiro em pacientes fumantes.

Objetivo da Pesquisa:

Objetivo Primário:

O propósito do presente estudo será avaliar clinicamente a efetividade, a genotoxicidade, a longevidade da cor e a sensibilidade dental de pacientes

fumantes e não fumantes submetidos ao clareamento caseiro com PC 10%.

Objetivo Secundário:

1-comparar a efetividade do clareamento dental caseiro utilizando PC 10% em pacientes fumantes e não fumantes;2-avaliar a segurança do

clareamento através da contagem de micronúcleos do epitélio gengival dos pacientes, antes e após o clareamento dental;

A estética dental é de grande importância para muitos pacientes. A demanda pública de odontologia estética, incluindo clareamento dental, tem

aumentado nos últimos anos (Kihn, 2007). Atualmente, existem algumas técnicas de clareamento dental disponíveis para uso clínico. Os

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clareamentos caseiro e de consultório são amplamente utilizados na prática odontológica (Auschill et al. 2005, Joiner 2006). Uma das vantagens do clareamento caseiro relatada é a sua eficácia, que é facilmente notada pelos pacientes (Grobler et al. 2010, Basting et al. 2012). Além disso, esta técnica apresenta menor custo e tempo clínico, quando comparada com a técnica de consultório.Apesar de o clareamento dental ser um procedimento efetivo, ele pode causar alterações na superfície do esmalte (Hosoya et al. 2003, Espina et al. 2008, Bodanezi et al. 2011), como por exemplo, o aumento da permeabilidade dental (Cândido et al.9 2005). Por isso, não é raro que os profissionais solicitem aos pacientes que evitem fumar durante a execução do tratamento clareador a fim de evitar a impregnação de manchas escuras (Watts e Addy 2001, Wasilewski et al. 2010, Bertoldo et al. 2011) na estrutura dental recém-clareada.Segundo Wasilewski et al. (2010) a fumaça do cigarro contém água, ar, monóxido de carbono (CO), dióxido de carbono (CO_2) e alcatrão. Durante a queima do cigarro, componentes como alcatrão, açúcares e cacau são transferidos para a fumaça devido ao aquecimento. E de acordo com Bazzi et al. (2012) estes componentes seriam provavelmente os responsáveis pelo manchamento nos dentes, pela sua tonalidade escura e capacidade de se aderir ao dente. Porém, o manchamento provocado pelo cigarro parece ser superficial e facilmente removido pela limpeza mecânica e clareamento dental (Bazzi et al. 2012).A ação genotóxica de substâncias que entram em contato com o epitélio bucal pode ser refletida em danos ao DNA tecidual, como por exemplo, formação de micronúcleos nas células epiteliais (Nerseyan et al. 2011, Bansal et al. 2012, Sivasankari et al. 2012). Para avaliar a segurança do clareamento dental caseiro, pode-se lançar mão do Teste de Micronúcleos, principalmente se tratando de pacientes fumantes (Martins e Filho 2003, Nerseyan et al. 2011, Sivasankari et al. 2012).Micronúcleos são estruturas que medem de 1/3 a 1/5 do tamanho do núcleo, possuem formato redondo ou oval e estão localizados próximos ao núcleo da célula (Schmid 1975). A sua causa é a destruição da molécula de DNA, dias ou semanas após agentes carcinógenos atuarem quando as células da camada basal estão em divisão (Stich e Rosin 1983, Stich et al. 1984). Portanto,

células micronucleadas podem representar a
incidência de eventos genotóxicos sobre a mucosa oral (Belliën et al. 1995).O dano causado por um agente ambiental no processo de divisão celular
nos tecidos pode refletir na presença de micronúcleos nas células, o que significa que houve alteração do DNA tecidual e, consequentemente há
uma maior predisposição de desenvolvimento de câncer bucal (Vine 1990). Portanto o Teste de Micronúcleos pode ser utilizado para a avaliação e
identificação de fatores que podem provocar danos ao DNA dos tecidos.Na Odontologia, a aplicação deste teste consiste em uma ferramenta
extremamente valiosa para avaliarmos a ação genotóxica de substâncias que entram em contato com o epitélio bucal, como por exemplo colutórios,
tabaco, bebidas alcoólicas, agentes clareadores caseiros, entre outros. Tais substâncias, assim como agentes físicos (trauma mecânico por

próteses, má oclusão, aparelhos ortodônticos) ou biológicos (biofilme dental), são exemplos de fatores de presença comum no ambiente bucal, e
que podem refletir uma potencial formação de micronúcleos às células do epitélio (Fenech et al. 1999).Outro efeito colateral causado pelo
clareamento dental externo é a sensibilidade dental, sendo considerado o efeito mais comum (Jorgensen e Caroll24 2002). Vários estudos têm
relatado sensibilidade dental utilizando diferentes concentrações de peróxido de carbamida (Leonard et al. 2007, Krause et al. 2008, Turkun et al.
2010, Cardoso et al. 2010, Basting et al. 2012, de Almeida et al. 2012, Rezende et al. 2013). Até o momento não há estudos que tenham avaliado a
efetividade do clareamento dental caseiro em pacientes fumantes, bem como e a segurança do mesmo e a sensibilidade dental. Portanto, o objetivo
do presente estudo será avaliar clinicamente a efetividade, segurança e sensibilidade causada pelo clareamento dental caseiro em pacientes
fumantes e não fumantes.3-avaliar a longevidade da cor nos períodos de 1 semana, 1 mês e 1 ano após o clareamento em ambos os grupos;4-avaliar a sensibilidade dental
durante o clareamento caseiro através da escala visual analógica (VAS) e da escala numérica analógica (NRS) em ambos os grupos.

