UNIVERSIDADE NOVE DE JULHO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOFOTÔNICA APLICADA ÀS CIÊNCIAS DA SAÚDE

RÚBIA GARCIA LOPES

ENSAIO CLÍNICO CONTROLADO DO USO DA TERAPIA FOTODINÂMICA EM ADOLESCENTES COM HALITOSE

São Paulo, SP
2014

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Dissertação apresentada à Universidade Nove de Julho, para obtenção do título de Mestre em Biofotônica aplicada às Ciências da Saúde

.

Orientadora: Profa. Dra. Sandra Kalil Bussadori

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Dissertação apresentada à Universidade Nove de Julho, para obtenção do título de Mestre em Biofotônica aplicada às Ciências da Saúde

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São Paulo, de de 2014.

DEDICATÓRIA

Dedico esse trabalho a quem me guiou no caminho da vida, ensinou a importância do buscar pela sabedoria e confiou sua vida em mim até o fim: Senhor Gera, meu tão saudoso pai.

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A toda minha família pelo incentivo e amor que sempre recebo.

RESUMO

A halitose é um termo utilizado para definir o odor desagradável e fétido que emana da boca, podendo apresentar origem sistêmica (10%) ou oral (90%). O mau odor é provocado principalmente por compostos sulforados voláteis, produzido pela ação de bactérias Gram-negativas. A luz acompanhada ou não de agentes químicos tem sido usada para induzir efeitos terapêuticos e antimicrobianos na terapia fotodinâmica (TFD), sendo que o efeito antimicrobiano fica confinado apenas às áreas cobertas pelo corante e irradiadas pela luz. O objetivo deste estudo foi avaliar o efeito antimicrobiano da TFD em adolescentes com halitose, pela análise da concentração de compostos sulforados voláteis, mensurado por cromatografia gasosa (OralChroma ^{TM}). Por meio de estudo clínico controlado, 45 adolescentes foram avaliados e divididos aleatoriamente em 3 grupos que receberam tratamentos distintos: grupo 1 tratamento com TFD aplicada na região de dorso e terço médio da língua, grupo 2 raspador lingual e grupo 3 tratamento combinado de raspador lingual e TFD. O diagnóstico de halitose foi realizado antes e depois do tratamento pela cromatorgrafia gasosa. Foi aplicado o teste de Kruskal-Wallis para comparação seguido do teste Student-Newman-Keuls. Para todas as análises foi considerado um nível de significância de $\alpha=0,05$. Após o tratamento houve redução estatisticamente significante para todos os grupos ($p < 0,001$), contudo a associação da terapia fotodinâmica ao raspador lingual mostrou ser mais eficiente na redução total de sulfidretos (mediana=0). Conclui-se portanto, que esse estudo traz uma nova opção de tratamento para halitose, com efeito imediato e sem agressão mecânica as papilas linguais comum ao tratamento convencional com raspadores.

PALAVRAS CHAVE: halitose, terapia fotodinâmica, adolescentes

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ABSTRACT

Halitosis is a term used to define the unpleasant breath that may have a systemic or oral origin. Volatile sulfur compounds produced by the Gram-negative bacteria mainly cause the bad breath. Using light - along with by chemical agents or not - is common to induce therapeutic and antimicrobial effects in the photodynamic therapy, and the antimicrobial effect happens only in the areas covered by the dye and irradiated by light. The aim of this study was to evaluate the antimicrobial effect of the photodynamic therapy in adolescent halitosis, analyzing the volatile sulfur compounds concentration, measured by gas chromatography (OralChroma^{TM}). 45 adolescents were assessed and randomly divided (through controlled clinical study) into 3 groups that received different treatments: group 1 treatment with photodynamic therapy applied on the back (dorsum) region and on the middle third of the tongue, group 2 tongue scraper and group 3 treatment with tongue scraper and photodynamic therapy. The halitosis diagnosis was performed before and after the OralChroma treatment. The Kruskal-Wallis test was applied and compared with the Student-Newman-Keuls test. The $\alpha = 0.05$ significance level was considered for all analysis. After the treatment, there was a statistically significant decline on all groups ($p < 0.001$); however, the photodynamic therapy and tongue scraper treatment proved to be more efficient to fully reduce the hydrogen sulfides (median = 0). This study provides a new option for treating adolescent halitosis with immediate effects without mechanical aggression to the lingual papillae, which is common in the conventional treatment with tongue scrapers.

Keywords: halitosis, photodynamic therapy, adolescents.

Clinical Trials Registration - NCT02007993

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SUMÁRIO

LISTA DE TABELAS E QUADROS

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

CSV – Compostos sulforados voláteis

SH_2 – Sulfidreto

CH_3SH – Metilmercaptanas

CH_3SCH_3 – Dimetilsulfeto

TFD – Terapia fotodinâmica

FS – fotossensibilizador

Ppb – parte por bilhão

1. Contextualização

A Halitose, também conhecida como mau hálito, é um termo utilizado para definir um odor desagradável e fétido que emana da boca, podendo apresentar origem local ou sistêmica (QUIRYNEN et al., 2004; ROSENBERG; MCCULLOCH, 1992; SHIMURA et al., 1997). É considerado um problema comum que afeta grande parte da população mundial e causa constrangimento tanto para quem a possui como para as pessoas com as quais o indivíduo convive, é um fator negativo importante na comunicação social, com impacto direto na qualidade de vida (KIZHNER; XU; KRESPI, 2011). Estudos sobre etiologia da halitose mostram que 2% dos casos estão relacionados a síndromes metabólicas, alterações renais, hepáticas, endocrinológicas e gastrointestinais (como infecções por *Helicobacter pylori* e obstrução intestinal), 8% por alterações respiratórias e otorrinolaringológicas como amigdalite aguda, presença de escorrimento nasal posterior e sinusites, 80-90% dos casos estão diretamente ligados as condições da cavidade oral, como a presença de doença periodontal (13%), saburra lingual (51%) ou a combinação de ambos (22%), pobre higiene oral, alterações salivares (mudança do pH e hiposialia), entre outras causas (estomatite, neoplasia intra-oral, exposição pulpar, feridas pós extração e apinhamento dentário) (AMIR; SHIMONOV; ROSENBERG, 1999; BOLLEN; BEIKLER, 2012; DAL RIO et al., 2006; MAROCCHIO; CONCEIÇÃO; TÁRZIA, 2009; QUIRYNEN et al., 2009).

O mau hálito é provocado principalmente por compostos sulforados voláteis (CSV), produzidos pela ação de bactérias Gram-negativas anaeróbias (*Fusobacterium nucleatum, Selenomonas, Treponema denticola, Prevotella intermedia, Tannerella Forsythensis, Porphyromonas gingivalis, Bacteroides forsythus and Eubacterium*) (LIU; ZHU; HUANG, 2009) sobre substratos contendo enxofre encontrados na boca (RAANGS; WINKEL; VAN WINKELHOFF, 2013; SALAKO; PHILIP, 2011). Os CSV produzidos a partir desse metabolismo são: sulfidreto (SH_2) - encontrados principalmente em dorso lingual - metilmercaptana (CH_3SH) - presentes no sulco gengival - e dimetilsulfeto (CH_3SCH_3) - origem extra-oral (CALIL; MARCONDES, 2006; SPRINGFIELD et al., 2001; TANGERMAN; WINKEL, 2008; TOLENTINO; CHINELLATO; TARZIA, 2011), e a concentração desses gases é usada como

indicador da halitose (ROSENBERG; MCCULLOCH, 1992; ROSENBERG et al., 1991). Recentemente a bactéria Gram-positiva anaeróbia *Solobacterium moorei* (conhecida como *Bulleidia moorei*) também foi associada a halitose pela produção SH_2 na presença de diferentes suplementações com aminoácidos, em especial a cisteína (HARASZTHY et al., 2008; TANABE; GRENIER, 2012). Pesquisas vêm demonstrando que a presença destas bactérias no dorso lingual, saliva e sulco periodontal podem desencadear além da halitose problemas sistêmicos como complicações na gravidez, doenças cardiovasculares e principalmente infecção respiratório baixa (SILVESTRI et al., 2014) considerada a terceira causa mais comum de mortalidade (BANSAL; KHATRI; TANEJA, 2013; CHRISTENSEN, 1998; QUIRYNEN et al., 2004; TANAKA et al., 2004; TARZIA, 2003).

Há dois principais métodos usados para avaliar o hálito: avaliação subjetiva (organoléptica) e avaliação objetiva (cromatografia gasosa e monitor de sulfeto) (KARA; TEZEL; ORBAK, 2006; KARA et al., 2008). Estudos realizados comparando a eficácia dos testes apontaram a cromatografia gasosa como método objetivo mais eficaz (BOLLEN; BEIKLER, 2012; TANGERMAN; WINKEL, 2008), e atualmente considerada padrão ouro da literatura (SALAKO; PHILIP, 2011). Porém, a maioria dos pesquisadores tem usado a combinação de ambos, outros apenas o organoléptico por ser o método mais barato e fácil de executar (KARA et al., 2008).

No teste organoléptico um juiz treinado e calibrado posicionado a distância de 10 cm, distingue o ar expirado pelo olfato e o resultado é determinado usando a tabela de Rosenberg (“0-5 Rosenberg scale”) (BOLLEN; BEIKLER, 2012; ROSENBERG; MCCULLOCH, 1992). Onde 0 representa ausência de odor, 1 odor dificilmente detectável, 2 odor leve, 3 odor moderado, 4 odor forte e 5 odor extremamente forte. O hálito também pode ser examinado por um monitor de sulfeto como o Halimeter (Interscan Corporation, Chatsworth, CA, USA) (DONALDSON et al., 2007; KIZHNER; XU; KRESPI, 2011; ROSENBERG; MCCULLOCH, 1992; ROSENBERG, 1990), que determina a quantidade total de CSV em partes por bilhão (ppb), em condições normais, e de acordo com o fabricante essa quantidade tem que ser inferior a 80 ppb, contudo, esse equipamento não é capaz de diferenciar a origem ou o tipo CSV, é mais sensível ao sulfidreto que a metilmercaptanas e insensível ao dimetilsulfeto

(FURNE et al., 2002; TANGERMAN; WINKEL, 2008). A cromatografia gasosa é o método mais apropriado para detectar a halitose, em 2004, um novo cromatógrafo gasoso, OralChroma™ (Abilit Corporation, Miyamae-KU Kawasaki-shi, Kanagawa, Japan), foi desenvolvido no Japão para mensuração individual dos 3 principais CSV (sulfidreto, metilmercapitana e dimetilsulfeto), permitindo avaliar a intensidade do hálito e sua origem (BOLLEN; BEIKLER, 2012; SALAKO; PHILIP, 2011; TANGERMAN; WINKEL, 2008).

A falta de padronização no protocolo para diagnóstico e tratamento de halitose dificulta a comparação dos dados epidemiológicos obtidos em diferentes países, mas acredita-se que hoje a população afetada por essa desordem é de aproximadamente 25% (BOLLEN; BEIKLER, 2012).

1.1. Terapia fotodinâmica

A terapia fotodinâmica (TFD) foi descoberta em 1900 por Oskar Raab e Hermann von Tappeiner, e na década de 1970 foi inicialmente desenvolvida como uma terapia para tratamento de câncer. Recentemente, a TFD antimicrobiana tem sido utilizada como uma alternativa para o tratamento das infecções localizadas (DAI et al., 2012).

A TFD engloba o uso de um corante sensível a luz (fotossensibilizador) e não tóxico combinado a uma luz visível de comprimento de onda apropriado para coincidir com o espectro de absorção do fotossensibilizador (FS), que após absorver os fótons atinge um estado de excitação reagindo com o oxigênio do meio, formando espécies reativas de oxigênio (reactive oxygen species - ROS). Essa reação fototóxica induz a destruição da célula bacteriana, porém o efeito antimicrobiano fica confinado apenas às áreas cobertas pelo corante e irradiadas pela luz agindo no organismos alvo rapidamente, dependendo da dose de energia de luz e a saída de potência usada (DAI et al., 2012; FONTANA et al., 2009; HOPE; WILSON, 2006; LIU; ZHU; HUANG, 2009; PERVAIZ; OLIVO, 2006; WILSON, 2004). Além disso, de acordo com Wainwright (WAINWRIGHT, 1998) a resistência bacteriana à TFD é improvável, pois o oxigênio singleto e os radicais livres formados interagem com várias estruturas celulares bacterianas e diferentes caminhos metabólico (HOPE; WILSON, 2006; WILSON, 2004).

A halitose está diretamente relacionada a qualidade de vida e ao convívio social, por ser uma doença com etiologia multifatorial porém relacionada a

presença de bactérias, Gram negativas principalmente (BOLLEN; BEIKLER, 2012). O tratamento convencional da halitose quando relacionado a alterações orais consiste na redução química dos microrganismos com enxaguatórios (clorexidina 0,2%, óleos essenciais, triclosan e água oxigenada), redução mecânica dos nutrientes intra-orais com raspador ou escova lingual, mascaramento do odor (gomas de mascar, tabletes de menta e spray) e transformação do CSV (Zinco associado a clorexidina) (BOLLEN; BEIKLER, 2012; QUIRYNEN; MONGARDINI; VAN STEENBERGHE, 1998; QUIRYNEN et al., 2004; RAANGS; WINKEL; VAN WINKELHOFF, 2013; SAAD; GREENMAN; SHAW, 2011; SAAD; HEWETT; GREENMAN, 2012; TOLENTINO; CHINELLATO; TARZIA, 2011). Por outro lado a redução da carga bacteriana é dificultada devido as características irregulares da superfície do dorso lingual (COLLINS L; DAWES, 1987; QUIRYNEN; MONGARDINI; VAN STEENBERGHE, 1998; QUIRYNEN et al., 2004), revestido por numerosas papilas que se apresentam de 4 diferentes formas: fungiformes, filiformes, circunvaladas e foliadas. As patologias linguais são determinadas pelas características papilares condições da superfície lingual (tamanho, formato, fixação e caraterísticas papilares): língua pilosa, língua revestida, língua fissurada, atrofia papilar, língua geográfica, glossite romboide mediana, língua crenada, macroglossia e anquiloglossia(AVCU; KANLI, 2003).

Sendo assim, frente a essas dificuldades e aos questionamentos referentes ao tratamento preciso da halitose, bem como a ausência de estudos relacionados diretamente ao efeito da TFD na saburra lingual, o objetivo desse estudo foi avaliar a efetividade da aplicação da TFD em dorso de língua, pela análise do nível de CSV em adolescentes com halitose.

2. Justificativa

A halitose está diretamente relacionada a qualidade de vida e ao convívio social (BOLLEN; BEIKLER, 2012), por ser um conjunto de sinais e sintomas com etiologia multifatorial relacionada a presença de bactérias, Gram negativas principalmente, o diagnóstico e padrão ouro da literatura é a cromatografia gasosa, capaz de medir a quantidade e os tipos de CSV presentes no ar expirado (BOLLEN; BEIKLER, 2012; TANGERMAN; WINKEL, 2008).

O tratamento para halitose proposto pela literatura é amplo, e de modo geral consiste na redução mecânica e química da saburra lingual (BOLLEN; BEIKLER, 2012), contudo o uso vigoroso de limpadores linguais pode causar micro hemorragias (SEEMAN et al., 2014). A terapia fotodinâmica é uma possível alternativa conservadora para resolução deste problema. Frente aos questionamentos referentes a diagnóstico e tratamento preciso, bem como a escassez de estudos relacionados diretamente ao efeito da TFD na halitose, propõe-se a avaliação da efetividade da aplicação da TFD no biofilme lingual de adolescentes com halitose, em especial por se tratar de um procedimento rápido, não invasivo, de efeito imediato e sem causar injurias as tecido tratado.

3. Objetivos

O objetivo deste estudo foi analisar a efetividade da aplicação da terapia fotodinâmica em terço médio e dorso lingual de adolescentes, pela avaliação do nível de formação de sulfidreto por meio de cromatografia gasosa.

4. Material e métodos

O estudo seguiu as normas regulamentadoras de pesquisa em seres humanos com parecer favorável do Comitê de Ética em Pesquisa da Universidade Nove de Julho número 313.779/2013 (anexo 1), e os responsáveis pelos participantes assinaram o termo de consentimento livre após esclarecimentos para autorização da participação na pesquisa (Anexo 2), de acordo com a resolução 196/96 do Conselho Nacional Saúde.

4.1. Delineamento

Tipo de Estudo: Estudo clínico, randomizado.

4.1.1. Hipótese

Hipótese nula: Não há alteração da halitose após o uso da terapia fotodinâmica associada ou não ao raspador de língua.
Hipótese experimental: Há diminuição da halitose após o uso da terapia fotodinâmica associada ou não ao raspador de língua.

4.2. Sujeitos da Pesquisa

Para este estudo foram avaliados os adolescentes de ambos os sexos, matriculados regularmente no Instituto Meninos de São Judas Tadeu – São Paulo.

4.2.1. Critérios de Inclusão

Foram incluídos nesta pesquisa: adolescentes na faixa etária de 13 a 18 anos, com termo de consentimento livre e esclarecido (Anexo 2) e autorização para diagnóstico e tratamento da halitose assinados pelo responsável (Anexo 3); e adolescentes com diagnóstico de halitose apresentando resultados Oralchroma com desafio da cisteína SH_2 ≥ 112 ppb (AIZAWA et al., 2005; PHAM et al., 2011; SALAKO; PHILIP, 2011; TANGERMAN; WINKEL, 2008).

4.2.2. Critérios de Exclusão

Faram excluídos do estudo indivíduos (CASEMIRO et al., 2008): com anomalias dentofaciais, em tratamento ortodôntico e/ou ortopédico, com dispositivo removível, implante e/ou prótese, com doença periodontal, com dentes cariados, em tratamento oncológico, com diabetes mellitus, alterações

sistêmicas (gastrointestinais, renais, hepáticas), otorrinolaringológicos e respiratórios, em tratamento com antibiótico até 1 mês antes da pesquisa, grávidas, (BOLLEN; BEIKLER, 2012) e com hipersensibilidade ao FS.

4.2.3. Procedimentos

Por se tratar de um estudo clínico randomizado e buscando uma maior transparência e qualidade dessa pesquisa, utilizamos as recomendações CONSORT (Consolidated Standards of Reporting Trials) (figura 1).