Avaliação dos Riscos e Benefícios:
Bem delineados no projeto

UNIVERSIDADE ESTADUAL DE PONTA GROSSA - UEPG

Comentários e Considerações sobre a Pesquisa:

Serão selecionados 60 voluntários que procurem atendimento nas clínicas odontológicas da Universidade Estadual de Ponta Grossa (UEPG) e que
se enquadrem nos critérios de inclusão e exclusão do estudo.Após a assinatura do termo de consentimento livre e esclarecido (TCLE) (Apêndice),
será realizada profilaxia dental com jato de bicarbonato de sódio (Profi Class, Ribeirão Preto, São Paulo, Brasil) nos dentes das arcadas superior e
inferior, para remoção das manchas extrínsecas, duas semanas antes do início do clareamento dental

Considerações sobre os Termos de apresentação obrigatória:

De acordo com as normas

Recomendações:

Não se aplica

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PONTA GROSSA, 02 de Junho de 2014

Assinado por:
ULISSES COELHO
(Coordenador)

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ANEXO B

Longevity and Effectiveness of Bleaching in Healthy and Smokers Patients (BLESMOK)

This study has been completed.

Sponsor:

University of Chile

Information provided by (Responsible Party):

Eduardo Fernandez, University of Chile

ClinicalTrials.gov Identifier:

NCT02017873

First received: December 5, 2013

Last updated: May 26, 2015

Last verified: May 2015

History of Changes

Tracking Information	
First Received Date ICMJE	December 5, 2013
Last Updated Date	May 26, 2015
Start Date ICMJE	December 2013
Primary Completion Date	January 2014 (final data collection date for primary outcome measure)
Current Primary Outcome Measures ICMJE **(submitted: February 20, 2014)**	Color [Time Frame: 1, 2, 3, 4, 8 weeks and 3, 6, 9 and 12 month] [Designated as safety issue: Yes] Objective (Vita Easy shade), Subjective measurement
Original Primary Outcome Measures ICMJE **(submitted: December 16, 2013)**	Color [Time Frame: 1, 2, 3, 4, 8 weeks and 3, 6, 9 and 12 month] [Designated as safety issue: Yes] Objetive (Vita Easy shade) , Subjetive measurement
Change History	Complete list of historical versions of study NCT02017873 on ClinicalTrials.gov Archive Site
Current Secondary Outcome Measures ICMJE **(submitted: December 16, 2013)**	Sensitivity [Time Frame: 1, 2, 3, 4, 8 weeks 3, 6, 9,12 month] [Designated as safety issue: Yes] VAS Scale 0-4 Pain Scale
Original Secondary Outcome Measures ICMJE	*Same as current*

Current Other Outcome Measures ICMJE	*Not Provided*
Original Other Outcome Measures ICMJE	*Not Provided*

Descriptive Information

Brief Title ICMJE	Longevity and Effectiveness of Bleaching in Healthy and Smokers Patients
Official Title ICMJE	"Evaluation of the Effectiveness and Longevity Post Whitening Carbamide Peroxide 10% in Smokers and Nonsmokers. Double-blind Multicenter Clinical Trial. "
Brief Summary	The main objective of this study is to evaluate the effectiveness and longevity of color and Tooth sensitivity of patients undergoing home whitening peroxide 10% carbamide (Whiteness Perfect , FGM , Joinville , Santa Catarina , Brazil) , and the relationship with the cigarette use for tooth whitening. They will be selected 120 patients with incisors darker than A2, higher plants will be divided into 2 groups per center (n = 30) , GE - Group Experimental (smoking) and GC - Group Control (non-smoking) . For the two groups will be used Carbamide peroxide 10% for 3 hours daily for a period of 3 weeks. Color will evaluated through the Vita Classical scale and Vita Easyshade Spectrophotometer in the periods: Home , for tooth whitening (1st , 2nd and 3rd week) and post- whitening (1 week and 1 month , 2 and 3 months). Patients recorded the perceived sensitivity through Numerical Analogue Scale (NRS) with values from 0 to 4, where 0 = no sensation, 1 = mild, 2 = moderate, 3 = severe and 4 = significant , also in the Visual Analogue Scale (VAS), with values from 0 to 10 where 0 = 10 = severe tenderness and sensitivity. for color analysis will be made two-way ANOVA (group vs. treatment time), being Over time the repeated measure (α = 0.05). Test will be held on Tukey to contrast the average (α = 0.05). The sensitivity will be evaluated by the Fisher exact test . It is expected that there is no difference on the effectiveness of home whitening and tooth sensitivity between smokers and nonsmokers.
Detailed Description	This study will be conducted under the CONSORT recommendations and respecting the principles of Helsinsky convention and

corresponds to a multicenter study in conjunction with the University of Ponta Grossa Brazil (Deputy resolution protocol and ethics committee (Appendix A and B) . Will be invited to participate in the study to patients who come to the clinic through FOUCH public posters. Subsequently 120 volunteers will be selected on the criteria that qualify inclusion and exclusion from the study will be reported all volunteers were examined not qualify under the criteria for inclusion as part of the initial n , according to CONSORT recommendations .

After signing the informed (TCLE) consent (Appendix 3) shall be made prophylaxis teeth of the upper and lower teeth , for the removal of extrinsic stains jet sodium bicarbonate (Profi class , Ribeirao Preto , Sao Paulo , Brazil) , two weeks before the beginning of tooth whitening.

Inclusion and exclusion criteria:

Patients included in this study must be over 18, in good general health and buccal, have teeth free of carious lesions and periodontal disease, which agree with the informed consent document . And the color of the anterior teeth higher is classified as A2 or greater value , according to the scale VITA Classical (Vita Zahnfabrik, Bad Sackingen, Germany) and the spectrophotometer Easyshade Vita (Vita Zahnfabrik, Bad Sackingen, Germany). Evaluation of color through the VITA scale Classical will be made independently by two calibrated investigators and blind.

Be excluded from the study patients: they have already made treatment tooth whitening, or dental prosthesis having options at the upper front teeth , who are pregnant or lactating , presenting gingival recession, sensitivity dental, endodontic treatment in anterior maxillary teeth, which have a coloring severe internal, if they have non-carious cervical lesions, are taking medications , using fixed orthodontic appliances, submit bruxism habits, which have visible teeth cranks and those who are not available to attend the controls.

Study Design Patients will be divided into two groups (n = 60), GC (control group) and GE (group experimental). At the initial consultation volunteers will be asked about smoking habits daily. Patients who do not smoke will be part of the GC, and heavy smokers (more than 10 day) will be part of GE.A cigarettes all smokers are complementary be given a booklet of tips for quitting smoking and damage, and sites where to find more information. (Annex C) The teeth whitening technique selected for this study, is the technique of

	home bleaching, validated for both groups. Treatment and follow-up will no cost to the patient . For the development of individual buckets, this will be through impression of the upper and lower arch of each patient Jeltrate Plus alginate (Dentply, Petrópolis, Rio de Janeiro, Brazil), the molds will be cast in plaster and immediately after printing. After obtaining this plaster model is cut and taken to the vacuum laminator (Protécni, Araraquara, Sao Paulo, Brazil) for making buckets individual vinyl acetate 1 mm thick (Plate Pail Whiteness - FGM, Joinville, Santa Catarina, Brazil). Cuvettes acetate be cut an inch margin on gingival . For both groups of PC gel will be used 10% (Whiteness Perfect, FGM, Joinville, Santa Catarina, Brazil), for a period of 3 hours a day for three weeks) . After the test cuvette acetate individual, the method of application of the product will be carefully explained to each patient in the study as follows : Dispense a drop in the region of the product corresponding to the buccal surface of each tooth in the tray. The amount of gel should be sufficient to remain in contact with the buccal tooth surface without covering the gingival third, preventing injuries from it. After this period, patients will be instructed to withdraw the tray with the whitening gel and perform vigorous mouth rinsed with water to complete removal of the product. Evaluation of Color Subjective method Color will be assessed by the scale VITA Classical (Vita Zahnfabrik, Bad Sackingen, Germany), consisting of 16 color guides, organized by value, higher value (B1) to low value (C4). Although this scale is not linear, it is organized according to a ranking range valor. Represent and the purpose of analysis. Two blind reviewers record the color of the right maxillary central incisor patients through the scale VITA Classical (Vita Zahnfabrik, Bad Sackingen, Germany). At the following times : initial, 1st week, 2nd week, 3rd week (active phase bleaching) and post- bleaching periods : 1 week, 1 month, 2 and 3 months. Evaluators always record the color independently in the same room with the same lighting. If there is a discrepancy in the color registration, a new evaluation will be conducted together until a consenso. The area chosen for color measurement is the middle third of the labial surface of the central incisor, according to the ADA specifications . The color change will be evaluated by means of varying scale Vita units (ΔUEV) organized by value.

	Objective method The color will also be measured with the spectrophotometer Easyshade (Vita Zahnfabrik, Bad Sackingen, Germany) according to the CIELab system of Vita. Calibration of equipment will always be made before each measurement, and three measurements for each tooth will be made. The evaluation will be conducted at the same times that the method subjective. For standardize the measuring of color, a mold of the teeth of the upper arch with heavy condensation silicone (Coltoflax profile and cub, Vigodent, Rio de Janeiro will be held, Brazil) for making a silicone matrix. The matrix was used to standardize the region of the tooth in which the color is measured with the spectrophotometer. The matrix will be drilled in the vestibular region, in the middle third, in the upper teeth, using a scalpel circular 6 mm in diameter, Biopsy punch (Miltex, York, PA USA), similar diameter to the tip of the spectrophotometer Vita Easyshade. Color recurrence begins the day the patient is bleached, the spectrophotometer used has a sufficient sensitivity to determine minimal color changes, is based on the construction algorithm for the detection of the dimensions h and b color . (Jadad et al. , 2011) Evaluation of the Dental Sensitivity In the initial clinical examination of patients , baseline sensitivity is measured by the vertical , horizontal drum , air jet application and probing of all teeth, so it can be compared with the sensitivity during the whitening dental.Durante whitening, patients record the presence or absence of tooth sensitivity, in a newspaper of tooth sensitivity using analog numerical scale (ENR), with values from 0 to 4, where: 0 = no tenderness, 1 = Slight, 2 = moderate 3 = considerably and 4 = severe. And on the visual analogue scale (VAS) with values from 0 to 10 where 0 = no sensitivity and 10 = severe. Patients mark a vertical line across the horizontal line of the scale corresponding to the intensity of tooth sensitivity. After measurement in millimeters are made with the aid of a millimeter ruler. Annex 5 and 6 values will be organized into two categories: percentage of patients with tooth sensitivity at some time during treatment (absolute risk sensitivity) and intensity of tooth sensitivity. Patients with severe sensitivity will be immediately assisted by investigators to reverse the painful picture using desensitizing and / or analgesics and anti-inflammatories for pain relief, the patient will be removed from study. If sensitivity is used desensitizing FMG KF 2% (nitrate Potassium and Sodium Fluoride 2%, Joinville, Brazil) , the