4.3. FLUXOGRAMA

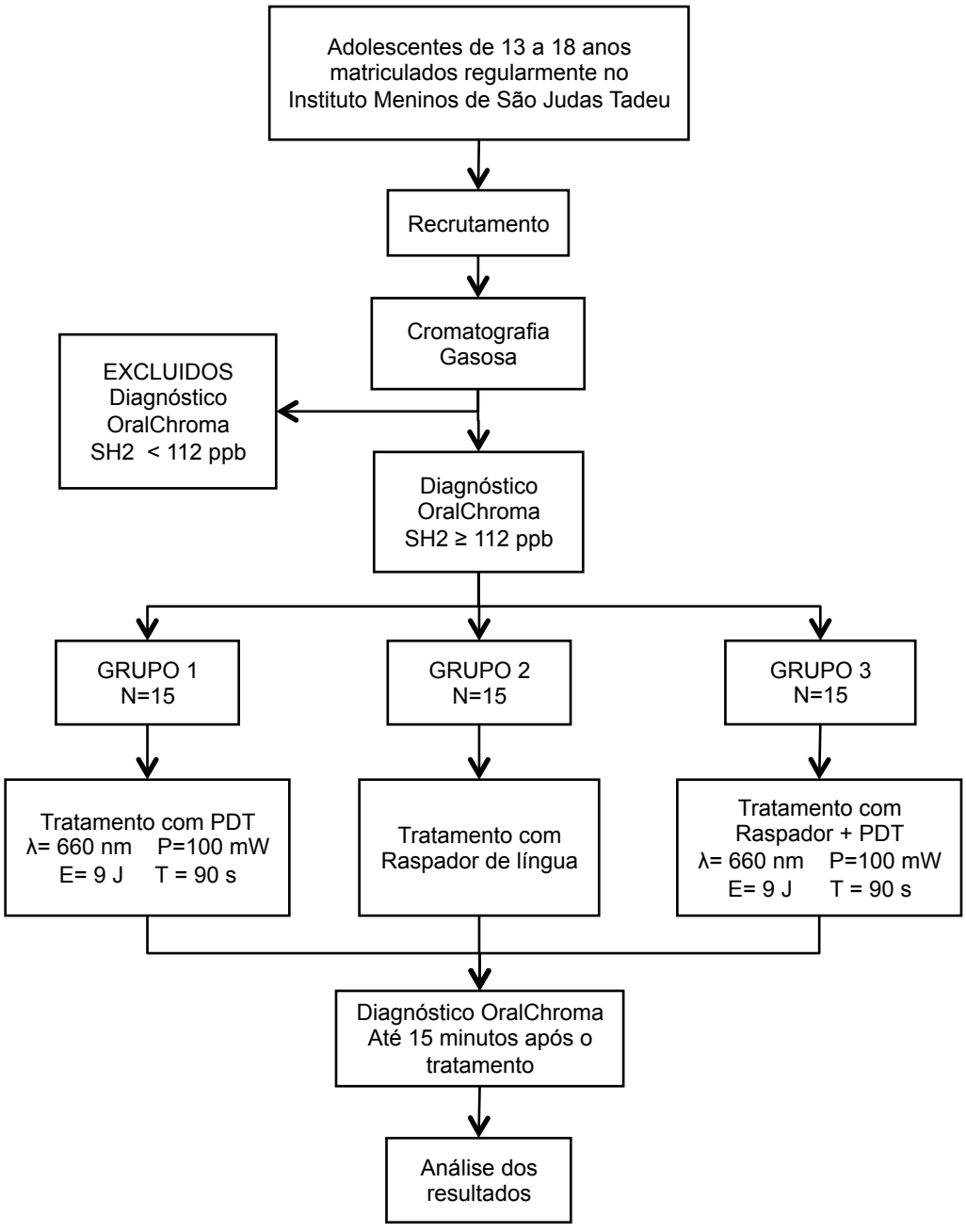

Figura 1 – fluxograma do estudo

Os sujeitos selecionados foram aleatoriamente divididos em 3 grupos por ordem de chegada, onde o primeiro participante foi direcionado ao grupo 1, o segundo ao grupo 2 e o terceiro ao grupo 3, dando continuidade a distribuição dos participantes aos 3 grupos seguindo a mesma ordem, e conforme descrito no quadro 1 todos foram submetidos à avaliação com OralChroma™ antes e depois de cada tratamento proposto.

Quadro 1: Resumo da condição experimental.

Grupo	N	Halitose	Tratamento
1	15	$SH_2 \geq 112$ ppb	TFD E = 9J T= 90s
2	15	$SH_2 \geq 112$ ppb	Raspador Lingual
3	15	$SH_2 \geq 112$ ppb	Raspador lingual + TFD E = 9J T= 90s

4.4. Avaliação do nível de halitose

A literatura descreve alguns métodos de mensuração de halitose, como a avaliação organoléptica do ar emanado da cavidade oral (ROSENBERG, 1990; ROSENBERG et al., 1991), por monitor de sulfeto (KARA et al., 2008; MOTTA L et al., 2011; ROSENBERG et al., 1991) e por cromatografia gasosa, considerado hoje padrão ouro na literatura (MOTTA L et al., 2011; SALAKO; PHILIP, 2011; VANDEKERCKHOVE et al., 2009). Como o teste organoléptico pode ser influenciado pela capacidade olfatória, estado emocional do examinador e por condições climáticas (ROSENBERG; MCCULLOCH, 1992), para este estudo foi utilizado o dispositivo portátil OralChroma™ (Abilit, Japan) (figura 2), que utiliza um sensor de gás semicondutor altamente sensível aos CSV e de fácil utilização.

Figura 2 – OralChroma®

A coleta do ar bucal seguiu as orientações do fabricante (OralChroma^{TM} Manual Instruction), onde o participante foi orientado a fazer bochecho com cisteína (10 mM) por 1 minuto para diferenciar a origem dos CSV e abranger as bactérias Gram-positiva anaeróbia *Solobacterium moorei*, e permanecer com a boca fechada mais 1 minuto. Em seguida foi introduzido na boca do paciente uma seringa estéril do mesmo fabricante, própria para coleta do ar bucal. Durante 1 minuto o paciente permaneceu de boca fechada, respirando pelo nariz, sem tocar na seringa com a língua. Após coleta do ar e limpeza da ponta da seringa foi acoplada uma agulha de injeção de gás e removido o excesso de ar para o conteúdo de 0,5ml e injetado na porta de entrada do aparelho com um movimento único (figura 3) (TANGERMAN; WINKEL, 2008).

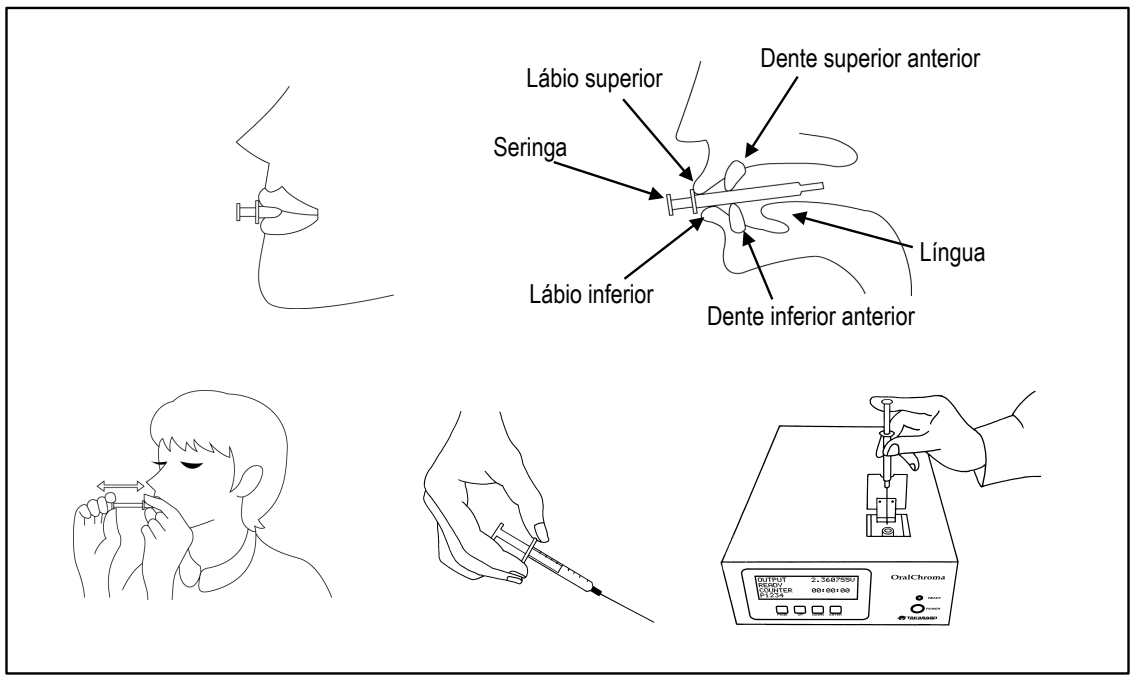

Figura 3 – Processo de realização da halimetria

O OralChroma^{TM}, conectado ao computador (com software especifico) permite a captura de um gráfico correspondente aos picos e valores de concentração dos gases, medindo os limiares dos CSV (de 0 a 2913 ppd), com muita precisão após 8 minutos (figura 4). Os resultados são armazenados tanto no programa quanto no próprio aparelho e podem ser resgatados e visualizados a qualquer momento para comparação antes, durante e após o tratamento.

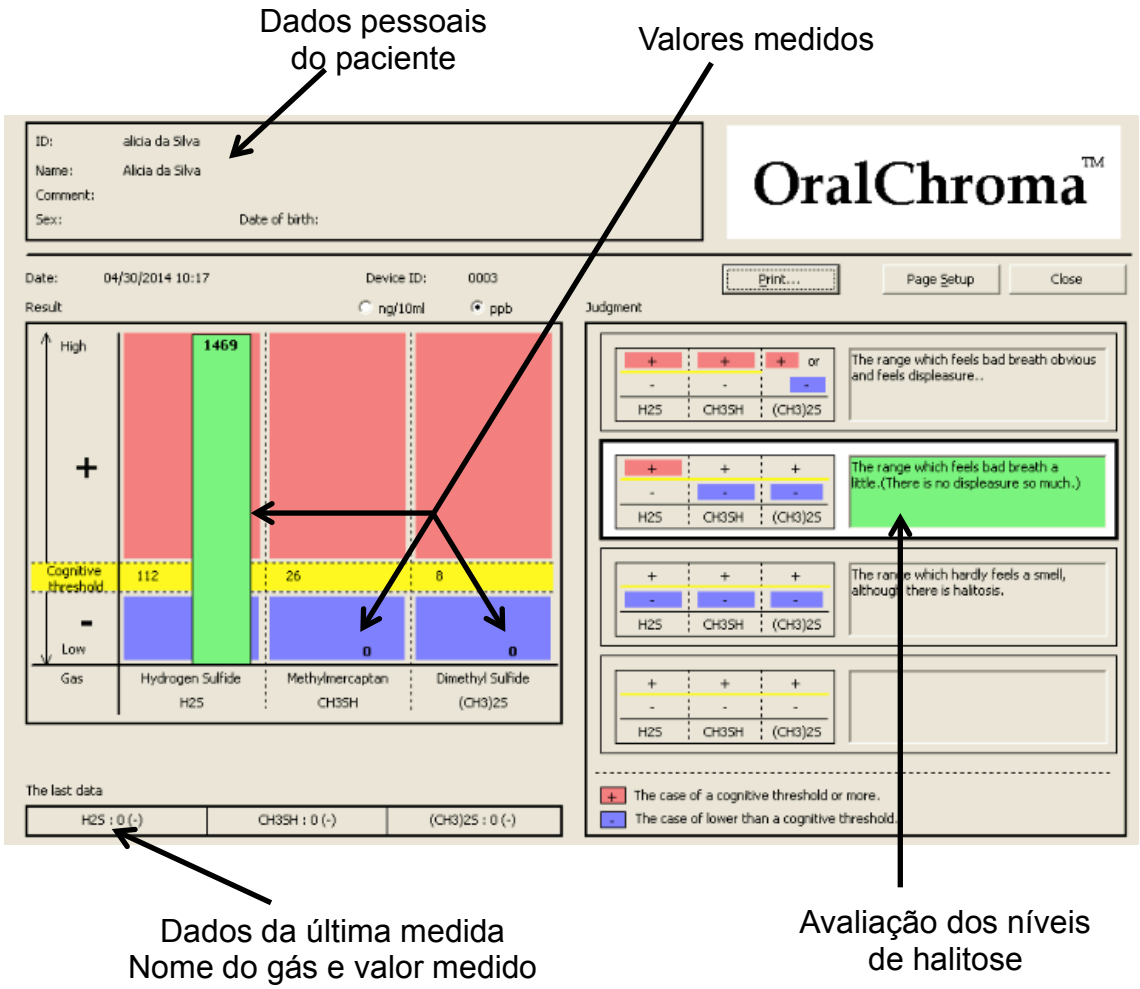

Figura 4 – Resultado obtido pelo software

Da análise dos CSV capturado pelo sistema, temos como indicadores e halitose:

- Sulfidreto: origem principalmente das bactérias presentes no dorso da língua. Valores acima de 112 ppb são indicadores de halitose.

- Metilmercaptana: predominantemente mais elevada nas bolsas periodontais. Valores até 26 ppb são considerados normais. A doença periodontal resulta tipicamente numa alta razão entre metilmercaptana/sulfidreto (>3:1)

- Dimetilsulfeto: tanto pode ser de origem periodontal como de origem sistêmica (intestinal, hepática, pulmonar). Há possibilidade de se fazer a distinção entre o dimetilsulfeto de origem bucal e o de origem sistêmica, através da comparação dos resultados da halimetria feita no oralchroma com e sem o desafio da cisteína (cisteína 10 mM, ou seja 16 mg de cisteína em 100 ml de água destilada – 16 mg%). Outros odores (não CSV) podem aparecer em um pico

anterior ao teoricamente primeiro pico que é o do sulfidreto (TANGERMAN; WINKEL, 2008).

Para evitar alterações na halimetria os participantes foram instruídos a seguir as seguintes orientações: 48 horas antes da avaliação evitar a ingestão de alimentos com alho, cebola e temperos fortes, consumo de álcool e uso de antisséptico bucal. No dia da avaliação, pela manhã, puderam alimentar-se até no máximo 2 horas antes do exame, abster-se de café, balas, goma de mascar, produtos de higiene oral e pessoal com perfume (pós-barba, desodorante, perfume, cremes e/ou tônico) e a escovação foi apenas com água (DONALDSON et al., 2007; QUIRYNEN et al., 2009).

4.5. Aplicação da TFD

Para a terapia fotodinâmica foi utilizado o aparelho THERAPY XT-EC® (DMC ABC Equipamentos Médicos e Odontológicos, SP, BR) (figura 5), com emissão de LASER vermelho (660nm) e infravermelho (810nm), e ponta afilada para uso odontológico, com diâmetro de 0,094 cm.

Figura 5 – Aparelho Therapy XT-EC - DMC ®

No momento da aplicação da TFD estavam presentes somente o voluntário a ser tratado e o profissional responsável, ambos utilizando óculos específicos para proteção ocular. A ponta ativa do laser foi revestida com plástico transparente descartável (PVC) e o profissional foi devidamente paramentado.

Foi realizada 1 sessão de TFD com FS azul de metileno manipulado na concentração de 0,005% (165 µM) e aplicado em quantidade suficiente para

cobrir o terço médio e dorso da língua com tempo de pré irradiação de 5 minutos, o excesso foi removido com sugador de forma a manter a superfície úmida com o próprio FS, sem utilização de água. Foram irradiados 6 pontos com distância de 1 cm entre os pontos, considerando o halo de espalhamento da luz e efetividade da TFD (figura 6). Com base em estudos desenvolvidos no tratamento da doença periodontal com a TFD (BERAKDAR et al., 2012; BRAUN et al., 2008; DILSIZ; CANAKCI; AYDIN, 2013; GIANNELLI et al., 2012; LUI; CORBET; JIN, 2011; LULIC M et al., 2009; POLANSKY et al., 2009) (quadro 2)(BERAKDAR et al., 2012; BRAUN et al., 2008; DILSIZ; CANAKCI; AYDIN, 2013; GIANNELLI et al., 2012; LUI; CORBET; JIN, 2011; LULIC M et al., 2009; POLANSKY et al., 2009) e estudo piloto realizado previamente (LOPES et al., 2014), o aparelho estava previamente calibrado com comprimento de onda 660nm, energia de 9J e potência de 100mW para os grupos 1 e 3 que foram irradiados durante 90 segundos por ponto, fluência de 320 J/cm^2 e irradiância de 3537mW/cm^2, com método de aplicação pontual e em contato direto com a língua.

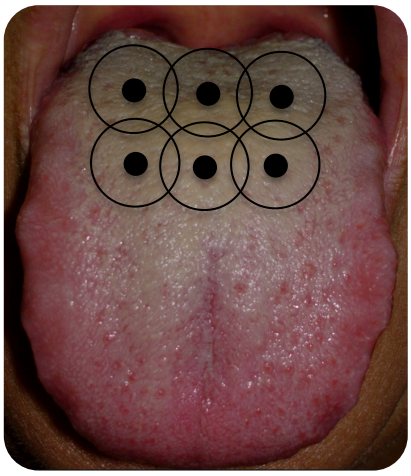

Figura 6 – Pontos de aplicação da TFD

Quadro 2: Características dos estudos da TFD no tratamento da periodontite.