	literature does not present any report about RAM, if for any reason there is any Medical Adverse Event the research team will be responsible for any additional medical or dental treatment required and its follow-up, in the wake of whitening procedure (restorations, endodontics, etc.) Statistical Analysis The color data obtained by the objective and subjective analysis will be evaluated by analysis of variance of two factors (ANOVA) for repeated measures (time vs. treatment groups) (α = 0.05). Tukey's test performed to test the means (α = 0.05). The absolute risk of sensitivity will be evaluated by the Fisher exact test . Sampling calculation was obtained by the G -Power 3.1 program considering a Beta error 0.8, and an alpha error 0.05, meaning a sample calculation of 25 patients per group per center, considering the drop-out reported in other published work (5%) was decided to increase to 30 the n sample size for each group per center. Coincident with the Odds Ratio of all clinical work whitening the past 10 years.
Study Type ICMJE	Interventional
Study Phase	Phase 4
Study Design ICMJE	Allocation: Non-Randomized Endpoint Classification: Bio-equivalence Study Intervention Model: Parallel Assignment Masking: Double Blind (Investigator, Outcomes Assessor) Primary Purpose: Treatment
Condition ICMJE	Dentin Sensitivity
Intervention ICMJE	Drug: Peroxide Carbamide 10% - Dental bleaching treatment During 3 hours in 3 weeks of bleaching in both groups in healthy patients and smokers patients Other Name: FGM Peroxide Carbamide (Joinville , Brazil)
Study Arm (s)	• Active Comparator: healthy patients bleaching Healthy patients Peroxide Carbamide 10% - Dental bleaching treatment Intervention: Drug: Peroxide Carbamide 10% - Dental bleaching treatment

	• Experimental: Smokers bleaching smokers patients Peroxide Carbamide 10% - Dental bleaching treatment Intervention: Drug: Peroxide Carbamide 10% - Dental bleaching treatment
Publications *	de Geus JL, Bersezio C, Urrutia J, Yamada T, Fernández E, Loguercio AD, Reis A, Kossatz S. Effectiveness of and tooth sensitivity with at-home bleaching in smokers: a multicenter clinical trial. J Am Dent Assoc. 2015 Apr;146(4):233-40. doi: 10.1016/j.adaj.2014.12.014.

*** Includes publications given by the data provider as well as publications identified by ClinicalTrials.gov Identifier (NCT Number) in Medline.**

Recruitment Information

Recruitment Status ICMJE	Completed
Enrollment ICMJE	120
Completion Date	January 2014
Primary Completion Date	January 2014 (final data collection date for primary outcome measure)
Eligibility Criteria ICMJE	Inclusion Criteria: Patients included in this study must be over 18 years with good general and oral health Free teeth having carious lesions and periodontal disease agree with the informed consent document The color of the upper anterior teeth is classified as A2 or greater value, according to the scale VITA Classical (Vita Zahnfabrik, Bad Sackingen, Germany) and the spectrophotometer Easyshade Vita (Vita Zahnfabrik, Bad Sackingen, Germany). Exclusion Criteria: Have already made treatment tooth whitening Dental prosthesis having options at the upper front teeth Who are pregnant or lactating

	Presenting gingival recession Tooth sensitivity Endodontic treatment in anterior maxillary teeth Which have a severe internal coloring Cervical lesions carious Taking medications (AINES) Fixed orthodontic appliances Submit bruxism habits Having visible cracks in teeth Those who are not available to attend the controls.
Gender	Both
Ages	18 Years and older (Adult, Senior)
Accepts Healthy Volunteers	Yes
Contacts ICMJE	*Contact information is only displayed when the study is recruiting subjects*
Listed Location Countries ICMJE	Brazil, Chile
Removed Location Countries	

Administrative Information

NCT Number ICMJE	NCT02017873
Other Study ID Numbers ICMJE	Nº 2013/41 FOUCH
Has Data Monitoring Committee	Yes
Plan to Share Data	*Not Provided*
IPD Description	*Not Provided*
Responsible Party	Eduardo Fernandez, University of Chile
Study Sponsor ICMJE	University of Chile
Collaborators ICMJE	*Not Provided*

Investigators ICMJE	Principal Investigator:	Eduardo Fernandez, Prof.	University of Chile
Information Provided By	University of Chile		
Verification Date	May 2015		

ICMJE **Data element required by the International Committee of Medical Journal Editors and the World Health Organization ICTRP**

ANEXO C

UNIVERSIDADE ESTADUAL DE PONTA GROSSA - UEPG

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Quantificação da nicotina em dentes submetidos à fumaça de cigarro - análise em GC/MS

Pesquisador: Stella Kossatz Pereira

Área Temática:

Versão: 1

CAAE: 44733215.4.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Universidade Estadual de Ponta Grossa

DADOS DO PARECER

Número do Parecer: 1.065.444

Data da Relatoria: 28/05/2015

Apresentação do Projeto:

O objetivo do estudo será quantificar a nicotina presente em dentes expostos à fumaça de cigarro, através da cromatografia gasosa. Sessenta
dentes serão submetidos à fumaça de cigarro em uma máquina própria para tal função. Os outros vinte dentes servirão como grupo controle. Os
dentes dentes expostos serão divididos em 3 grupos: expostos (G1), expostos + profilaxia (G2) e expostos + clareamento dental (G3). Os dentes
serão pulverizados em um moinho de bolas. Vinte miligramas do pó dos dentes serão adicionados a 1 mL de NaOH, em aquecimento, durante 30
min. A solução será submetida a um processo de extração em fase sólida. Um volume de 3 mL serrá injetado no sistema GC-MS acoplado a um
espectrômetro de massas. Os íons selecionados para cada compostos serão: m / z 84, 133 e 161 para a nicotina e m / z 168 e 169 para a
difenilamina, que será usado como padrão interno. Espera-se encontrar uma quantidade significativamente maior de nicotina em dentes expostos à
fumaça de cigarro, quando comparados àqueles não expostos, e que a profilaxia e o clareamento dental diminuam a quantidade de nicotina

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Telefone: (42)3220-3108 **E-mail:** coep@uepg.br

presente nos dentes.

Objetivo da Pesquisa:

Objetivo Primário:

Quantificar a nicotina em dentes expostos à fumaça de cigarro através da cromatografia gasosa.

Objetivo Secundário:

Validação do método de quantificação da nicotina em dentes utilizando a cromatografia gasosa.

Avaliação dos Riscos e Benefícios:

Riscos:

Durante a exposição dos dentes à fumaça de cigarro, o operador pode inalar a fumaça do cigarro. Por isso ele deve estar paramentado e utilizando
máscara e os outros componentes do EPI.

Benefícios:

Este estudo abrirá portas para utilizar o GC-MS como metodologia de quantificação de diversas substâncias em dentes.

Comentários e Considerações sobre a Pesquisa:

Espera-se encontrar uma quantidade significativamente maior de nicotina em dentes expostos à fumaça de cigarro, quando comparados àqueles
não expostos. Espera-se que a profilaxia e o clareamento dental diminuam a quantidade de nicotina presente nos dentes dos grupos expostos.

Considerações sobre os Termos de apresentação obrigatória:

Em anexo

Recomendações:

Não se aplica

Conclusões ou Pendências e Lista de Inadequações:

Aprovado

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

UNIVERSIDADE ESTADUAL DE PONTA GROSSA - UEPG

Continuação do Parecer: 1.065.444

PONTA GROSSA, 15 de Maio de 2015

Assinado por:
ULISSES COELHO
(Coordenador)

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ANEXO D

UNIVERSITY of York
Centre for Reviews and Dissemination

NHS
National Institute for Health Research

PROSPERO International prospective register of systematic reviews

Review title and timescale

1 Review title
Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.
Does the smoking habit increase the mucronuclei frequency in the oral mucosa of adults compared to non-smokers? A systematic review.

2 Original language title
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.
O hábito de fumar aumenta a frequência de micronúcleos na mucosa oral de adultos comparados com não-fumantes? Uma revisão sistemática

3 Anticipated or actual start date
Give the date when the systematic review commenced, or is expected to commence.
10/12/2014

4 Anticipated completion date
Give the date by which the review is expected to be completed.
15/07/2015

5 Stage of review at time of this submission
Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Juliana de Geus

7 Named contact email
Enter the electronic mail address of the named contact.
ju_degeus@hotmail.com

8 Named contact address
Enter the full postal address for the named contact.
Barbosa Lima Street, 50. Uvaranas, Ponta Grossa, Paran, Brazil. ZIP CODE: 84.020-180

9 Named contact phone number
Enter the telephone number for the named contact, including international dialing code.
(+5542) 8402-3103

10 Organisational affiliation of the review

Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.
none

Website address:
none

11 Review team members and their organisational affiliations
Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Miss	Juliana	de Geus	State University of Ponta Grossa
Miss	Letícia Maíra	Wambier	State University of Ponta Grossa
Professor	Alessandra	Reis	State University of Ponta Grossa
Professor	Stella	Kossatz	State University of Ponta Grossa

12 Funding sources/sponsors
Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.
none

13 Conflicts of interest
List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
Are there any actual or potential conflicts of interest?
None known

14 Collaborators
Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details
Professor	Ana Cláudia	Chibinski	State University of Ponta Grossa

Review methods

15 Review question(s)
State the question(s) to be addressed / review objectives. Please complete a separate box for each question.
Does the smoking habit increase the mucronuclei frequency in the oral mucosa in adults compared to non-smokers?

16 Searches
Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.
A systematic review will be performed to evaluate the frequency of micronuclei in the oral mucosa of smokers and non-smokers in adult patients. Methods: A comprehensive search will be carried out in the MEDLINE via PubMeb, Scopus, Web of Science, LILACS, BBO and Cochrane Library and SIGLE without restrictions. The annual conference of the IADR abstracts (1990–2014), and unpublished and ongoing trials registry will be also searched. Dissertations and theses will be searched using the ProQuest Dissertations and Periodicos Capes Theses Databases.

17 URL to search strategy
If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

I give permission for this file to be made publicly available
No

18 Condition or domain being studied

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Smokers and non-smokers who underwent exfoliative cytology in the oral mucosa to assess DNA damage.