REFERÊNCIA	ANALISE CLINICA	IRRADIAÇÃO	FOTOSSENSIBILIZADOR	MICROBIOLOGICO	RESULTADO
Braun et al. (2008)	CAL BOP PD REC	λ = 660 nm P = 100 mW (HELBO) T = 10s	Cloreto de fenotiazina por 3 min (Helbo Photodynamic Syztems)	Não analisado	SRP+aPDT positivo não significante para REC
Lulic et al. (2009)	CAL BOP PD PI	λ = 670 nm P = 75 mW/cm2 T = 60 s repetiu 1, 2, 7 e 14 dias após	Cloreto de fenotiazina por 3 min (Helbo blue photosensitizer, Helbo Photodynamic Syztems)	Não analisado	positivo após 3 e 6 meses
Polansky et al.(2009)	CAL BOP PD	λ = 680 nm P = 75 mW T = 60 s	Cloreto de fenotiazina (Helbo blue photosensitizer, Helbo Photodynamic Syztems)	P.g. T.f. T.d	P.g.- reduziu signif. T.f. e T.d. - não teve redução significativa BOP - não teve diferença significativa Clinicamente - positivo
Lui et al. (2011)	BOP PD PI REC	comprimento de pulso = 0,05 ms intervalo de pulso = 0,2 ms P = 1W (pico de 5W) T = 30 s	AM 1% por 3 min	P.g. T.f. T.d.	positivo para todos os parâmetros
Berakdar et al. (2012)	CAL BOP PD PI GI REC	λ = 670 nm P = 150 mW T = 60 s	AM 0,005%	Não analisado	SRP+aPDT positivo não significante para PD
Giannelli et al. (2012)	CAL BOP PD	Dentro da bolsa λ = 635 nm P = 100 mW 100mW De = 6,7 J/cm2 J/cm2 T = 60 s Externo λ = 635nm P = De = 3,8 T = 60 s	AM 0,3% por 5 min	Polimorfonuclear Eritrocitos Celulas epiteliais (Cocos, bacilos, espiroquetas)	positivo para todos os parâmetros
Dilsiz et al. (2013)	CAL BOP PD PI GI	λ = 808 nm P = 100 mW T = 60 s E = 6J	AM 1% por 3 min	Não analisado	Não apresentou resultado significativo

Legenda

CAL - nível de inserção clinica
BOP - sangramento a sondagem
PD - profundidade de bolsa
PI - índice de placa
REC - recessão gengicval
BGI - índice de sagramento gengival

λ - comprimento de onda
P - potência
T - tempo
E - energia
De - densidade de Energia

AM - azul de metileno

P.g. - *Porphyromonas gingivalis*
T.f. - *Tannerella forsythia*
T.d. - *Treponema denticola*

SRP - alisamento radicular
aPDT - terapia fotodinâmica antimicrobiana

Quadro 3: Parâmetros do Laser

PARÂMETROS	LASER VERMELHO
Comprimento de onda [nm]	660
Largura espectral (FWHM) [nm]	5
Modo de funcionamento	Contínuo
Potência [mW]	100
Polarização	Random
Diâmetro de abertura [cm]	0,094
Irradiância na abertura [mW/cm^2]	3537
Perfil do feixe	Multimodo
Área do feixe [cm^2]	0,02827
Irradiância no alvo [mW/cm^2]	3537
Tempo de exposição [s]	90
Fluência [J/cm^2]	320
Energia [J]	9
Número de pontos irradiados	6
Área irradiada [cm^2]	0,169
Técnica de aplicação	Contato
Número de sessões e frequência	1 sessão
Energia total irradiada [J]	54

4.6. Cálculo da amostra

Apesar de os dados desse estudo já indicarem uma diferença estatisticamente significativa, o cálculo do tamanho amostral foi aprofundado para considerar também o poder do teste.

Baseado nos dados do estudo, foi feita uma simulação do poder do teste (Kruskal Wallis ANOVA) em função do tamanho amostral, conforme pode ser observar na figura 07.

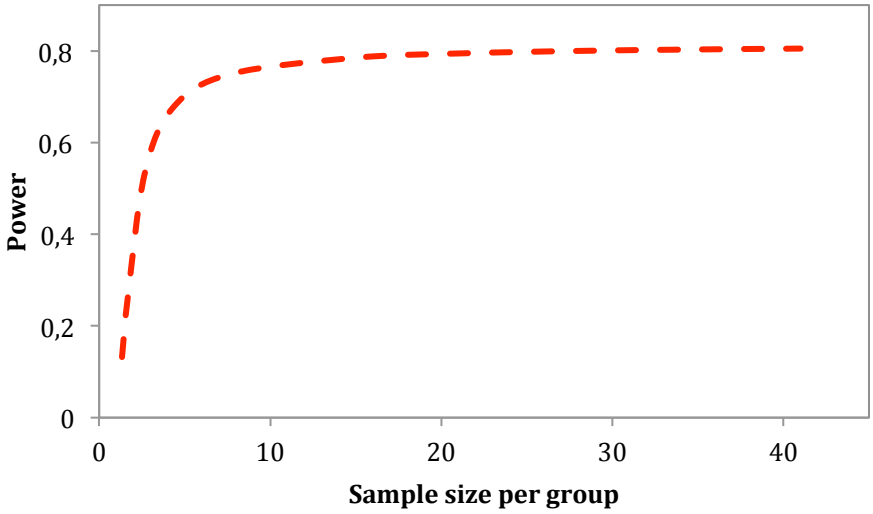

figura 07: poder do teste em função do tamanho amostral

A figura 07 mostra que, para um tamanho amostra N = 15 por grupo, o poder do teste foi de 80%.

4.7. Organização e Tratamento Estatístico dos Dados

Os dados oriundos do OralChorma foram analisados pelo teste de Shapiro – Wilk e a hipótese de normalidade foi rejeitada. Para análise estatística comparativa entre os grupos foram utilizados os testes de Kruskal-Wallis seguido pelo teste de Student-Newman-Keuls. A análise dos resultados de cada tratamento nos dois períodos do estudo foi feita pelo teste de Wilcoxon. Para todas as análises foi considerado um nível de significância $\alpha=0,05$.

5. Resultados

5.1. Artigo 1

Lopes RG, Santi MESO, Franco BE, Deana AM, Prates RA, França CM, Fernandes KPS, Mesquita-Ferrari RA, Bussadori SK. Photodynamic Therapy as Novel Treatment for Halitosis in Adolescents: A Case Series Study. **J Laser Med Sci.** 2014;5(3):146–52.

Photodynamic Therapy as Novel Treatment for Halitosis in Adolescents: A Case Series Study

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Abstract

Introduction: Halitosis is a common problem that affects a large portion of the population worldwide. The origin of this condition is oral in 90% of cases and systemic in 10% of cases. The foul odor is caused mainly by volatile sulfur compounds produced by Gram-negative bacteria. However, it has recently been found that anaerobic Gram-positive bacteria also produce hydrogen sulfide (H_2S) in the presence of amino acids, such as cysteine. Light with and without the combination of chemical agents has been used to induce therapeutic and antimicrobial effects. In photodynamic therapy, the antimicrobial effect is confined to areas covered by the photosensitizing dye. The aim of the present case series study was to evaluate the antimicrobial effect of photodynamic therapy on halitosis in adolescents through the analysis of volatile sulfur compounds measured using a sulfide meter (Halimeter®).

Methods: Five adolescents aged 14 to 16 years were evaluated using a sulfide meter before and one hour after photodynamic therapy, which involved the use of methylene blue 0.005% on the middle third and posterior thirds of the dorsum of the tongue and nine points of laser irradiation in the red band (660 nm) with an energy dose of 9 J, power output of 100 mW and 90-seconds exposure time.

Results: A 31.8% reduction in the concentration of volatile sulfur compounds was found in the comparison of the initial and final readings. The statistically significant reduction ($p = 0.0091$) led to an absence of halitosis following treatment (mean: 58.2 ppb).

Conclusion: Photodynamic therapy seems to be effective on reduction the concentration of volatile sulfur compounds. Considering the positive effects of photodynamic therapy in this case series, further studies involving microbiological analyses should be conducted to allow comparisons of the results.

Conclusion:

Key words: photodynamic therapy; laser; adolescent.

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Introduction

Halitosis (bad breath) is a term used to define a foul, unpleasant odor that emanates from the mouth stemming from either a local or systemic origin.[1-3] This common problem affects a large portion of the population worldwide and causes considerable embarrassment. Halitosis therefore has a negative impact on social communication and quality of life.[4] While the lack of standardization in the protocol for the diagnosis and treatment of halitosis hinders the comparison of data from epidemiological studies carried out in different countries, it is believed that 25% of the population are affected by this condition.[6]

Studies on the etiology of this condition report that 2% of cases stem from renal, metabolic, hepatic, endocrinologic and gastrointestinal disorders (such as infection by *Helicobacter pylori* and intestinal blockage), 8% due to conditions of the respiratory system and conditions of the ears, nose and throat (ENT), such as acute tonsillitis, postnasal drip, sinusitis and tonsilloliths, and 80 to 90% are directly linked to conditions of the oral cavity, such as periodontal disease, coated tongue, poor oral hygiene, salivary abnormalities (change in pH and hyposialy), stomatitis, intra-oral neoplasm, pulp exposure, extraction wounds and crowding of the teeth.[5-8]

Bad breath is mainly caused by volatile sulfur compounds (VSCs) produced by the action of anaerobic Gram-negative bacteria (*Fusobacterium nucleatum*, *Selenomonas*, *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Bacteroides forsythus* and

Eubacterium) found in the oral cavity on substrates containing sulfur.[9-11] The VSCs produced by the metabolism of these bacteria are hydrogen sulfide (H_2S), found mainly on the dorsum of the tongue, methanethiol (CH_3SH) in gingival pockets and dimethyl sulfide (CH_3SCH_3), which has an extra-oral origin.[12-15] The concentration of these compounds is used as an indicator of halitosis.[3,16] Recently, the anaerobic Gram-positive bacterium *Solobacterium moorei* (also known as *Bulleidia moorei*) has been associated to halitosis due to the production of H_2S in the presence of different supplements with amino acids, especially cysteine.[17,18] Studies have demonstrated that the presence of these bacteria on the dorsum of the tongue as well as in saliva and periodontal pockets can lead to both halitosis and systemic problems, such as complications during pregnancy, cardiovascular disease and chronic lower respiratory infection,[19] which is considered the third most common cause of death.[2,20-23]

Detection

Two main methods are used to evaluate oral malodor: a subjective (organoleptic) evaluation and an objective evaluation (quantitative measure of VSC, GC gas chromatography and monitor analysis).[24,25] Studies comparing the efficacy of these methods report gas chromatography (GC) to be the most objective and efficacious[6,15] and this method is currently considered the gold standard in the literature.[11] However, the majority of researchers have used a combination of both subjective and objective evaluations, whereas others have only used an organoleptic evaluation due to its ease of execution and low cost.[24]

Organoleptic evaluation

For the organoleptic evaluation, a trained and calibrated rater positioned at a distance of 10 cm distinguishes the breath through the olfactory sense and a score is attributed using the 0 to 5-point Rosenberg scale[3,6] (0 = absence of odor; 1 = nearly undetectable odor; 2 = mild odor; 3 = moderate odor; 4 = strong odor; and 5 = extremely strong odor).

Portable gas analysis

Mouth air can be analyzed using a sulfide monitor, such as the Halimeter (Interscan Corporation, Chatsworth, CA, USA),[3,4,26,27] which determines the total amount of VSCs in parts per billion (ppb) under normal conditions. According to the manufacturer, this quantity should be less than 80 ppb. However, the equipment is unable to differentiate the origin or type of VSC, is more sensitive to H_2S than CH_3SH and is insensitive to CH_3SCH_3.[15,28]

Gas chromatography

GC is the most appropriate method for detecting halitosis. In 2004, an new GC denominated Oral Chroma™ (Abilit Corporation) was developed in Japan for the individual determination of H_2S, CH_3SH and CH_3SCH_3, allowing the evaluation of both the intensity of bad breath and its origin.[6,11,15]

Photodynamic therapy

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raaband

Hermannvon Tappeiner. In the 1970s, PDT was used for the treatment of cancer. Recently, antimicrobial PDT has been used as a treatment option for localized infections.[29] PDT involves the use of a non-toxic light-sensitive photosensitizer combined with visible light at the appropriate wavelength to coincide with the absorption spectrum of the photosensitizer, which reaches a state of excitation after absorbing the photons, reacting with the oxygen in the medium to form reactive oxygen species (ROS). This phototoxic reaction induces the destruction of bacterial cells, but the antimicrobial effect is confined to areas covered by the light-activated photosensitizer, quickly acting on the target organisms when the appropriate energy dose and output power are used.[9,29-33] According to Wainwright (1998),[34] bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways.[32,33]
As a condition with a multifactor etiology but related to bacteria, especially Gram-negative bacteria, halitosis exerts a direct impact on social interactions and quality of life.[6] The conventional treatment of halitosis related to oral conditions consists of the chemical reduction of microorganisms with a mouthwash, such as chlorhexidine (CHX) 0.2%, essential oils, triclosan and hydrogen peroxide, the mechanical removal of nutrients with a tongue scraper or brush, the masking of odor with chewing gum, mints and breath spray and the transformation of VSC using zinc plus CHX.[2,6,10,12,35-37] However, the irregular characteristics of the surface of the dorsum of the tongue make the adequate reduction in bacterial load a particular challenge.[2,36,38] Considering the issues regarding the precise treatment of halitosis and the scarcity of studies addressing the effect of PDT on coated tongue, the aim of the present study was to evaluate the effectiveness of PDT on the dorsum of the tongue in adolescents with halitosis through an analysis of VSCs.

Methods

This study was carried out in compliance with the norms regulating research involving human subjects and was approved by the ethics committee of the University Nove de Julho (Brazil) under process number 037315/2013. After receiving clarifications regarding the objectives and procedures, all legal guardians who agreed to the participation of their adolescent son or daughter signed a statement of informed consent in compliance with Resolution 196/96 of the Brazilian National Health Board.

Male and female adolescents enrolled at the dental clinic of the university were recruited for the study. Those aged 14 to 16 years with a diagnosis of halitosis and Halimeter results above 80 ppb during the cysteine challenge[11,15,39,40] were included. The following were the exclusion criteria:[41] dentofacial anomalies; currently undergoing orthodontic or orthopedic treatment; current use of a removable appliance, implant or dentures; periodontal disease; teeth with carious lesions; currently undergoing cancer treatment; diabetes mellitus;

systemic (gastrointestinal, renal or hepatic disorder); ENT conditions; respiratory condition; antibiotic therapy in the previous month; current pregnancy; and hypersensitivity to the photosensitizer. The recommendations of the Consolidated Standards of Reporting Trials (CONSORT) were used to ensure greater transparency and quality.

Evaluation of halitosis

The literature describes a number of methods for measuring halitosis, such as an organoleptic evaluation of the air emanated from the oral cavity,[16,26] the use of a sulfide meter[16,24,42] and GC. Although the latter is currently considered the gold standard,[11,42,43] its high cost can be prohibitive. The organoleptic test can be influenced by the olfactory capacity and emotional state of the examiner as well as climatic conditions.[3] Thus, the portable Halimeter™ (Interscan Corporation, Chatsworth, CA, USA) was employed in the present study, which uses a sensor that is highly sensitive to the VSC to be evaluated (H_2S), is inexpensive and easy to use. The readings were performed following the manufacturer's instructions (Halimeter® Instruction Manual). The participant was instructed to keep his/her mouth closed for three minutes prior to the exam. A disposable plastic tube was inserted into the mouth over the dorsum of the tongue without touching the oral or lingual mucosa. The mouth was maintained slightly open without breathing as the equipment performed the reading. The highest score during the reading was recorded. The same procedure was performed three times at three-minute intervals, resulting in three Halimeter® readings, the mean of which was calculated by the equipment itself.[27] An hour after the treatment the same halimeter measurement was performed. To standardize the halimetric readings, the participants were instructed to avoid the consumption of garlic, onion, strong spices and alcohol as well the use of an antiseptic mouthwash 48 hours prior to the evaluation. On the day of the evaluation, the most recent meal had to be consumed at least two hours prior and the participant was to avoid coffee, cigarettes, breath mints, chewing gum, oral hygiene product and personal products, such as perfume/cologne, aftershave lotion, deodorant, creams and tonics, and was to brush the teeth with water alone.[27,44]

Photodynamic therapy

The THERAPY XT-EC® device (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) was used for PDT, with laser emission in the red (660 nm) and infrared (810 nm) range and the tip tapered for dental use (diameter: 0.094 cm). A single session of PDT was held with the Chimiolux® methylene blue photosensitizer (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) at a concentration of 0.005% (165 µm) applied immediately after de last halimeter measurement to the middle third and posterior thirds of the dorsum of the tongue. After five minutes of pre-irradiation time for incubation, the excess was removed with an aspirator to maintain the

surface moist with the photosensitizer alone (without the use of water). Before the application of the laser, the participant and researchers present put on protective eyewear and the equipment was encased in a plastic protector. Nine points were irradiated with a distance of 1 cm between points, considering the light scattering halo and effectiveness of PDT. Based on previous studies developed for the treatment of periodontal disease with PDT,[45-51] the device was previously calibrated to operate with a wavelength of 660 nm, energy dose of 9 J, power output of 100 mW, 90-seconds exposure time per point, fluency of 320 J/cm^2 and irradiance of 3537 mW/cm^2. The punctual method was used in direct contact with the tongue.

Statistical analysis

The data were tabulated and processed using the BioEstat 5.0 program. The Shapiro-Wilk test was used to determine the distribution of the data (normal or non-normal). The paired t-test was used for the comparisons of the evaluation times, with the level of significance set to 5% ($p < 0.05$).

Results

Five individuals were evaluated (2 males and 3 females; mean age: 15 years). Table 1 displays the descriptive statistics of the readings before and after treatment.

Table 1. Descriptive statistics of individuals evaluated

	Pre-treatment	Post-treatment
Mean	85.4 ppb	58.2 ppb
Standard deviation	7.9	11.7
Standard error	3.5	5.2
Shapiro-Wilk *p*-value	0.8625	0.6884

Since the data exhibited approximately normal distribution, the differences before and after treatment were determined using the paired t-test. Although only a pilot study with a sample size of n = 5, the test power was greater than 80%. A statistically significant difference was found in halimeter readings (Figure 1), with a mean of 85.4 ppb prior to treatment and 58.2 ppb after treatment ($p = 0.0091$).

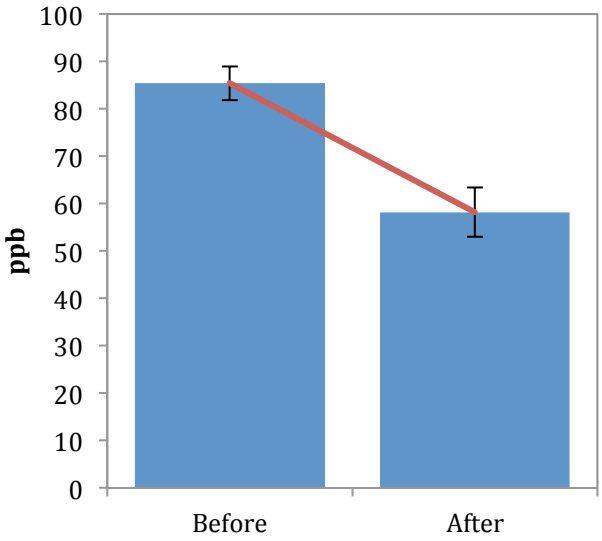

Figure 1. Mean (± SEM) halimeter measures before and after treatment

Discussion

In this study, the effectiveness of PDT for the treatment of halitosis in adolescence was evaluated through the analysis of the concentration of VSCs, measured by a sulfide monitor in a single session. PDT applied to the dorsum of the tongue eliminated bad odors by reducing the concentration of VSCs, as demonstrated by the Halimeter®, which is highly sensitive to H_2S.[11,37,44] Despite the lack of a microbiological analysis, the bacteria in the condition of coated tongue were likely affected by PDT, as these bacteria are associated with the production of high concentrations of H_2S,[9-11] especially in the presence of cysteine, as demonstrated in both *in vivo* and *invitro* models.[11,52]

The effectiveness of PDT on microorganisms has been extensively investigated using different combinations of light and photosensitizers. The degree of photodamage depends on the type and concentration of the photosensitizer, the fluence and fluence-rate of the light as well as the genera of the microorganisms.[53] Most microorganisms tested have proven to be susceptible to PDT and *C. albicans* requires a higher dose.[54] Moreover, Kormerik states that PDT is the best treatment option for localized, superficial oral infections.[55] Based on the present findings, one may hypothesize that PDT caused the direct elimination of pathogens that colonized the dorsum of the tongue, thereby leading to a reduction in halitosis. The microorganisms were submitted to high concentrations of ROS due to irradiation of the photosensitizer. Although no evaluation was performed of the microbiological content in the sites treated, microorganisms are considered responsible for the metabolism of substrates and the production of volatile compounds in patients.

The application of punctual PDT on the tongue alone is in line with a previous study involving 2000 patients in whom coated tongue was scored based on a visual inspection: 0 = absence; 1 = 1/3 of the tongue with thin coating; 2 = more

than 1/3 with thin coating or 1/3 with thick coating; and 3 = more than 1/3 with thick coating; the findings demonstrated that 43.4% of cases of halitosis stemmed from coated tongue, as demonstrated by the organoleptic test and Halimeter®, whereas 7.4% stemmed from periodontal disease and nearly 2% had an ENT cause.[44] Over the years, studies have demonstrated a small, long-term reduction in the amount of bacteria in coated tongue with the use of a tongue scraper with or without a concomitant mouthwash.[2,56] This limited reduction in bacteria is related to the irregular characteristics of the surface of the tongue,[38] which underscores the need for daily oral hygiene control to maintain a low level of bacterial proliferation. The penetration of light and the flow of the photosensitizing agent were not affected by the posterior papillae. Thus, PDT can achieve promising results in the treatment of halitosis, as suggested by the present study. However, it is possible that the combination of both methods would achieve the best results, as reported in studies involving PDT in conjunction with conventional periodontal treatment methods.[24,49,57,58]

Due to the lack of previous studies involving PDT for the treatment of coated tongue, the parameters employed in the present study were based on papers describing the treatment of periodontal disease with PDT,[45,46,48-50] in which the use of methylene blue and laser at wavelengths ranging from 635 to 670 nm proved successful in reducing the amount of the bacteria analyzed (*Porphyromonas gingivalis*, *Tannerella forsythensis* and *Treponema denticola*),[48,49] which are also found in coated tongue.

Although no microbiological analysis was performed in the present study, the reduction in VSCs was likely associated to the reduction in the amount of bacteria.[11,37] The ease of applicability of PDT is believed to favor the control of oral infection in adolescence, which is a period of intensive hormonal transformations that exert an influence on the gingival inflammation process, facilitating the formation of coated tongue due to the increase in the shedding of the gingival epithelial tissue.[59,60] This method may also be effective in adolescents who exhibit the mouth-breathing habit, which causes changes in salivary flow and the amount of mucin, thereby favoring the formation of coated tongue and an increase in halitosis.[42,61] Children with postnasal drip may also benefit from this method, as a study involving individuals aged five to 14 years found a significant association between oral mal odor and postnasal drip,[5] which leads to direct contact between the mucus of the nasal sinuses and the dorsum of the tongue.[6]

Considering the easy application of the photosensitizing agent associated with the tapered tip of the laser equipment (THERAPY XT-EC®) in areas of difficult access as the posterior region of the tongue, made PDT a valuable choice for treatment.. However, some limitations should be addressed. The irradiation time per point caused patient discomfort and avoidance responses. Thus, the dose should be altered in further studies or a device should be manufactured to allow the single application over a larger surface. Moreover, these measures should

be combined to educational counseling regarding the cleaning of the tongue.

Conclusion

Photodynamic therapy applied to the dorsum of the tongue demonstrated positive results and could be suggested as conservative, noninvasive, fast, effective treatment for halitosis in adolescents. As a preliminary study involving only the analysis of the effect of PDT on the concentration of VSCs, the findings motivate the researchers to develop further studies for the acquisition of more detailed data on this innovating treatment for the treatment of a common problem that affects a large portion of the population.

Conflict of Interests

The authors declare no conflicts of interest.

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5.2. Artigo 2

Lopes RG, de Godoy CH, Deana AM, de Santi ME, Prates RA, França CM, Fernandes KP, Mesquita-Ferrari RA, Bussadori SK. Photodynamic Therapy as a Novel Treatment for Halitosis in Adolescents: study protocol for a randomized controlled trial. **Trials.** 2014;15(1):443.

Photodynamic therapy as a novel treatment for halitosis in adolescents: study protocol for a randomized controlled trial

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Abstract

Background

Halitosis is a common problem that affects a large portion of the population worldwide. The origin of this condition is oral in 90% and systemic in 10% of cases. The unpleasant odor is mainly the result of volatile sulfur compounds produced by Gram-negative bacteria. However, it has recently been found that anaerobic Gram-positive bacteria also produce hydrogen sulfide (H_2S) in the presence of amino acids, such as cysteine. Light, both with and without the use of chemical agents, has been used to induce therapeutic and antimicrobial effects. In photodynamic therapy, the antimicrobial effect is confined to areas covered by photosensitizing dye. The aim of the present study is to evaluate the antimicrobial effect of photodynamic therapy on halitosis in adolescents through the analysis of volatile sulfur compounds measured using gas chromatography and microbiological analysis of coated tongue.

Methods/Design

A quantitative clinical trial will be carried out involving 60 adolescents randomly divided into the following groups: group 1 will receive treatment with a tongue scraper, group 2 will receive photodynamic therapy applied to the posterior two-thirds of the dorsum of the tongue, and group 3 will receive combined treatment (tongue scraper and photodynamic therapy). Gas chromatography (OralChroma^{TM}) and microbiological analysis will be used for the diagnosis of halitosis at the beginning of the study. Post-treatment evaluations will be conducted at one hour and 24 hours after treatment. The statistical analysis will include the Shapiro-Wilk test for the determination of the distribution of the data. If normal distribution is demonstrated, analysis of variance followed by Tukey's test will be used to compare groups. The Kruskal-Wallis test followed by the Student-Newman-Keuls test will be used for data with non-normal distribution. Either the paired *t*-test or the Wilcoxon test will be used to compare data before and after treatment, depending on the distribution of the data.

Discussion

The results of this trial will determine the efficacy of using photodynamic therapy alone or in combination with a tongue scraper to treat bad breath in adolescents.

Trial registration

The protocol for this study was registered with Clinical Trials (registration number NCT02007993) on 10 December 2013.

Keywords

Halitosis, Photodynamic therapy, Adolescent

Background

Halitosis is a term used to define an unpleasant odor that emanates from the mouth, stemming from either a local or systemic origin [1-3]. This common problem affects a large portion of the population worldwide and causes considerable embarrassment. Therefore, halitosis has a negative impact on social communication and quality of life [4]. The lack of standardization in the protocol for the diagnosis and treatment of halitosis hinders the comparison of data from epidemiological studies conducted in different countries and yet it is believed that 25% of the population are affected by this condition [5].

Studies on the etiology of halitosis report that 2% of cases stem from renal, metabolic, hepatic, endocrinological and gastrointestinal disorders (such as infection by *Helicobacter pylori* and intestinal blockage), and 8% are due to conditions of the respiratory system and conditions of the ears, nose and throat (ENT), such as acute tonsillitis, postnasal drip, sinusitis and tonsillolith. The majority of cases (80 to 90%) are directly linked to conditions of the oral cavity, such as periodontal disease, coated tongue, poor oral hygiene, salivary abnormalities (change in pH and hyposialy), stomatitis, intra-oral neoplasm, pulp exposure, extraction wounds and crowding of the teeth [5-8].

Bad breath mainly stems from volatile sulfur compounds (VSCs) produced by the action of anaerobic Gram-negative bacteria (*Fusobacterium nucleatum*, *Selenomonas*, *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythyia*, *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Eubacterium*) found in the oral cavity on substrates containing sulfur [9-11]. The VSCs produced by the metabolism of these bacteria are hydrogen sulfide (H_2S), found mainly on the dorsum of the tongue, methanethiol (CH_3SH) in gingival pockets and dimethyl sulfide (CH_3SCH_3), which has an extra-oral origin [12-15]. The concentration of these compounds is used as an indicator of halitosis [3, 16].

Recently, the anaerobic Gram-positive bacterium *Solobacterium moorei* (also known as *Bulleidia moorei*) has been associated with halitosis due to the production of H_2S in the presence of different supplements containing amino acids, especially cysteine [17, 18]. Studies have demonstrated that the presence of these bacteria on the dorsum of the tongue, as well as in saliva and periodontal pockets, can lead to both halitosis and systemic problems such as complications during pregnancy, cardiovascular disease and chronic lower respiratory infection [19], which is considered the third most common cause of death [2, 20-23].

Detection

Two main methods are used to evaluate halitosis: a subjective (organoleptic) evaluation and an objective evaluation (quantitative measure of VSCs, gas chromatography (GC) and monitor analysis) [24-27]. Studies comparing the efficacy of these methods report GC to be the most objective and efficacious method for the individual detection of H_2S, CH_3SH and CH_3SCH_3, allowing the evaluation of both the intensity of bad breath and its origin [5, 15]. Indeed, GC is currently considered the gold standard for the detection of halitosis [11]. However, the majority of researchers have used a combination of both subjective and objective evaluations, whereas others have only used an organoleptic

evaluation due to its ease of execution and low cost [24]. Halitosis can be analyzed using a sulfide monitor, such as the Halimeter (Interscan Corporation, Chatsworth, California, United States) [3, 4, 26-28], which determines the total amount of VSCs in parts per billion (ppb) under normal conditions.

Photodynamic therapy

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raab and Hermann von Tappeiner. In the 1970s, PDT began to be used for the treatment of cancer. Recently, antimicrobial PDT has been used as a treatment option for localized infections [29]. PDT involves the use of a non-toxic light-sensitive photosensitizer combined with visible light at the appropriate wavelength to coincide with the absorption spectrum of the photosensitizer, which reaches a state of excitation after absorbing the photons, reacting with the oxygen in the medium to form reactive oxygen species. This phototoxic reaction induces the destruction of bacterial cells. The antimicrobial effect is confined to areas covered by the light-activated photosensitizer, quickly acting on the target organisms when the appropriate energy dose and output power are used [9, 29-33]. According to Wainwright [34], bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways [32, 33].

The conventional treatment of halitosis related to oral conditions consists of the chemical reduction of microorganisms with a mouthwash, such as 0.2% chlorhexidine, essential oils, triclosan and hydrogen peroxide, the mechanical removal of nutrients with a tongue scraper or brush, the masking of odor with chewing gum, mints and breath spray, and the transformation of VSCs using zinc plus chlorhexidine [2, 5, 10, 12, 35-37]. However, the irregular characteristics of the surface of the dorsum of the tongue make the adequate reduction in bacterial a particular challenge [2, 36, 38].

Considering the scarcity of studies addressing the effect of PDT on tongue biofilm, the aim of the present study was to evaluate the effectiveness of PDT on the dorsum of the tongue in adolescents with halitosis by an analysis of VSCs and microbiological analysis of the tongue.

Methods/Design

This study will be carried out in compliance with regulatory norms governing research involving human subjects. Approval was obtained from the Human Research Ethics Committee of University Nove de Julho (Brazil) under process number 037315/2013, and the study is registered with the United States National Institutes of Health (Clinical Trials.gov registration number: NCT02007993). The guardians of the participants will be informed regarding the procedures and will sign a statement of informed consent authorizing the participation of their sons and daughters in compliance with Resolution 196/96 of the Brazilian National Health Board.

Male and female adolescents enrolled at the dental clinic of the university will be recruited for the study. Those aged between 13 and 18 years, with a diagnosis of halitosis and OralChroma^{TM} results of $H_2S \geq 112$ ppb during the cysteine challenge [11, 15, 39, 40] will be included. The exclusion criteria will be dentofacial anomalies, currently undergoing orthodontic or orthopedic treatment, current use of a removable

appliance, implant or dentures, periodontal disease, teeth with carious lesions, currently undergoing cancer treatment, diabetes mellitus, systemic (gastrointestinal, renal or hepatic disorder) conditions, ear, nose or throat conditions, respiratory conditions, antibiotic therapy in the previous month, current pregnancy [5] or hypersensitivity to the photosensitizer. As this is a randomized clinical trial, the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) will be used to ensure greater transparency and quality (Figure 1).

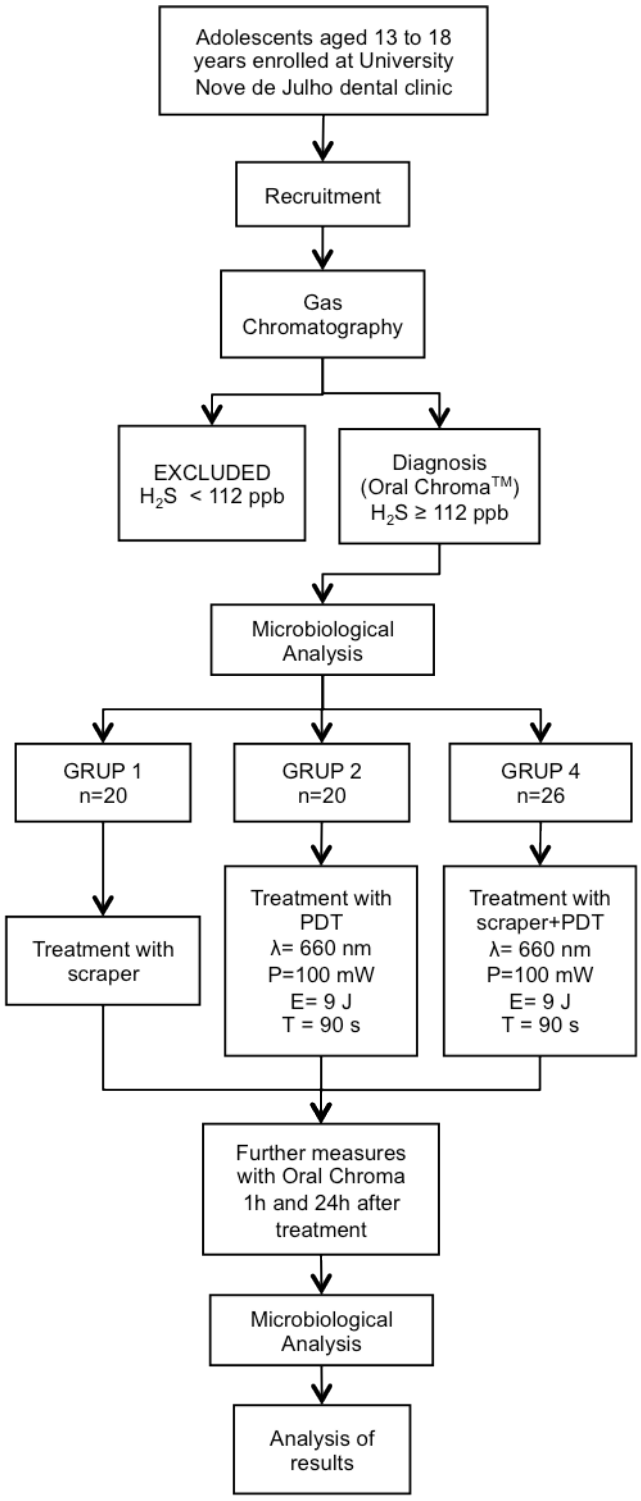

Figure 1 Flowchart of study. PDT, photodynamic therapy.

The subjects selected will be randomly allocated to three groups (Table 1). All individuals will be submitted to evaluations with OralChroma™ before and after treatment.

Table 1 Summary of experimental conditions

Group	Halitosis	Treatment
1	$H_2S \geq 112$ ppb	Tongue scraper
2	$H_2S \geq 112$ ppb	PDT
		E = 9 J T = 90 s
3	$SH_2 \geq 112$ ppb	Tongue scraper + PDT
		E = 9 J T = 90 s

Microbiological analysis

Microbiological analyses of coated tongue will be performed before and after treatment using a 1-μl inoculation loop for the collection of biofilm samples from the dorsum of the tongue. The samples will be transferred to 1.5-ml vials with reduced transport fluid and placed in a vortex mixer (Prolab, São Paulo – Brazil) for approximately 30 seconds for homogenization. Ten-fold serial dilution will be prepared in 180 μl of sterile phosphate buffered saline (Probac, São Paulo – Brazil) and aliquots of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} will be transferred to plates with brain-heart infusion agar (Probac, São Paulo – Brazil). As the main bacteria responsible for the production of VSCs are Gram-negative, the plates will be incubated in anaerobic jar for 72 hours at 37°C, following by the quantification of colony-forming units [10, 42].

Halitosis detection

The literature describes a number of methods for measuring halitosis, such as an organoleptic evaluation of the air emanating from the oral cavity [16, 26] using a sulfide monitor [16, 25, 43] or GC [11, 43, 44]. However, it has been demonstrated that the organoleptic test can be influenced by the olfactory capacity and emotional state of the examiner, as well as climatic conditions [3]. Therefore, the portable OralChroma™ device Abilit Corporation, Chuo-ku, Osaka - Japan) will be employed. This device uses a highly sensitive gas semiconductor sensor.