19 Participants/population
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Inclusion criteria: smokers and non-smokers adults. Exclusion criteria: adults that use other types of drugs, children.

20 Intervention(s), exposure(s)
Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
The smoking habit.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
Adults who don't have the smoking habit.

22 Types of study to be included initially
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
We will include cross-sectional and prospective studies.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
Frequency of micronuclei in oral mucosa.

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
Other types of DNA damage, for example binucleated, karyorrhectic, karyolytic, pycnotic, and condensed chromatin cells.

Give information on timing and effect measures, as appropriate.

26 Data extraction, (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.
The titles and/or abstracts of studies retrieved using the search strategy and those from additional sources will be screened independently by two review authors to identify studies that potentially meet the inclusion criteria outlined above. The full text of these potentially eligible studies will be retrieved and independently assessed for eligibility by two review team members. Any disagreement between them over the eligibility of particular studies will be resolved through discussion with a third reviewer.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
Article quality assessment (Newcastle-Ottawa scale) The internal quality of included studies was assessed using the Newcastle-Ottawa scale, which evaluates the design and quality of nonrandomized studies, and also facilitates incorporation of assessments of quality in the interpretation of the meta-analysis results, albeit not used as a criterion for inclusion or exclusion of articles. The evaluation of each article is given a score consisting in a number of stars from three perspectives: a) selection (maximum: four stars), b) comparability (maximum: two stars), and c) results (maximum: three stars). Thus, when processing the article quality analysis, a maximum of nine stars can be obtained for high-quality studies. Lower-quality studies receive fewer stars.

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.
The extracted data will be analyzed using EndNote Web (Thomson Reuters, NY, USA). Data from eligible studies will be continuous (prevalence of micronuclei).

29 Analysis of subgroups or subsets
Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.
None planned

Review general information

30 Type of review
Select the type of review from the drop down list.
Epidemiologic

31 Language
Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.
English

Will a summary/abstract be made available in English?
Yes

32 Country
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.
Brazil

33 Other registration details
Give the name of any organisation where the systematic review title or protocol is registered together with any unique identification number assigned. If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here.

34 Reference and/or URL for published protocol
Give the citation for the published protocol, if there is one.
Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

I give permission for this file to be made publicly available
No

35 Dissemination plans
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
Do you intend to publish the review on completion?
Yes

36 Keywords
Give words or phrases that best describe the review. (One word per box, create a new box for each term)
Micronucleus tests

Smoking

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Ongoing

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

ANEXO E

QUALITY ASSESSMENT TOOL FOR QUANTITATIVE STUDIES

EPHPP
Effective Public Health Practice Project

COMPONENT RATINGS

A) SELECTION BIAS

(Q1) Are the individuals selected to participate in the study likely to be representative of the target population?

1 Very likely
2 Somewhat likely
3 Not likely
4 Can't tell

(Q2) What percentage of selected individuals agreed to participate?

1 80 - 100% agreement
2 60 – 79% agreement
3 less than 60% agreement
4 Not applicable
5 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

B) STUDY DESIGN

Indicate the study design

1 Randomized controlled trial
2 Controlled clinical trial
3 Cohort analytic (two group pre + post)
4 Case-control
5 Cohort (one group pre + post (before and after))
6 Interrupted time series
7 Other specify _____
8 Can't tell

Was the study described as randomized? If NO, go to Component C.

No Yes

If Yes, was the method of randomization described? (See dictionary)

No Yes

If Yes, was the method appropriate? (See dictionary)

No Yes

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

C) CONFOUNDERS

(01) **Were there important differences between groups prior to the intervention?**

1 Yes
2 No
3 Can't tell

The following are examples of confounders:

1 Race
2 Sex
3 Marital status/family
4 Age
5 SES (income or class)
6 Education
7 Health status
8 Pre-intervention score on outcome measure

(02) **If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g. stratification, matching) or analysis)?**

1 80 – 100% (most)
2 60 – 79% (some)
3 Less than 60% (few or none)
4 Can't Tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

D) BLINDING

(01) **Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants?**

1 Yes
2 No
3 Can't tell

(02) **Were the study participants aware of the research question?**

1 Yes
2 No
3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

E) DATA COLLECTION METHODS

(01) **Were data collection tools shown to be valid?**

1 Yes
2 No
3 Can't tell

(02) **Were data collection tools shown to be reliable?**

1 Yes
2 No
3 Can't tell

RATE THIS SECTION	STRONG	MODERATE	WEAK
See dictionary	1	2	3

F) WITHDRAWALS AND DROP-OUTS

(Q1) Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group?

1 Yes
2 No
3 Can't tell
4 Not Applicable (i.e. one time surveys or interviews)

(Q2) Indicate the percentage of participants completing the study. (If the percentage differs by groups, record the lowest).

1 80 -100%
2 60 - 79%
3 less than 60%
4 Can't tell
5 Not Applicable (i.e. Retrospective case-control)

RATE THIS SECTION	STRONG	MODERATE	WEAK	
See dictionary	1	2	3	Not Applicable

G) INTERVENTION INTEGRITY

(Q1) What percentage of participants received the allocated intervention or exposure of interest?

1 80 -100%
2 60 - 79%
3 less than 60%
4 Can't tell

(Q2) Was the consistency of the intervention measured?

1 Yes
2 No
3 Can't tell

(Q3) Is it likely that subjects received an unintended intervention (contamination or co-intervention) that may influence the results?

4 Yes
5 No
6 Can't tell

H) ANALYSES

(Q1) Indicate the unit of allocation (circle one)

community organization/institution practice/office individual

(Q2) Indicate the unit of analysis (circle one)

community organization/institution practice/office individual

(Q3) Are the statistical methods appropriate for the study design?

1 Yes
2 No
3 Can't tell

(Q4) Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

1 Yes
2 No
3 Can't tell

GLOBAL RATING

COMPONENT RATINGS

Please transcribe the information from the gray boxes on pages 1-4 onto this page. See dictionary on how to rate this section.

A	**SELECTION BIAS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
B	**STUDY DESIGN**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
C	**CONFOUNDERS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
D	**BLINDING**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
E	**DATA COLLECTION METHOD**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	
F	**WITHDRAWALS AND DROPOUTS**	**STRONG**	**MODERATE**	**WEAK**	
		1	2	3	Not Applicable

GLOBAL RATING FOR THIS PAPER (circle one):

1 STRONG (no WEAK ratings)
2 MODERATE (one WEAK rating)
3 WEAK (two or more WEAK ratings)

With both reviewers discussing the ratings:

Is there a discrepancy between the two reviewers with respect to the component (A-F) ratings?

No Yes

If yes, indicate the reason for the discrepancy

1 Oversight
2 Differences in interpretation of criteria
3 Differences in interpretation of study

Final decision of both reviewers (circle one):

1 STRONG
2 MODERATE
3 WEAK

ANEXO F

Quality Assessment Tool for Quantitative Studies Dictionary

EPHPP
Effective Public Health Practice Project

The purpose of this dictionary is to describe items in the tool thereby assisting raters to score study quality. Due to under-reporting or lack of clarity in the primary study, raters will need to make judgements about the extent that bias may be present. When making judgements about each component, raters should form their opinion based upon information contained in the study rather than making inferences about what the authors intended.

A) SELECTION BIAS

(Q1) Participants are more likely to be representative of the target population if they are randomly selected from a comprehensive list of individuals in the target population (score very likely). They may not be representative if they are referred from a source (e.g. clinic) in a systematic manner (score somewhat likely) or self-referred (score not likely).

(Q2) Refers to the % of subjects in the control and intervention groups that agreed to participate in the study before they were assigned to intervention or control groups.

B) STUDY DESIGN

In this section, raters assess the likelihood of bias due to the allocation process in an experimental study. For observational studies, raters assess the extent that assessments of exposure and outcome are likely to be independent. Generally, the type of design is a good indicator of the extent of bias. In stronger designs, an equivalent control group is present and the allocation process is such that the investigators are unable to predict the sequence.

Randomized Controlled Trial (RCT)

An experimental design where investigators randomly allocate eligible people to an intervention or control group. A rater should describe a study as an RCT if the randomization sequence allows each study participant to have the same chance of receiving each intervention and the investigators could not predict which intervention was next. If the investigators do not describe the allocation process and only use the words 'random' or 'randomly', the study is described as a controlled clinical trial.

See below for more details.

Was the study described as randomized?

Score YES, if the authors used words such as random allocation, randomly assigned, and random assignment.

Score NO, if no mention of randomization is made.

Was the method of randomization described?

Score YES, if the authors describe any method used to generate a random allocation sequence.

Score NO, if the authors do not describe the allocation method or describe methods of allocation such as alternation, case record numbers, dates of birth, day of the week, and any allocation procedure that is entirely transparent before assignment, such as an open list of random numbers of assignments.
If NO is scored, then the study is a controlled clinical trial.

Was the method appropriate?

Score YES, if the randomization sequence allowed each study participant to have the same chance of receiving each intervention and the investigators could not predict which intervention was next. Examples of appropriate approaches include assignment of subjects by a central office unaware of subject characteristics, or sequentially numbered, sealed, opaque envelopes.

Score NO, if the randomization sequence is open to the individuals responsible for recruiting and allocating participants or providing the intervention, since those individuals can influence the allocation process, either knowingly or unknowingly.

If NO is scored, then the study is a controlled clinical trial.

Controlled Clinical Trial (CCT)
An experimental study design where the method of allocating study subjects to intervention or control groups is open to individuals responsible for recruiting subjects or providing the intervention. The method of allocation is transparent before assignment, e.g. an open list of random numbers or allocation by date of birth, etc.

Cohort analytic (two group pre and post)
An observational study design where groups are assembled according to whether or not exposure to the intervention has occurred. Exposure to the intervention is not under the control of the investigators. Study groups might be non-equivalent or not comparable on some feature that affects outcome.

Case control study
A retrospective study design where the investigators gather 'cases' of people who already have the outcome of interest and 'controls' who do not. Both groups are then questioned or their records examined about whether they received the intervention exposure of interest.

Cohort (one group pre + post (before and after)
The same group is pretested, given an intervention, and tested immediately after the intervention. The intervention group, by means of the pretest, act as their own control group.

Interrupted time series
A time series consists of multiple observations over time. Observations can be on the same units (e.g. individuals over time) or on different but similar units (e.g. student achievement scores for particular grade and school). Interrupted time series analysis requires knowing the specific point in the series when an intervention occurred.

C) CONFOUNDERS

By definition, a confounder is a variable that is associated with the intervention or exposure and causally related to the outcome of interest. Even in a robust study design, groups may not be balanced with respect to important variables prior to the intervention. The authors should indicate if confounders were controlled in the design (by stratification or matching) or in the analysis. If the allocation to intervention and control groups is randomized, the authors must report that the groups were balanced at baseline with respect to confounders (either in the text or a table).