The participant will first rinse with cysteine (Fórmula & Ação, São Paulo – Brazil) for one minute (cysteine 10 mM - 16 mg of cysteine in 100 ml of distilled water - 16 %mg). A syringe will be placed in the participant's mouth with the plunger completely inserted. The participant will close his or her mouth, breathe through the nose and remain still with the mouth closed for one minute. The participant will be instructed not to touch the tip of the syringe with his or her tongue. The plunger will then be withdrawn, pushed back in to empty the air into the participant's mouth and will be withdrawn again to fill the syringe with the breath sample. The tip of the syringe will be cleaned to remove saliva and a gas injection needle will be placed on the syringe. The plunger will be adjusted to 0.5 ml and the contents will be injected into the input of the device in a single motion (Figure 2) [15].

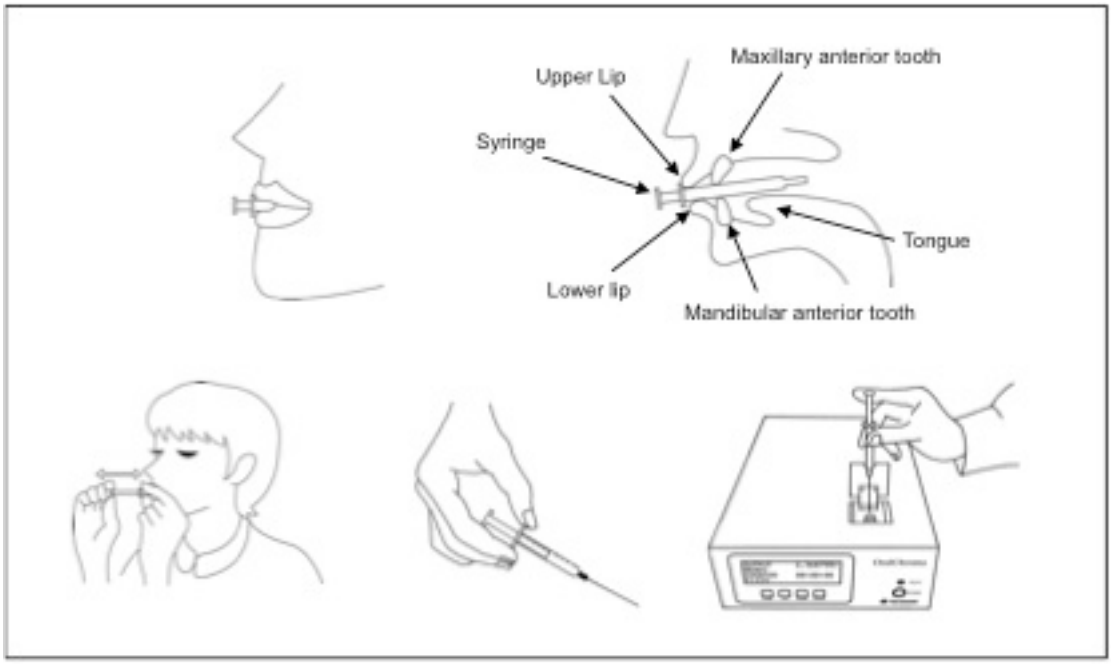

Figure 2 Process for the acquisition of the sample for the halimetric. OralChroma (Abiliti Corporation, Chuo-ku, Osaka – Japan)

The OralChroma™ will be connected to the computer with a specific software program that allows the creation of a graph corresponding to the peaks and concentrations of VSCs (0 to 2913 ppb) with considerable precision after eight minutes. The results are stored in the program, as well as in the device itself, and can be retrieved at any time for comparisons of the readings before, during and after treatment.

Analysis of VSCs

OralChroma™ (Abilit Corporation) was developed in Japan for the individual determination of H_2S, CH_3SH and CH_3SCH_3, allowing for the evaluation of both the intensity of bad breath and its origin [5, 11, 15]. H_2S originates mainly from bacteria on the dorsum of the tongue. Values greater than 112 ppb indicate halitosis. CH_3SH is found in greater concentration in periodontal pockets. Values up to 26 ppb are considered normal. Periodontal disease typically results in a high CH_3SH:H_2S ratio (>3:1). CH_3SCH_3 may have a periodontal or systemic (intestine, liver or lung) origin and may also be temporarily caused by the ingestion of certain foods and beverages. The distinction between CH_3SCH_3 of an oral or systemic origin can be made through the comparison of the results of the halimetric (OralChroma™) with and without a cysteine challenge. The perception threshold for CH_3SCH_3 is very low (8 ppb). Other non-VSC odors may appear in a peak prior to the theoretical first peak, which is H_2S [15].

To maximize the standardization of the readings, the exam will be carried out in the morning and the participants will be instructed to avoid the ingestion of foods with garlic, onion or strong spices, as well as the consumption of alcohol and the use of an antiseptic mouthwash. On the morning of the exam, more than two hours should have passed since any food intake and the participants are to abstain from coffee, hard candy, chewing gum, oral hygiene products and personal care items containing fragrances

(aftershave, deodorant, perfume and creams). Brushing will be performed with water alone [27, 45].

Photodynamic therapy

The THERAPY XT-ES™ (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) with a red (660 nm) and infrared (810 nm) laser and a fine tip (for regions of difficult access) will be used. Only the volunteer and operator will be present at the time of PDT and both will be wearing protective eyewear. The active point of the laser will be covered with disposable clear plastic wrap (PVC) for hygiene purposes and to avoid cross-contamination. The operator will use the appropriate clothing.

A single session of PDT will be performed with the Chimiolux™ methylene blue photosensitizer (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) at a concentration of 0.005% (165 μm) applied to the middle and posterior thirds of the dorsum of the tongue. After five minutes of pre-irradiation time for incubation, the excess will be removed with an aspirator to maintain the surface moist with the photosensitizer alone (without the use of water). A total of six points will be irradiated (Figure 3). Based on studies developed for the treatment of periodontal disease with PDT [46-52] and a previous pilot study [53], the device will be calibrated with a wavelength of 660 nm, power output of 100 mW, fluency of 320 J/cm^2, irradiance of 3537 mW/cm^2 and an energy dose of 9 joules for 90 seconds per point in groups 2 and 3. The punctual application method will be used with the conventional tip in contact with the tongue (Table 2).

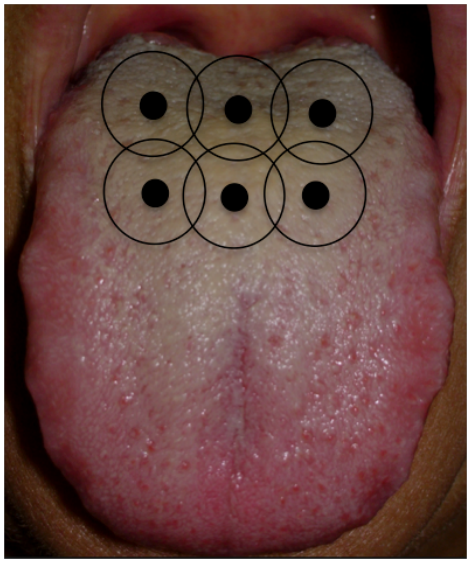

Figure 3 Points of photodynamic therapy application.

Table 2 Parameters of laser

Parameter	Red laser
Center wavelength (nm)	660
Spectral bandwidth (FWHM) (nm)	5
Operating mode	Continuous wave
Average radiant power (mW)	100
Polarization	Random
Aperture diameter (cm)	0.094
Irradiance at aperture (mW/cm^2)	3537
Beam profile	Multimode
Beam spot size at target (cm^2)	0.02827
Irradiance at target (mW/cm^2)	3537
Exposure duration (s)	90/120
Radiant exposure (J/cm^2)	320/428
Radiant energy (J)	9/12
Number of points irradiated	9
Area irradiated (cm^2)	0.254
Application technique	Contact
Number and frequency of treatment sessions	1 session
Total radiant energy (J)	81/108

Tongue scraping intervention

A Halicare^{TM} tongue scraper (Odomed, São Paulo, Brazil) will be used for the removal of biofilm. The participant will be instructed to divide the tongue into two parts and scrape each side 10 times (Figure 4).

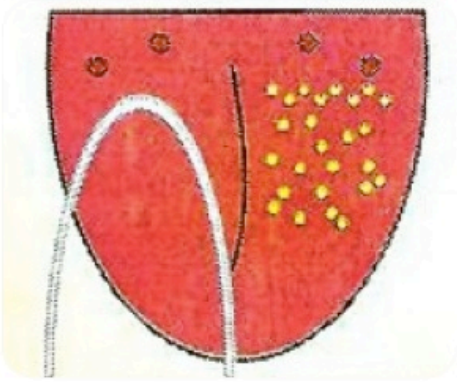

Figure 4 Diagram of tongue scraper use.

Calculation of sample size

The error was established as $err = \left|\overline{x_1} - \overline{x_2}\right|$, in which $\overline{x_1}$ and $\overline{x_2}$ are the means of groups 1 and 2. Assuming both samples as having the same size ($n_1 = n_2$), the sample size is obtained from the following equation:

$$n_1 = n_2 = \frac{err}{Z\sqrt{\sigma_1^2 + \sigma_2^2}} \quad \textbf{(Equation 1)}$$

in which σ_1^2 and σ_1^2 are the variances in groups 1 and 2, respectively. As more than two groups will be studied, the decision was made to employ the largest error found in the literature [54] to estimate the sample size. Assuming all groups as having normal or approximately normal distribution, and that the sample will be large enough for a significance level of α =0.05, the *Z* value was determined to be 1.96. However, for the sample size, the test power was established as 1-β = 0.80. In case the hypothesis of normality in the samples was rejected, the sample size was corrected by 5%. Based on the experimental groups in the study by Tsai *et al*. [54] it was determined that each group should contain 26 participants (n =26).

Outcome measures

Only participants with H_2S ≥112 ppb, CH_3SH ≤26 ppb and CH_3SCH_3 ≤8 ppb will be part of the research, which limits participation to individuals with halitosis caused by coated tongue alone. Immediately after treatment, a second halimetry test will be performed and the results will be analyzed.

Hypothesis

Our null hypothesis is that there will be no change in halitosis following the use of PDT. Our experimental hypothesis is that there will be a reduction in halitosis following the use of PDT alone or in combination with a tongue scraper.

Organization and statistical analysis of data

The Shapiro-Wilk test will be used to determine the distribution of the halimetricdata. If the data presents with normal distribution, analysis of variance (ANOVA) followed by the Tukey test will be used to evaluate the correlation between each of the proposed treatments and halitosis. The paired *t*-test will be used to compare the data before and after each treatment and determine whether the treatments reduced the degree of halitosis. The Kruskal-Wallis test followed by the Student-Newman-Keuls test will be used for data with non-normal distribution, and the Wilcoxon test will be used to analyze the data before and after each treatment. Microbiological data presents with log-normal distribution and will therefore be analyzed using the methods described for data with normal distribution. A significance level of α = 0.05 will be used.

Discussion

The main objective of the proposed study is to evaluate the effect of PDT with and without the use of a tongue scraper for the treatment of halitosis in adolescents. This objective has two aspects: the evaluation of VSC levels before and after treatment through a quantitative analysis of H_2S using GC, and a microbiological analysis of the effect of PDT on coated tongue. The findings are expected to provide convincing evidence that PDT is more effective for the treatment of halitosis.

In the literature, the treatment of halitosis is performed using a tongue scraper with or without a mouthwash [2], which leads to a small, long-term reduction in the amount of bacteria on the tongue [38]. Thus, daily oral hygiene is needed to maintain a low level of bacterial proliferation. As the penetration of light and spreading of the photosensitizer do not seem to be affected by the posterior papillae of the tongue, treatment with PDT is promising and may achieve satisfactory results, especially when combined with conventional treatment.

Trial status

The authors are currently recruiting participants. It begun on March of 2014 and we pretend to go until August of 2015.

Abbreviations

CH_3SCH_3, Dimethyl sulfide; CH_3SH, Methanethiol; GC, Gas chromatography; H_2S, Hydrogen sulfide; PDT, Photodynamic therapy; VSC, Volatile sulfur compounds.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RGL participated in the conception and design of the study, data collection and drafting of the present manuscript. CHLG helped draft the manuscript and participated in the data collection. AMD performed the statistical analysis. RAP contributed to the design of the study. MESOS, CMF, KPSF and RAMF critically reviewed the manuscript for intellectual content. SKB conceived the study and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

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5.3. Artigo 3

Lopes RG, Soares CTA, Tarzia O, Deana AM, Prates RA, França CM, Fernandes KP, Mesquita-Ferrari RA, Bussadori SK. Efeito da Terapia Fotodinâmica no Tratamento da Halitose em Adolescentes – Ensaio Clínico Controlado. **Submetido à Journal of Breath Research (Fator de Impacto = 3,590)**

EFEITO DA TERAPIA FOTODINÂMICA NO TRATAMENTO DA HALITOSE EM ADOLESCENTES – ENSAIO CLÍNICO CONTROLADO

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Resumo

A luz acompanhada ou não de agentes químicos tem sido usada para induzir efeitos terapêuticos e antimicrobianos. Na terapia fotodinâmica o efeito antimicrobiano fica confinado apenas às áreas cobertas pelo corante e irradiadas pela luz. O objetivo deste estudo foi avaliar o efeito antimicrobiano da terapia fotodinâmica em adolescentes com halitose, pela análise da concentração de compostos sulforados voláteis em especial o nível de sulfidretos. Por meio de estudo clínico controlado, 45 adolescentes foram avaliados e divididos aleatoriamente em 3 grupos: grupo 1 terapia fotodinâmica aplicada na região de dorso e terço médio da língua, grupo 2 tratamento com limpador de língua e grupo 3 tratamento combinado de limpador de língua e terapia fotodinâmica. O diagnóstico de halitose foi realizado antes e depois do tratamento por meio de cromatografia gasosa. Foi aplicado o teste de Kruskal-Wallis para comparação seguido do teste Student-Newman-Keuls. Para todas as análises foi considerado um nível de significância de $\alpha=0,05$. Após o tratamento houve redução estatisticamente significante para todos os grupos ($p < 0,001$), contudo a associação da terapia fotodinâmica ao raspador lingual mostrou ser mais eficiente na redução total de sulfidretos (mediana=0).Esse estudo traz uma nova opção de tratamento para halitose em adolescentes com efeito imediato sem agressão mecânica as papilas linguais comum ao tratamento convencional.

Trial Registration: The protocol for this study has been submitted to Clinical Trials – registration number NCT02007993, registered on 10 December 2013.

Palavras-chave: Halitosis, photodynamic therapy, adolescent.

INTRODUÇÃO

A terapia fotodinâmica (TFD) foi descoberta em 1900 por Oskar Raab e Hermann von Tappeiner, e na década de 1970 foi inicialmente desenvolvida como uma terapia para tratamento de câncer. Recentemente a TFD antimicrobiana tem sido utilizada como uma alternativa para o tratamento das infecções localizadas (1).

A TFD engloba o uso de um corante sensível a luz (fotossensibilizador) e não tóxico combinado a uma luz visível de comprimento de onda apropriado para coincidir com o espectro de absorção do fotossensibilizador (FS), que após absorver os fótons atinge um estado de excitação reagindo com o oxigênio do meio, formando espécies reativas de oxigênio (reactive oxygen species - ROS). Essa reação fototóxica induz a destruição da célula bacteriana, porém o efeito antimicrobiano fica confinado apenas às áreas cobertas pelo corante e irradiadas pela luz agindo no organismo alvo rapidamente, dependendo da dose de energia de luz e a saída de potência usada (1–6). Além disso, de acordo com Wainwright (7) a resistência bacteriana à TFD é improvável, pois o oxigênio singleto e os radicais livres formados interagem com várias estruturas celulares bacterianas e diferentes caminhos metabólico (5,6).

A Halitose, também conhecida como mau hálito, é um termo utilizado para definir um odor desagradável e fétido que emana da boca, podendo apresentar origem local ou sistêmica (8–10). É considerado um problema comum que afeta grande parte da população mundial e causa constrangimento tanto para quem a possui como para as pessoas com as quais o indivíduo convive, é um fator negativo importante na comunicação social, com impacto direto na qualidade de vida (11). Estudos sobre etiologia da halitose mostram que 2% dos casos estão relacionados a síndromes metabólicas, alterações renais, hepáticas, endocrinológicas e gastrointestinais (como infecções por Helicobacter pylori e obstrução intestinal), 8% por alterações respiratórias e otorrinolaringológicas como amigdalite aguda, presença de escorrimento nasal posterior e sinusites, 80-90% dos casos estão diretamente ligados as condições da cavidade oral, como a presença de doença periodontal (13%), saburra lingual (51%) ou a combinação de ambos (22%), pobre higiene oral, alterações salivares (mudança do pH e hiposialia), entre outras causas (estomatite, neoplasia intra-oral, exposição pulpar, feridas pós extração e

apinhamento dentário). (12–16)

O mau hálito é provocado principalmente por compostos sulforados voláteis (CSV), produzidos pela ação de bactérias Gram-negativas anaeróbias (*Fusobacterium nucleatum, Selenomonas, Treponema denticola, Prevotella intermedia, Tannerella Forsythensis, Porphyromonas gingivalis, Bacteroides forsythus* and *Eubacterium*)(2) sobre substratos contendo enxofre encontrados na boca (17,18). Os CSV produzidos a partir desse metabolismo são: sulfidreto (SH_2) - encontrados principalmente em dorso lingual - metilmercaptana (CH_3SH) - presentes no sulco gengival - e dimetilsulfeto (CH_3SCH_3) - origem extra-oral (19–22), e a concentração desses gases é usada como indicador da halitose (10,23). Recentemente, a bactéria Gram-positiva anaeróbia *Solobacterium moorei* (conhecida como *Bulleidia moorei*) também foi associada a halitose pela produção SH_2 na presença de diferentes suplementações com aminoácidos, em especial a cisteína (24,25).

Há dois principais métodos usados para avaliar o hálito: avaliação subjetiva (organoléptica) e avaliação objetiva (cromatografia gasosa e monitor de sulfeto) (26,27). Pesquisas realizadas comparando a eficácia dos testes apontou a cromatografia gasosa como método mais objetivo e eficaz (13,22), e atualmente, considerada padrão ouro da literatura (18). Porém, a maioria dos pesquisadores tem usado a combinação de ambos, outros apenas o organoléptico por ser o método mais barato e fácil de executar (26).

O tratamento convencional da halitose quando relacionado a alterações orais consiste na redução química dos microrganismos com enxaguatórios (clorexidina 0,2%, óleos essenciais, triclosan e água oxigenada), redução mecânica dos nutrientes intra-orais com raspador ou escova lingual, mascaramento do odor (gomas de mascar, tabletes de menta e spray) e transformação do CSV (Zinco associado a clorexidina) (9,13,17,19,28–30). Por outro lado, a redução da carga bacteriana é dificultada devido às características irregulares da superfície lingual (9,29,31), revestido por numerosas papilas que se apresentam de 4 diferentes formas: fungiformes, filiformes, circunvaladas e folhadas. As patologias linguais são determinadas pelas características papilares condições da superfície lingual (tamanho, formato, fixação e caraterísticas papilares): língua pilosa (hairy tongue), língua revestida (coated tongue), língua fissurada (fissured tongue), atrofia papilar

(papillary atrophy), língua geográfica (geographic tongue), glossite romboide mediana (Median rhomboid glossitis), língua crenada (crenation tongue), macroglossia (macroglossia) e anquiloglossia (ankyloglossia)(32).

Sendo assim, frente a essas dificuldade e aos questionamentos referentes ao tratamento preciso da halitose, bem como a escassez de estudos relacionados diretamente ao efeito da TFD na saburra lingual, o objetivo desse estudo foi avaliar a efetividade da aplicação da TFD em dorso de língua, pela análise do nível de CSV em adolescentes com halitose.

MATERIAL E MÉTODOS

O estudo seguiu as normas regulamentadoras de pesquisa em seres humanos com parecer favorável do Comitê de Ética em Pesquisa da Universidade Nove de Julho número 313.779/2013, e os responsáveis pelos participantes assinaram o termo de consentimento livre após esclarecimentos para autorização da participação na pesquisa, de acordo com a resolução 196/96 do Conselho Nacional Saúde. Foi submetido aprovado pela Clinical Trials em 10 de dezembro de 2103 sob o registro número NCT02007993 e recebeu auxilio FAPESP para desenvolvimento da pesquisa (n^{o} 2013/13032-8) com publicação do protocolo na revista Trials (33).

Foram avaliados 45 adolescentes de ambos os sexos, na faixa etária de 13 a 18 anos, matriculados regularmente no Instituto Meninos de São Judas Tadeu – São Paulo. O diagnóstico de halitose foi realizado por meio de cromatografia gasosa com desafio da cisteína antes e depois do tratamento e participaram do estudos apenas quem apresentou resultados de $SH_2 \geq 112$ ppb (18,22,34,35).

Indivíduos com anomalias dentofaciais, em tratamento ortodôntico e/ou ortopédico, com dispositivo removível, implante e/ou prótese, com doença periodontal, com dentes cariados, em tratamento oncológico, com diabetes mellitus, alterações sistêmicas (gastrointestinais, renais, hepáticas), otorrinolaringológicos e respiratórios, em tratamento com antibiótico até 1 mês antes da pesquisa, grávidas, (36)(13) e com hipersensibilidade ao FS, foram excluídos do estudo.

Diagnóstico de Halitose

A coleta do ar bucal seguiu as orientações do fabricante (Oral Chroma^{TM} Manual Instruction), o participante foi orientado a fazer bochecho com cisteína

(10 mM - 16 mg de cisteína em 100 ml de água destilada – 16 mg%)(22) por 1 minuto, e permanecer com a boca fechada mais 1 minuto. Foi introduzido na boca do paciente uma seringa do mesmo fabricante para coleta de 5ml do ar bucal, em seguida com uma agulha apropriada injetado na porta de entrada do equipamento com movimento único (22).

Por meio de um software específico o Oral Chroma^{TM}, conectado ao computador permite a captura de um gráfico correspondente aos picos e valores de concentração dos gases (SH_2, CH_3SH, CH_3SCH_3), medindo os limiares dos CSV (de 0 a 2913 ppd), com muita precisão após 8 minuto.

Da análise dos CSV capturado pelo sistema, são indicadores de halitose medidas de SH_2 acima de 112 ppb, CH_3SH acima de 26 ppb e CH_3SCH_3 acima de 8 ppb(22).

Para evitar alterações na coleta do hálito os participantes foram instruídos a seguir as seguintes orientações: 48 horas antes da avaliação não ingerir alimentos com alho, cebola e temperos fortes, evita o consumo de álcool e uso de antisséptico bucal. No dia da avaliação puderam alimentar-se até no máximo 2 horas antes do exame, abster-se de café, balas, goma de mascar, produtos de higiene oral e pessoal com perfume (pós-barba, desodorante, perfume, cremes e/ou tônico) e a escovação foi apenas com água (37,16).

Os participantes foram divididos em 3 grupos por meio da aleatorização por bloco: grupo 1 tratamento com TFD (n=16), grupo 2 tratamento com raspador lingual (n=15) e grupo 3 tratamento com a associação do raspador lingual e TFD (n=14).

Aplicação da TFD

Para a terapia fotodinâmica foi utilizado o aparelho THERAPY XT-EC® (DMC ABC Equipamentos Médicos e Odontológicos, SP, BR). No momento da aplicação da TFD todos os presentes utilizaram óculos específicos para proteção ocular. A ponta ativa do laser foi revestida com plástico transparente descartável (PVC) (evitando contaminações e por motivo de higiene) e o profissional estava devidamente paramentado.

Foi realizada 1 sessão de TFD com FS azul de metileno manipulado na concentração de 0,005% (165 μm) e aplicado quantidade suficiente para cobrir o terço médio e dorso da língua por 5 minutos para incubação, o excesso foi

removido com sugador de forma a manter a superfície úmida com o próprio FS, sem utilização de água. Foram irradiados 6 pontos com distância de 1 cm entre os pontos, considerando o halo de espalhamento da luz e efetividade da TFD (figura 1). Com base em estudos desenvolvidos no tratamento da doença periodontal com a TFD (38–44) e estudo piloto realizado previamente (45), o aparelho estava previamente calibrado com comprimento de onda 660nm, energia de 9J e potência de 100mW para os grupos 2 e 3 que foram irradiados durante 90 segundos por ponto, fluência de 320 J/cm^2 e irradiância de 3537mW/cm^2. Foi utilizado o método de aplicação pontual, em contato direto com a língua (Figura 2). O grupo 1 foi tratado apenas com raspador lingual.

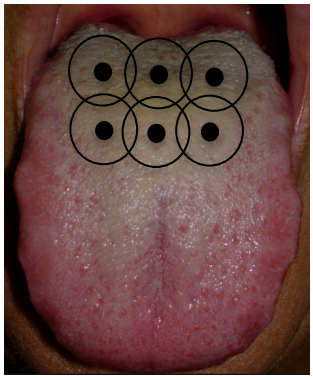

Figura 1 – Pontos de aplicação da TFD

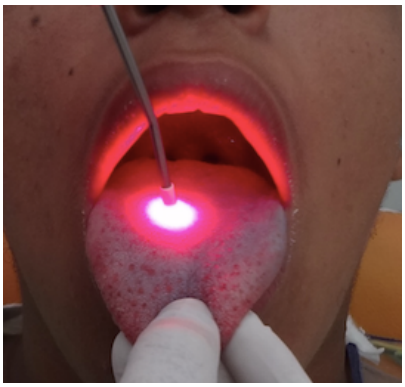

Figura 2 – Aplicação da TFD

Organização e Tratamento Estatístico dos Dados

Os dados obtidos pelo OralChorma foram analisados quanto à sua normalidade pelo teste de Shapiro – Wilk. Como a hipótese de normalidade foi rejeitada ($p < 0.05$ para todos os grupos), foi utilizado o teste Kruskal-Wallis seguido pelo teste de Student-Newman-Keuls como post hoc. Para analisar os resultados de cada tratamento nos dois períodos do estudo foi utilizado o teste de Wilcoxon. Para todas as análises foi considerado um nível de significância $\alpha=0,05$.

RESULTADOS

Um total de 45 participantes foram incluídos nesse estudo, com idade mínima de 13 anos e máxima de 17 anos (média de idade de 13,96 anos, com desvio padrão de 1,43) e 42% do gênero masculino (n=19) e 58% do gênero feminino

(n=26). Após halimetria inicial foi constatado que todos os participantes estavam com halitose proveniente do biofilme lingual, com nível de SH_2 acima de 112 ppb, sem diferença significativa entre os grupos (p=0,258), grupo 1 mediana SH_2=791 ppb, grupo 2 mediana SH_2=466 ppb e grupo 3 SH_2=1508 ppb (tabela 1).

Tabela 1 – Mediana de valores obtidos de SH_2 na halimetria inicial.

	N	Mediana (SH_2 em ppb)	Mínima	Máxima
Grupo 1	**16**	**791**	**130**	**2913**
Grupo 2	**15**	**466**	**170**	**2913**
Grupo 3	**14**	**1508**	**170**	**2913**

Abreviações: SH2 – sulfidreto; ppb – partes por bilhão.

Comparando os grupos depois do tratamento a análise de Kruskal – Wallis mostra diferença significativa entre os grupos (p=0,0008). Na análise estatística intergrupos antes e depois do tratamento a redução de SH_2 foi significativa quando comparadas as medianas antes e depois do tratamento, o grupo 1 reduziu em 88,6% (TFD p=0,0004), o grupo 2 reduziu 97% (raspador lingual p=0,0007) e o grupo 3 redução de 100% do nível de SH_2 (TFD+Raspador lingual p=0,001) (tabela – 2).

Tabela 2 – Mediana de valores obtidos de SH_2 na halimetria final.

	N	Mediana (ppb)	Mínima	Máxima	Outlayer
Grupo 1	**16**	**20**	**0**	**332**	**332**
Grupo 2	**15**	**53**	**0**	**238**	**238**
Grupo 3	**14**	**0**	**0**	**73**	**73**

Por se tratar de dados não paramétricos os resultados foram distribuídos em Boxplot (gráfico 1), contudo é possível visualizar o limiar para halitose em ppb (112 ppb) e a diferença entre os grupos antes (a) e depois (b e c) do tratamento (all p <0,001).

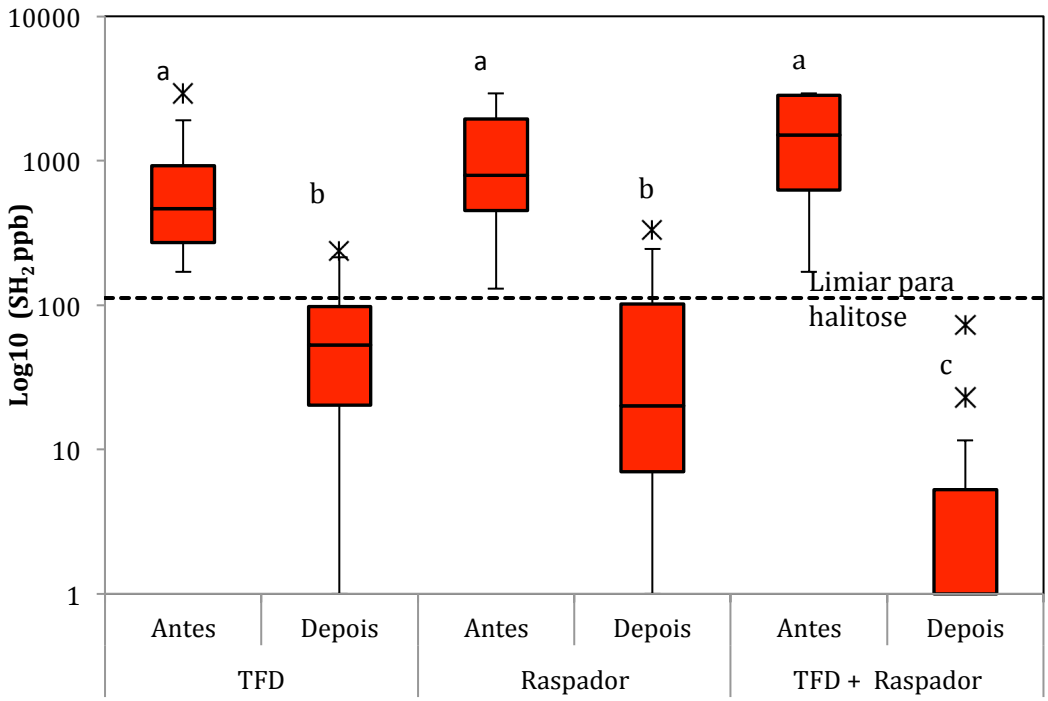

Figura 3 – Gráfico comparativo entre grupos e inter grupos antes e depois do tratamento.

(Abreviações: TFP – terapia fotodinâmica; SH_2 – sulfidreto; a – antes do tratamento; b – pós tratamento; c – pós tratamento com máxima redução; * – outlier)

DISCUSSÃO

Possivelmente este seja o primeiro estudo da literatura a avaliar a efetividade da TFD no tratamento da halitose em adolescentes pela análise da concentração de CSV especificamente de SH2. A cromatografia gasosa realizada pelo equipamento OralChroma®, considerada padrão ouro na literatura para análise objetiva do nível de halitose(18,30,16), demonstrou que o tratamento em sessão única eliminou o mal odor pela redução dos níveis de SH_2. A TFD aplicada no dorso lingual apresentou resultado semelhante ao tratamento com raspador lingual, porém com a vantagem de não ter efeito agressivo causado pela estimulação mecânica da língua, o que de acordo com levantamento realizado na literatura(46) pode induzir a ruptura da membrana plasmática de células da língua além de causar micro hemorragia, sugerindo assim uma limpeza lingual com baixo vigor e de forma cuidadosa(46).

Ao longo dos anos, alguns estudos clínicos randomizados mostraram que a geografia da língua fornece um refúgio para as espécies microbianas e funciona como um reservatório de detritos orais dificultando a ação do fluxo salivar e força mastigatória, e os resultados desses estudos que avaliam o impacto da escovação lingual sozinha, associada ao raspador lingual ou a enxaguatórios, indicam que a flora bacteriana da língua é resistente a intervenção mecânica, o que corrobora com os resultados obtidos no grupo 2 do nosso estudo; os resultados da associação da escova a outros produtos apresenta maior redução bacteriana, contudo essa diferenças é pequena (9,31,47,48). Ao utilizar a TFD junto ao raspador lingual a redução do mal odor foi total firmando a alta eficiência da técnica desenvolvida.

A ação da TFD em microorganismo tem sido extensamente investigada usando diferentes combinações de luz e fotossensibilizadores, e o grau de dano causado pela luz depende do tipo e concentração do FS, fluência e taxa de fluência da luz bem como o gênero das espécies (49), e a maioria dos microorganismos testados mostram ser susceptíveis a TFD(50). Em pesquisa realizada com TFD, Kormerik conclui que é a melhor opção de tratamento para infecções orais superficiais e localizadas(51). No presente experimento, a hipótese que pode explicar a diminuição da halitose dos pacientes dos grupos 1 e 3 é que a TFD atuou na eliminação direta dos patógenos que colonizavam a região superficial de dorso da língua e entre as papilas respectivamente. O efeito fototóxico ocorreu pois estes microorganismos foram submetidos a altas concentrações de espécies reativas de oxigênio gerados pela irradiação do FS. Contudo, a espessura do biofilme lingual não permite a penetração total do FS entre as papilas gustativas, por isso há semelhante nos resultados do grupo 1 e grupo 2.

A aplicação pontual da TFD apenas na língua corrobora a pesquisa realizada com 2000 pacientes adultos, a qual comprova que 43,4% dos casos de halitose tem origem da saburra lingual quando avaliados por teste organoléptico, Halimeter® e Oral Chroma^{TM}(16), e resultados semelhantes foram obtidos na avaliação da origem da halitose em crianças de 5 a 14 anos(12). Pela ausência de estudos utilizando TFD na saburra lingual, os parâmetros utilizados nesse estudo foram baseados em artigos de tratamento da doença periodontal associado a TFD (38,39,41–43), onde a utilização do azul de metileno

associado a comprimentos de onda que variaram de 635nm a 670nm obtiveram sucesso para bactérias analisadas (*Porphyromonas gingivalis, Tannerella forsythensis e Treponema denticola*) (41,42), presentes também na saburra lingual.

Durante a pesquisa a penetração da luz emitida e o escoamento do fotossensibilizador não foram afetados pelas papilas linguais, apenas pela espessura do biofilme lingual. O tratamento com a TFD teve resposta expressiva, entretanto a associação de ambas técnicas mostrou melhor resultado final, como também evidenciam pesquisas com uso da TFD em conjunto às técnicas convencionas de tratamento da doença periodontal (26,42,52,53). O resultado efetivo observado no grupo 3 provavelmente tenha sido alcançado devido a associação de tratamentos, promovida pela redução inicial da carga bacteriana realizada com o raspador lingual, que diminuiu a espessura do biofilme da língua e possivelmente, permitiu melhor penetração do fotossensibilizador e consequente ação da TFD nos microrganismos restantes presentes entre as papilas. Contudo, acredita-se que as bactérias presentes no dorso lingual, provenientes da saburra, foram afetadas de alguma maneira pela TFD, uma vez que as mesmas estão associadas à produção de altas concentrações de SH_2 (2,17,18), principalmente na presença de aminoácidos como a cisteína, o que demonstram modelos de estudos *in vivo* e *in vitro* (18,54).

A facilidade de aplicabilidade da técnica e a resposta imediata fazem com que o tratamento proposto pela pesquisa favoreça o controle de infecção oral em pacientes jovens, onde as intensas transformações hormonais influenciam no processo inflamatório gengival, facilitando a formação de saburra pelo aumento da descamação do epitélio gengival (55,56). Ou ainda em adolescentes com respiração oral cuja alterações do fluxo salivar e quantidade de mucina propiciam a formação da biofilme lingual e consequente aumento da halitose(57,58), e em crianças com gotejamento nasal posterior, onde estudos comprovam relação significativa do mal odor oral com contato direto do muco dos seios paranasais com o dorso lingual(12,13).

Portanto, a fácil aplicação do corante em regiões de difícil acesso e a ponta afilada do equipamento favoreceu a técnica escolhida e o tratamento na região

posterior da língua, contudo consideramos como limitação do estudo o tempo de irradiação por ponto que gerou desconforto ao paciente e isso sugere a realização de mais estudos alterando doses e/ou a fabricação de um equipamento de aplicação única em superfícies maiores.