D) BLINDING

(Q1) Assessors should be described as blinded to which participants were in the control and intervention groups. The purpose of blinding the outcome assessors (who might also be the care providers) is to protect against detection bias.

(Q2) Study participants should not be aware of (i.e. blinded to) the research question. The purpose of blinding the participants is to protect against reporting bias.

E) DATA COLLECTION METHODS

Tools for primary outcome measures must be described as reliable and valid. If 'face' validity or 'content' validity has been demonstrated, this is acceptable. Some sources from which data may be collected are described below:

Self reported data includes data that is collected from participants in the study (e.g. completing a questionnaire, survey, answering questions during an interview, etc.).

Assessment/Screening includes objective data that is retrieved by the researchers. (e.g. observations by investigators).

Medical Records/Vital Statistics refers to the types of formal records used for the extraction of the data.

Reliability and validity can be reported in the study or in a separate study. For example, some standard assessment tools have known reliability and validity.

F) WITHDRAWALS AND DROP-OUTS

Score **YES** if the authors describe BOTH the numbers and reasons for withdrawals and drop-outs.

Score **NO** if either the numbers or reasons for withdrawals and drop-outs are not reported.

The percentage of participants completing the study refers to the % of subjects remaining in the study at the final data collection period in all groups (i.e. control and intervention groups).

G) INTERVENTION INTEGRITY

The number of participants receiving the intended intervention should be noted (consider both frequency and intensity). For example, the authors may have reported that at least 80 percent of the participants received the complete intervention. The authors should describe a method of measuring if the intervention was provided to all participants the same way. As well, the authors should indicate if subjects received an unintended intervention that may have influenced the outcomes. For example, co-intervention occurs when the study group receives an additional intervention (other than that intended). In this case, it is possible that the effect of the intervention may be over-estimated. Contamination refers to situations where the control group accidentally receives the study intervention. This could result in an under-estimation of the impact of the intervention.

H) ANALYSIS APPROPRIATE TO QUESTION

Was the quantitative analysis appropriate to the research question being asked?

An intention-to-treat analysis is one in which all the participants in a trial are analyzed according to the intervention to which they were allocated, whether they received it or not. Intention-to-treat analyses are favoured in assessments of effectiveness as they mirror the noncompliance and treatment changes that are likely to occur when the intervention is used in practice, and because of the risk of attrition bias when participants are excluded from the analysis.

Component Ratings of Study:

For each of the six components A – F, use the following descriptions as a roadmap.

A) SELECTION BIAS

Strong: The selected individuals are very likely to be representative of the target population (Q1 is 1) **and** there is greater than 80% participation (Q2 is 1).

Moderate: The selected individuals are at least somewhat likely to be representative of the target population (Q1 is 1 or 2); **and** there is 60 - 79% participation (Q2 is 2). 'Moderate' may also be assigned if Q1 is 1 or 2 and Q2 is 5 (can't tell).

Weak: The selected individuals are not likely to be representative of the target population (Q1 is 3); **or** there is less than 60% participation (Q2 is 3) **or** selection is not described (Q1 is 4); and the level of participation is not described (Q2 is 5).

B) DESIGN

Strong: will be assigned to those articles that described RCTs and CCTs.

Moderate: will be assigned to those that described a cohort analytic study, a case control study, a cohort design, or an interrupted time series.

Weak: will be assigned to those that used any other method or did not state the method used.

C) CONFOUNDERS

Strong: will be assigned to those articles that controlled for at least 80% of relevant confounders (Q1 is 2); **or** (Q2 is 1).

Moderate: will be given to those studies that controlled for 60 – 79% of relevant confounders (Q1 is 1) **and** (Q2 is 2).

Weak: will be assigned when less than 60% of relevant confounders were controlled (Q1 is 1) **and** (Q2 is 3) **or** control of confounders was not described (Q1 is 3) **and** (Q2 is 4).

D) BLINDING

Strong: The outcome assessor is not aware of the intervention status of participants (Q1 is 2); **and** the study participants are not aware of the research question (Q2 is 2).

Moderate: The outcome assessor is not aware of the intervention status of participants (Q1 is 2); **or** the study participants are not aware of the research question (Q2 is 2); **or** blinding is not described (Q1 is 3 and Q2 is 3).

Weak: The outcome assessor is aware of the intervention status of participants (Q1 is 1); **and** the study participants are aware of the research question (Q2 is 1).

E) DATA COLLECTION METHODS

Strong: The data collection tools have been shown to be valid (Q1 is 1); **and** the data collection tools have been shown to be reliable (Q2 is 1).

Moderate: The data collection tools have been shown to be valid (Q1 is 1); **and** the data collection tools have not been shown to be reliable (Q2 is 2) **or** reliability is not described (Q2 is 3).

Weak: The data collection tools have not been shown to be valid (Q1 is 2) **or** both reliability and validity are not described (Q1 is 3 and Q2 is 3).

F) WITHDRAWALS AND DROP-OUTS - a rating of:

Strong: will be assigned when the follow-up rate is 80% or greater (Q2 is 1).

Moderate: will be assigned when the follow-up rate is 60 – 79% (Q2 is 2) **OR** Q2 is 5 (N/A).

Weak: will be assigned when a follow-up rate is less than 60% (Q2 is 3) or if the withdrawals and drop-outs were not described (Q2 is 4).