ACKNOWLEDGMENTS

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6. Considerações finais

Nossos resultados demonstraram que a aplicação da TFD em saburra lingual presentes no terço médio e dorso lingual teve efeito imediato estatisticamente significativo, acabando com o mal odor bucal ao diminuir os níveis de sulfidretos (SH_2<112ppb). Acredita-se que houve uma diminuição da carga bacteriana para todos os tipos de tratamentos avaliados pois o SH_2 é um tipo de CSV produzido por bactérias encontradas principalmente no biofilme lingual.
A TFD pode ser uma alternativa de tratamento para substituir o uso de raspadores linguais em situações de impossibilidade de remoção mecânica da saburra lingual e por não causar injurias as papilas, ou ainda, pelo fato da associação de ambos tratamentos apresentar nível zero de SH_2 torna-se uma opção para casos que se faz necessário maior redução da carga bacteriana dessa região.
São necessários mais estudos envolvendo análise microbiológica para que haja melhor compreensão do efeito da TFD nas bactérias do biofilme lingual em adolescentes com halitose, bem como pesquisas alterando o tempo de irradiação do laser.

6.1. Limitações do estudo

Tempo de aplicação da TFD deixou o paciente cansado de ficar com a língua exposta.

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ANEXOS

ANEXO 1 - CEP

UNIVERSIDADE NOVE DE JULHO - UNINOVE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ENSAIO CLÍNICO CONTROLADO DO USO DA TERAPIA FOTODINÂMICA EM ADOLESCENTES COM HALITOSE

Pesquisador: Rubia Garcia Lopes

Área Temática:

Versão: 2

CAAE: 17482113.1.0000.5511

Instituição Proponente: Universidade Nove de Julho - UNINOVE

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 313.779

Data da Relatoria: 24/06/2013

ANEXO 2 - TCLE

UNIVERSIDADE NOVE DE JULHO
DIRETORIA DA SAÚDE
MESTRADO EM BIOFOTÔNICA APLICADA ÀS CIÊNCIAS DA SAÚDE

Termo de Consentimento para Participação em Pesquisa Clínica:

Nome do Voluntário:_____
Nome do Responsável:_____
Endereço:_____
Telefone para contato:_____Cidade:_____CEP:_____
Email:_____

As informações contidas neste prontuário foram fornecidas pela aluna Rubia Garcia Lopes (mestranda em Biofotônica Aplicada às Ciências da Saúde da Universidade Nove de Julho) e Profª. Drª Sandra Kalil Bussadori, objetivando firmar acordo escrito mediante o qual, o voluntário da pesquisa autoriza sua participação com pleno conhecimento da natureza dos procedimentos e riscos a que se submeterá, com a capacidade de livre arbítrio e sem qualquer coação.

1. Título do Trabalho Experimental: Terapia Fotodinâmica No Tratamento Da Halitose Em Adolescentes
2. Objetivos: avaliar o efeito antimicrobiano da terapia fotodinâmica (TFD) no nível de halitose em adolescentes de 14 a 18 anos.
3. Justificativa: diante da ausência de relatos na literatura relacionandos a utilização da terapia fotodinâmica no tratamento da halitose, faz-se necessário avaliar esta relação, assim como identificar o nível e o tipo de halitose presente em adolescentes e um protocolo eficaz de tratamento.
4. Procedimentos da Fase Experimental: A pesquisa será realizada com pacientes de ambos os sexos matriculados regularmente na Clínica de Odontologia da Universidade Nove de Julho – São Paulo. Primeiramente será verificada a presença ou não de halitose por meio do dispositivo portátil OralChroma, onde, coloca-se a seringa própria para a coleta do ar bucal na boca do paciente, com o êmbolo completamente inserido. O paciente fecha a boca, respira pelo nariz e aguarda com a boca fechada por 1 minuto. Pede-se ao paciente que não toque a ponta da seringa com a língua. Puxa-se o êmbolo para fora, volta-se a esvaziar o ar da seringa na boca do paciente e novamente puxa o êmbolo para encher a seringa com a amostra do hálito. Para o tratamento da halitose, o grupo 1 receberá raspador de língua com cerdas, o grupos 2 e 3 terapia fotodinâmica na língua (região de dorso e terço médio), e grupos 4 e 5 ambos tratamentos.
5. Desconforto ou Riscos Esperados: Os voluntários não serão submetidos a riscos durante o período experimental. Nenhum desconforto é esperado para análise dos níveis de halitose. No tratamento com a TFD o paciente pode sentir náusea quando o profissional manipular a língua.
6. Informações: O voluntário tem garantia que receberá respostas a qualquer pergunta ou esclarecimento de qualquer dúvida quanto aos procedimentos, riscos benefícios e outros assuntos relacionados com pesquisa. Também os pesquisadores supracitados

assumem o compromisso de proporcionar informação atualizada obtida durante o estudo, ainda que esta possa afetar a vontade do indivíduo em continuar participando.
Qualquer duvida os responsáveis poderão ser contatados pelos fones: Dra. Sandra (11) 98381-7453 ou Rubia (11) 98593-9878. Dúvidas sobre questões éticas deverão ser encaminhadas ao Comitê de ética e pesquisa da Uninove através do email comiteetica@uninove.br.
7. Métodos Alternativos Existentes: A pesquisa citada dispensa qualquer método alternativo.
8. Retirada do Consentimento: o voluntário tem a liberdade de retirar seu consentimento a qualquer momento e deixar de participar do estudo.
9. Aspecto Legal: Elaborados de acordo com as diretrizes e normas regulamentadas de pesquisa envolvendo seres humanos atendendo à Resolução n.º 196, de 10 de outubro de 1996, do Conselho Nacional de Saúde do Ministério de Saúde – Brasília – DF.
10. Garantia do Sigilo: Os pesquisadores asseguram a privacidade dos voluntários quanto aos dados confidenciais envolvidos na pesquisa.
11. Formas de Ressarcimento das Despesas decorrentes da participação na Pesquisa: Serão ressarcidas despesas com eventuais deslocamentos.
12. Local da Pesquisa: A pesquisa será desenvolvida na Universidade Nove de Julho localizado à Rua Vergueiro, 235 – Liberdade, São Paulo, SP.
Endereço do Comitê de Ética em Pesquisa da Uninove: Rua. Vergueiro nº 235/249 – Liberdade – SP - CEP. 01504-001 -1º andar
13. Nome Completo e telefones dos pesquisadores para contato:
Orientador: Profª Drª Sandra Kalil Bussadori – Tel (11) 98381-7453
Pesquisadora: Rubia Garcia Lopes – Tel (11) 985939878
14. Consentimento pós-informação:

Eu, _____, responsável pelo menor _____, após leitura e compreensão deste termo de informação e consentimento, entendo que a participação é voluntária e que é permitido se retirar do estudo a qualquer momento, sem prejuízo algum. Confirmo que recebi cópia deste termo de consentimento, e autorizo a execução do trabalho de pesquisa e a divulgação dos dados obtidos neste estudo no meio científico.

* Não assine este termo se ainda tiver alguma dúvida a respeito.

São Paulo, _____de_____ de 2013.

Nome do responsável (por extenso): _____

Assinatura:_____

1ª via: Instituição

2ª via: Voluntário

ANEXO 3 – AUTORIZAÇÃO

UNIVERSIDADE NOVE DE JULHO
DIRETORIA DA SAÚDE
MESTRADO EM BIOFOTÔNICA APLICADA ÀS CIÊNCIAS DA SAÚDE

AUTORIZAÇÃO PARA TRATAMENTO COM LASER DE BAIXA POTÊNCIA

Os lasers de baixa potência possuem efeito analgésico, antinflamatório e biomodulador, sendo utilizados como nos casos de aftas, herpes labial, queilite angular, trismos, parestesia, hipersensibilidade dentinária, pós-cirurgias, pós-intervenções endodônticas. Como efeitos da laserterapia pode-se citar os aumentos da microcirculação local e da velocidade da cicatrização, além da analgesia temporária e da mudança na polarização celular, permitindo diminuir estados e hiper e de hiposensibilidade. É capaz de auxiliar na resposta imunológica do organismo de forma local e sistêmica.
Riscos: Se todas as normas de segurança para a aplicação da luz laser de baixa intensidade forem corretamente respeitadas, não existe nenhum risco ao paciente, operador e equipe, durante e após o procedimento clínico.
Benefícios: Tratamento menos agressivo e mais rápido, preservando tecidos saudáveis.
Alternativas: tratamento odontológico convencional adequado para cada caso.

Eu,_____ portador do RG:_____, CPF:_____, responsável legal do menor _____, concordo com a terapia com laser de baixa intensidade. Eu tive a oportunidade de questionar o(a) operador(a) sobre os riscos, benefícios e alternativas para o tratamento. Eu também tive a oportunidade de questionar sobre as atuais pesquisas e sobre a importância desse procedimento. Não me foram feitas promessas ou garantias em relação aos procedimentos em obter resultados miraculosos, existem hipóteses e resultados clínicos e experimentais que têm sido satisfatórios. Eu dou a permissão para que o tratamento seja documentado com fotografias e radiografias com finalidade didática e profissional.
Eu dou a permissão para realização da laserterapia.

Resp. Legal: _____
(nome legível)

_____
(assinatura)

Operador(a): _____
(nome legível)

_____
(assinatura)

_____, _____ de _____ de 20_____.

ANEXO 4 – ARTIGO PUBLICADO 1

Case Series

Photodynamic Therapy as Novel Treatment for Halitosis in Adolescents: A Case Series Study

Rubia Garcia Lopes[1], Maria Eugenia Simões Onofre de Santi[1], Bruno Edin Franco[2], Alessandro Melo Deana[1] Renato Araujo Prates[1], Cristiane Miranda França[1], Kristianne Porta Santos Fernandes[1] Raquel Agnelli Mesquita Ferrari[1], Sandra Kalil Bussadori[1]

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Abstract:

Introduction: Halitosis is a common problem that affects a large portion of the population worldwide. The origin of this condition is oral in 90% of cases and systemic in 10% of cases. The foul odor is caused mainly by volatile sulfur compounds produced by Gram-negative bacteria. However, it has recently been found that anaerobic Gram-positive bacteria also produce hydrogen sulfide (H_2S) in the presence of amino acids, such as cysteine. Light with and without the combination of chemical agents has been used to induce therapeutic and antimicrobial effects. In photodynamic therapy, the antimicrobial effect is confined to areas covered by the photosensitizing dye. The aim of the present case series study was to evaluate the antimicrobial effect of photodynamic therapy on halitosis in adolescents through the analysis of volatile sulfur compounds measured using a sulfide meter (Halimeter®).

Methods: Five adolescents aged 14 to 16 years were evaluated using a sulfide meter before and one hour after photodynamic therapy, which involved the use of methylene blue 0.005% on the middle third and posterior thirds of the dorsum of the tongue and nine points of laser irradiation in the red band (660 nm) with an energy dose of 9 J, power output of 100 mW and 90-seconds exposure time.

Results: A 31.8% reduction in the concentration of volatile sulfur compounds was found in the comparison of the initial and final readings. The statistically significant reduction ($p = 0.0091$) led to an absence of halitosis following treatment (mean: 58.2 ppb).

Conclusion: Photodynamic therapy seems to be effective on reduction the concentration of volatile sulfur compounds.Considering the positive effects of photodynamic therapy in this case series, further studies involving microbiological analyses should be conducted to allow comparisons of the results.

Keywords: photodynamic therapy; laser; adolescent.

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Introduction

Halitosis (bad breath) is a term used to define a foul, unpleasant odor that emanates from the mouth stemming from either a local or systemic origin.[1-3] This common problem affects a large portion of the population worldwide and causes considerable embarrassment. Halitosis therefore has a negative impact on social communication and quality of life.[4] While the lack of standardization in the protocol for the diagnosis and

treatment of halitosis hinders the comparison of data from epidemiological studies carried out in different countries, it is believed that 25% of the population are affected by this condition.[6]

Studies on the etiology of this condition report that 2% of cases stem from renal, metabolic, hepatic, endocrinologic and gastrointestinal disorders (such as infection by *Helicobacter pylori* and intestinal blockage), 8% due to conditions of the respiratory system and conditions of the ears, nose and throat (ENT), such as acute tonsillitis, postnasal drip, sinusitis and tonsilloliths, and 80 to 90% are directly linked to conditions of the oral cavity, such as periodontal disease, coated tongue, poor oral hygiene, salivary abnormalities (change in pH and hyposialy), stomatitis, intra-oral neoplasm, pulp exposure, extraction wounds and crowding of the teeth.[5-8]

Bad breath is mainly caused by volatile sulfur compounds (VSCs) produced by the action of anaerobic Gram-negative bacteria (*Fusobacterium nucleatum*, *Selenomonas*, *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Eubacterium*) found in the oral cavity on substrates containing sulfur.[9-11] The VSCs produced by the metabolism of these bacteria are hydrogen sulfide (H_2S), found mainly on the dorsum of the tongue, methanethiol (CH_3SH) in gingival pockets and dimethyl sulfide (CH_3SCH_3), which has an extra-oral origin.[12-15] The concentration of these compounds is used as an indicator of halitosis.[3,16] Recently, the anaerobic Gram-positive bacterium *Solobacterium moorei* (also known as *Bulleidia moorei*) has been associated to halitosis due to the production of H_2S in the presence of different supplements with amino acids, especially cysteine.[17,18] Studies have demonstrated that the presence of these bacteria on the dorsum of the tongue as well as in saliva and periodontal pockets can lead to both halitosis and systemic problems, such as complications during pregnancy, cardiovascular disease and chronic lower respiratory infection,[19] which is considered the third most common cause of death.[2,20-23]

Detection

Two main methods are used to evaluate oral malodor: a subjective (organoleptic) evaluation and an objective evaluation (quantitative measure of VSC, GC gas chromatography and monitor analysis).[24,25] Studies comparing the efficacy of these methods report gas chromatography (GC) to be the most objective and efficacious[6,15] and this method is currently considered the gold standard in the literature.[11] However, the majority of researchers have used a combination of both subjective and objective evaluations, whereas others have only used an organoleptic evaluation due to its ease of execution and low cost.[24]

Organoleptic evaluation

For the organoleptic evaluation, a trained and calibrated rater positioned at a distance of 10 cm distinguishes the breath through the olfactory sense and a score is attributed using the 0 to 5-point Rosenberg scale[3,6] (0 = absence of odor; 1 = nearly undetectable odor; 2 = mild odor; 3 = moderate odor; 4 = strong odor; and 5 = extremely strong odor).

Portable gas analysis

Mouth air can be analyzed using a sulfide monitor, such as the Halimeter (Interscan Corporation, Chatsworth, CA, USA),[3,4,26,27] which determines the total amount of VSCs in parts per billion (ppb) under normal conditions. According to the manufacturer, this quantity should be less than 80 ppb. However, the equipment is unable to differentiate the origin or type of VSC, is more sensitive to H_2S than CH_3SH and is insensitive to CH_3SCH_3.[15,28]

Gas chromatography

GC is the most appropriate method for detecting halitosis. In 2004, an new GC denominated Oral Chroma™ (Abilit Corporation) was developed in Japan for the individual determination of H_2S, CH_3SH and CH_3SCH_3, allowing the evaluation of both the intensity of bad breath and its origin.[6,11,15]

Photodynamic therapy

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raaband Hermannvon Tappeiner. In the 1970s, PDT was used for the treatment of cancer. Recently, antimicrobial PDT has been used as a treatment option for localized infections.[29] PDT involves the use of a non-toxic light-sensitive photosensitizer combined with visible light at the appropriate wavelength to coincide with the absorption spectrum of the photosensitizer, which reaches a state of excitation after absorbing the photons, reacting with the oxygen in the medium to form reactive oxygen species (ROS). This phototoxic reaction induces the destruction of bacterial cells, but the antimicrobial

effect is confined to areas covered by the light-activated photosensitizer, quickly acting on the target organisms when the appropriate energy dose and output power are used.[9,29-33] According to Wainwright (1998),[34] bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways.[32,33]

As a condition with a multifactor etiology but related to bacteria, especially Gram-negative bacteria, halitosis exerts a direct impact on social interactions and quality of life.[6] The conventional treatment of halitosis related to oral conditions consists of the chemical reduction of microorganisms with a mouthwash, such as chlorhexidine (CHX) 0.2%, essential oils, triclosan and hydrogen peroxide, the mechanical removal of nutrients with a tongue scraper or brush, the masking of odor with chewing gum, mints and breath spray and the transformation of VSC using zinc plus CHX.[2,6,10,12,35-37] However, the irregular characteristics of the surface of the dorsum of the tongue make the adequate reduction in bacterial load a particular challenge.[2,36,38] Considering the issues regarding the precise treatment of halitosis and the scarcity of studies addressing the effect of PDT on coated tongue, the aim of the present study was to evaluate the effectiveness of PDT on the dorsum of the tongue in adolescents with halitosis through an analysis of VSCs.

Methods

This study was carried out in compliance with the norms regulating research involving human subjects and was approved by the ethics committee of the University Nove de Julho (Brazil) under process number 037315/2013. After receiving clarifications regarding the objectives and procedures, all legal guardians who agreed to the participation of their adolescent son or daughter signed a statement of informed consent in compliance with Resolution 196/96 of the Brazilian National Health Board.

Male and female adolescents enrolled at the dental clinic of the university were recruited for the study. Those aged 14 to 16 years with a diagnosis of halitosis and Halimeter results above 80 ppb during the cysteine challenge[11,15,39,40] were included. The following were the exclusion criteria:[41] dentofacial anomalies; currently undergoing orthodontic or orthopedic treatment; current use of a removable appliance, implant or dentures; periodontal disease; teeth with carious lesions; currently undergoing cancer treatment; diabetes mellitus; systemic (gastrointestinal, renal or hepatic disorder); ENT conditions; respiratory condition; antibiotic therapy in the previous month; current pregnancy; and hypersensitivity to the photosensitizer. The recommendations of the Consolidated Standards of Reporting Trials (CONSORT) were used to ensure greater transparency and quality.

Evaluation of halitosis

The literature describes a number of methods for measuring halitosis, such as an organoleptic evaluation of the air emanated from the oral cavity,[16,26] the use of a sulfide meter[16,24,42] and GC. Although the latter is currently considered the gold standard,[11,42,43] its high cost can be prohibitive. The organoleptic test can be influenced by the olfactory capacity and emotional state of the examiner as well as climatic conditions.[3] Thus, the portable Halimeter™ (Interscan Corporation, Chatsworth, CA, USA) was employed in the present study, which uses a sensor that is highly sensitive to the VSC to be evaluated (H_2S), is inexpensive and easy to use. The readings were performed following the manufacturer's instructions (Halimeter® Instruction Manual). The participant was instructed to keep his/her mouth closed for three minutes prior to the exam. A disposable plastic tube was inserted into the mouth over the dorsum of the tongue without touching the oral or lingual mucosa. The mouth was maintained slightly open without breathing as the equipment performed the reading. The highest score during the reading was recorded. The same procedure was performed three times at three-minute intervals, resulting in three Halimeter® readings, the mean of which was calculated by the equipment itself.[27]An hour after the treatment the same halimeter measurment was performed. To standardize the halimetric readings, the participants were instructed to avoid the consumption of garlic, onion, strong spices and alcohol as well the use of an antiseptic mouthwash 48 hours prior to the evaluation. On the day of the evaluation, the most recent meal had to be consumed at least two hours prior and the participant was to avoid coffee, cigarettes, breath mints, chewing gum, oral hygiene product and personal products, such as perfume/cologne, aftershave lotion, deodorant, creams and tonics, and was to brush the teeth with water alone.[27,44]

Photodynamic therapy

The THERAPY XT-EC® device (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) was used for PDT, with laser emission in the red (660 nm) and infrared (810 nm) range and the tip tapered

for dental use (diameter: 0.094 cm). A single session of PDT was held with the Chimiolux® methylene blue photosensitizer (DMC ABC Equipamentos Médicos e Odontológicos, SP, Brazil) at a concentration of 0.005% (165 μm) applied immediately after de last halimeter measurement to the middle third and posterior thirds of the dorsum of the tongue. After five minutes of pre-irradiation time for incubation, the excess was removed with an aspirator to maintain the surface moist with the photosensitizer alone (without the use of water). Before the application of the laser, the participant and researchers present put on protective eyewear and the equipment was encased in a plastic protector. Nine points were irradiated with a distance of 1 cm between points, considering the light scattering halo and effectiveness of PDT. Based on previous studies developed for the treatment of periodontal disease with PDT,[45-51] the device was previously calibrated to operate with a wavelength of 660 nm, energy dose of 9 J, power output of 100 mW, 90-seconds exposure time per point, fluency of 320 J/cm^2 and irradiance of 3537 mW/cm^2. The punctual method was used in direct contact with the tongue.

Statistical analysis

The data were tabulated and processed using the BioEstat 5.0 program. The Shapiro-Wilk test was used to determine the distribution of the data (normal or non-normal). The paired t-test was used for the comparisons of the evaluation times, with the level of significance set to 5% ($p < 0.05$).

Results

Five individuals were evaluated (2 males and 3 females; mean age: 15 years). Table 1 displays the descriptive statistics of the readings before and after treatment.

Since the data exhibited approximately normal distribution, the differences before and after treatment were determined using the paired t-test. Although only a pilot study with a sample size of n = 5, the test power was greater than 80%. A statistically significant difference was found in halimeter readings (Figure 1), with a mean of 85.4 ppb prior to treatment and 58.2 ppb after treatment ($p = 0.0091$).

Table 1. Descriptive statistics of individuals evaluated

	Pre-treatment	Post-treatment
Mean	**85.4 ppb**	**58.2 ppb**
Standard deviation	7.9	11.7
Standard error	3.5	5.2
Shapiro-Wilk *P*-value	0.8625	0.6884

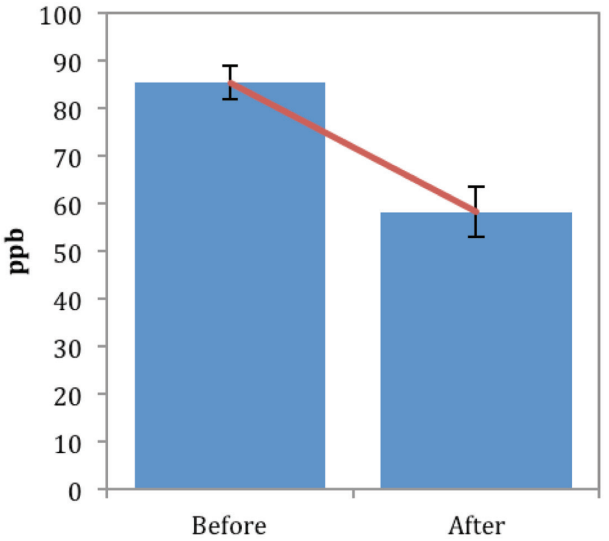

Figure 1. Mean (± SEM) halimeter measures before and after treatment

Discussion

In this study, the effectiveness of PDT for the treatment of halitosis in adolescence was evaluated through the analysis of the concentration of VSCs, measured by a sulfide monitor in a single session. PDT applied to the dorsum of the tongue eliminated bad odors by reducing the concentration of VSCs, as demonstrated by the Halimeter®, which is highly sensitive to H_2S.[11,37,44] Despite the lack of a microbiological analysis, the bacteria in the condition of coated tongue were likely affected by PDT, as these bacteria are associated with the production of high concentrations of H_2S,[9-11] especially in the presence of cysteine, as demonstrated in both *in vivo* and *invitro* models.[11,52]

The effectiveness of PDT on microorganisms has been extensively investigated using different combinations of light and photosensitizers. The degree of photodamage depends on the type and concentration of the photosensitizer, the fluence and fluence-rate of the light as well as the genera of the microorganisms.[53] Most microorganisms tested have proven to be susceptible to PDT and *C. albicans* requires a higher dose.[54] Moreover, Kormerik states that PDT is the best treatment option for localized, superficial oral infections.[55] Based on the present findings, one may hypothesize that PDT caused the direct elimination of pathogens that colonized the dorsum of the tongue, thereby leading to a reduction

in halitosis. The microorganisms were submitted to high concentrations of ROS due to irradiation of the photosensitizer. Although no evaluation was performed of the microbiological content in the sites treated, microorganisms are considered responsible for the metabolism of substrates and the production of volatile compounds in patients.

The application of punctual PDT on the tongue alone is in line with a previous study involving 2000 patients in whom coated tongue was scored based on a visual inspection: 0 = absence; 1 = 1/3 of the tongue with thin coating; 2 = more than 1/3 with thin coating or 1/3 with thick coating; and 3 = more than 1/3 with thick coating; the findings demonstrated that 43.4% of cases of halitosis stemmed from coated tongue, as demonstrated by the organoleptic test and Halimeter®, whereas 7.4% stemmed from periodontal disease and nearly 2% had an ENT cause.[44] Over the years, studies have demonstrated a small, long-term reduction in the amount of bacteria in coated tongue with the use of a tongue scraper with or without a concomitant mouthwash.[2,56] This limited reduction in bacteria is related to the irregular characteristics of the surface of the tongue,[38] which underscores the need for daily oral hygiene control to maintain a low level of bacterial proliferation. The penetration of light and the flow of the photosensitizing agent were not affected by the posterior papillae. Thus, PDT can achieve promising results in the treatment of halitosis, as suggested by the present study. However, it is possible that the combination of both methods would achieve the best results, as reported in studies involving PDT in conjunction with conventional periodontal treatment methods.[24,49,57,58]

Due to the lack of previous studies involving PDT for the treatment of coated tongue, the parameters employed in the present study were based on papers describing the treatment of periodontal disease with PDT,[45,46,48-50] in which the use of methylene blue and laser at wavelengths ranging from 635 to 670 nm proved successful in reducing the amount of the bacteria analyzed (*Porphyromonas gingivalis*, *Tannerella forsythensis* and *Treponema denticola*),[48,49] which are also found in coated tongue.

Although no microbiological analysis was performed in the present study, the reduction in VSCs was likely associated to the reduction in the amount of bacteria.[11,37] The ease of applicability of PDT is believed to favor the control of oral infection in adolescence, which is a period of intensive hormonal transformations that exert an influence on the gingival inflammation process, facilitating the formation of coated tongue due to the increase in the shedding of the gingival epithelial tissue.[59,60] This method may also be effective in adolescents who exhibit the mouth-breathing habit, which causes changes in salivary flow and the amount of mucin, thereby favoring the formation of coated tongue and an increase in halitosis.[42,61] Children with postnasal drip may also benefit from this method, as a study involving individuals aged five to 14 years found a significant association between oral mal odor and postnasal drip,[5] which leads to direct contact between the mucus of the nasal sinuses and the dorsum of the tongue.[6]

Considering the easy application of the photosensitizing agent assosiated with the tapered tip of the laser equipment (THERAPY XT-EC®) in areas of difficult access as the posterior region of the tongue, PDT can be considered a valuable choice for treatment. However, some limitations should be addressed. The irradiation time per point caused patient discomfort and avoidance responses. Thus, the dose should be altered in further studies or a device should be manufactured to allow the single application over a larger surface. Moreover, these measures should be combined to educational counseling regarding the cleaning of the tongue.

Conclusion

Photodynamic therapy applied to the dorsum of the tongue demonstrated positive results and could be suggested as conservative, noninvasive, fast, effective treatment for halitosis in adolescents. As a preliminary study involving only the analysis of the effect of PDT on the concentration of VSCs, the findings motivate the researchers to develop further studies for the acquisition of more detailed data on this innovating treatment for the treatment of a common problem that affects a large portion of the population.

Conflict of Interests

The authors declare no conflicts of interest.

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ANEXO 5 - ARTIGO PUBLICADO 2

Lopes et al. Trials 2014, 15:443
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TRIALS

STUDY PROTOCOL Open Access

Photodynamic therapy as a novel treatment for halitosis in adolescents: study protocol for a randomized controlled trial

Rubia Garcia Lopes, Camila Haddad Leal de Godoy, Alessandro Melo Deana, Maria Eugenia Simões Onofre de Santi, Renato Araujo Prates, Cristiane Miranda França, Kristianne Porta Santos Fernandes, Raquel Agnelli Mesquita-Ferrari and Sandra Kalil Bussadori*

Abstract

Background: Halitosis is a common problem that affects a large portion of the population worldwide. The origin of this condition is oral in 90% and systemic in 10% of cases. The unpleasant odor is mainly the result of volatile sulfur compounds produced by Gram-negative bacteria. However, it has recently been found that anaerobic Gram-positive bacteria also produce hydrogen sulfide (H_2S) in the presence of amino acids, such as cysteine. Light, both with and without the use of chemical agents, has been used to induce therapeutic and antimicrobial effects. In photodynamic therapy, the antimicrobial effect is confined to areas covered by photosensitizing dye. The aim of the present study is to evaluate the antimicrobial effect of photodynamic therapy on halitosis in adolescents through the analysis of volatile sulfur compounds measured using gas chromatography and microbiological analysis of coated tongue.

Methods/Design: A quantitative clinical trial will be carried out involving 60 adolescents randomly divided into the following groups: group 1 will receive treatment with a tongue scraper, group 2 will receive photodynamic therapy applied to the posterior two-thirds of the dorsum of the tongue, and group 3 will receive combined treatment (tongue scraper and photodynamic therapy). Gas chromatography (OralChroma™) and microbiological analysis will be used for the diagnosis of halitosis at the beginning of the study. Post-treatment evaluations will be conducted at one hour and 24 hours after treatment. The statistical analysis will include the Shapiro-Wilk test for the determination of the distribution of the data. If normal distribution is demonstrated, analysis of variance followed by Tukey's test will be used to compare groups. The Kruskal-Wallis test followed by the Student-Newman-Keuls test will be used for data with non-normal distribution. Either the paired *t*-test or the Wilcoxon test will be used to compare data before and after treatment, depending on the distribution of the data.

Discussion: The results of this trial will determine the efficacy of using photodynamic therapy alone or in combination with a tongue scraper to treat bad breath in adolescents.

Trial registration: The protocol for this study was registered with Clinical Trials (registration number NCT02007993) on 10 December 2013.

Keywords: Halitosis, Photodynamic therapy, Adolescent

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Background

Halitosis is a term used to define an unpleasant odor that emanates from the mouth, stemming from either a local or systemic origin [1-3]. This common problem affects a large portion of the population worldwide and causes considerable embarrassment. Therefore, halitosis has a negative impact on social communication and quality of life [4]. The lack of standardization in the protocol for the diagnosis and treatment of halitosis hinders the comparison of data from epidemiological studies conducted in different countries and yet it is believed that 25% of the population are affected by this condition [5].

Studies on the etiology of halitosis report that 2% of cases stem from renal, metabolic, hepatic, endocrinological and gastrointestinal disorders (such as infection by *Helicobacter pylori* and intestinal blockage), and 8% are due to conditions of the respiratory system and conditions of the ears, nose and throat (ENT), such as acute tonsillitis, postnasal drip, sinusitis and tonsillolith. The majority of cases (80 to 90%) are directly linked to conditions of the oral cavity, such as periodontal disease, coated tongue, poor oral hygiene, salivary abnormalities (change in pH and hyposialy), stomatitis, intra-oral neoplasm, pulp exposure, extraction wounds and crowding of the teeth [5-8].

Bad breath mainly stems from volatile sulfur compounds (VSCs) produced by the action of anaerobic Gram-negative bacteria (*Fusobacterium nucleatum*, *Selenomonas*, *Treponema denticola*, *Prevotella intermedia*, *Tannerella forsythyia*, *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Eubacterium*) found in the oral cavity on substrates containing sulfur [9-11]. The VSCs produced by the metabolism of these bacteria are hydrogen sulfide (H_2S), found mainly on the dorsum of the tongue, methanethiol (CH_3SH) in gingival pockets and dimethyl sulfide (CH_3SCH_3), which has an extra-oral origin [12-15]. The concentration of these compounds is used as an indicator of halitosis [3,16].

Recently, the anaerobic Gram-positive bacterium *Solobacterium moorei* (also known as *Bulleidia moorei*) has been associated with halitosis due to the production of H_2S in the presence of different supplements containing amino acids, especially cysteine [17,18]. Studies have demonstrated that the presence of these bacteria on the dorsum of the tongue, as well as in saliva and periodontal pockets, can lead to both halitosis and systemic problems such as complications during pregnancy, cardiovascular disease and chronic lower respiratory infection [19], which is considered the third most common cause of death [2,20-23].

Detection

Two main methods are used to evaluate halitosis: a subjective (organoleptic) evaluation and an objective evaluation (quantitative measure of VSCs, gas chromatography (GC) and monitor analysis) [24-27]. Studies comparing the efficacy of these methods report GC to be the most objective and efficacious method for the individual detection of H_2S, CH_3SH and CH_3SCH_3, allowing the evaluation of both the intensity of bad breath and its origin [5,15]. Indeed, GC is currently considered the gold standard for the detection of halitosis [11]. However, the majority of researchers have used a combination of both subjective and objective evaluations, whereas others have only used an organoleptic evaluation due to its ease of execution and low cost [24]. Halitosis can be analyzed using a sulfide monitor, such as the Halimeter (Interscan Corporation, Chatsworth, California, United States) [3,4,26-28], which determines the total amount of VSCs in parts per billion (ppb) under normal conditions.

Photodynamic therapy

Photodynamic therapy (PDT) was discovered in 1900 by Oskar Raab and Hermann von Tappeiner. In the 1970s, PDT began to be used for the treatment of cancer. Recently, antimicrobial PDT has been used as a treatment option for localized infections [29]. PDT involves the use of a non-toxic light-sensitive photosensitizer combined with visible light at the appropriate wavelength to coincide with the absorption spectrum of the photosensitizer, which reaches a state of excitation after absorbing the photons, reacting with the oxygen in the medium to form reactive oxygen species. This phototoxic reaction induces the destruction of bacterial cells. The antimicrobial effect is confined to areas covered by the light-activated photosensitizer, quickly acting on the target organisms when the appropriate energy dose and output power are used [9,29-33]. According to Wainwright [34], bacterial resistance to PDT is unlikely, as the singlet oxygen and free radicals formed interact with different bacterial cell structures and different metabolic pathways [32,33].

The conventional treatment of halitosis related to oral conditions consists of the chemical reduction of microorganisms with a mouthwash, such as 0.2% chlorhexidine, essential oils, triclosan and hydrogen peroxide, the mechanical removal of nutrients with a tongue scraper or brush, the masking of odor with chewing gum, mints and breath spray, and the transformation of VSCs using zinc plus chlorhexidine [2,5,10,12,35-37]. However, the irregular characteristics of the surface of the dorsum of the tongue make the adequate reduction in bacterial a particular challenge [2,36,38].

Considering the scarcity of studies addressing the effect of PDT on tongue biofilm, the aim of the present study was to evaluate the effectiveness of PDT on the dorsum of the tongue in adolescents with halitosis by an analysis of VSCs and microbiological analysis of the tongue.

Methods/Design

This study will be carried out in compliance with regulatory norms governing research involving human subjects. Approval was obtained from the Human Research Ethics Committee of University Nove de Julho (Brazil) under process number 037315/2013, and the study is registered with the United States National Institutes of Health (Clinical Trials.gov registration number: NCT02007993). The guardians of the participants will be informed regarding the procedures and will sign a statement of informed consent authorizing the participation of their sons and daughters in compliance with Resolution 196/96 of the Brazilian National Health Board.

Male and female adolescents enrolled at the dental clinic of the university will be recruited for the study. Those aged between 13 and 18 years, with a diagnosis of halitosis and OralChroma™ results of H_2S ≥112 ppb during the cysteine challenge [11,15,39,40] will be included. The exclusion criteria will be [41] dentofacial anomalies, currently undergoing orthodontic or orthopedic treatment, current use of a removable appliance, implant or dentures, periodontal disease, teeth with carious lesions, currently undergoing cancer treatment, diabetes mellitus, systemic (gastrointestinal, renal or hepatic disorder) conditions, ear, nose or throat conditions, respiratory conditions, antibiotic therapy in the previous month, current pregnancy [5] or hypersensitivity to the photosensitizer. As this is a randomized clinical trial, the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) will be used to ensure greater transparency and quality (Figure 1).

The subjects selected will be randomly allocated to three groups (Table 1). All individuals will be submitted to evaluations with OralChroma™ before and after treatment.

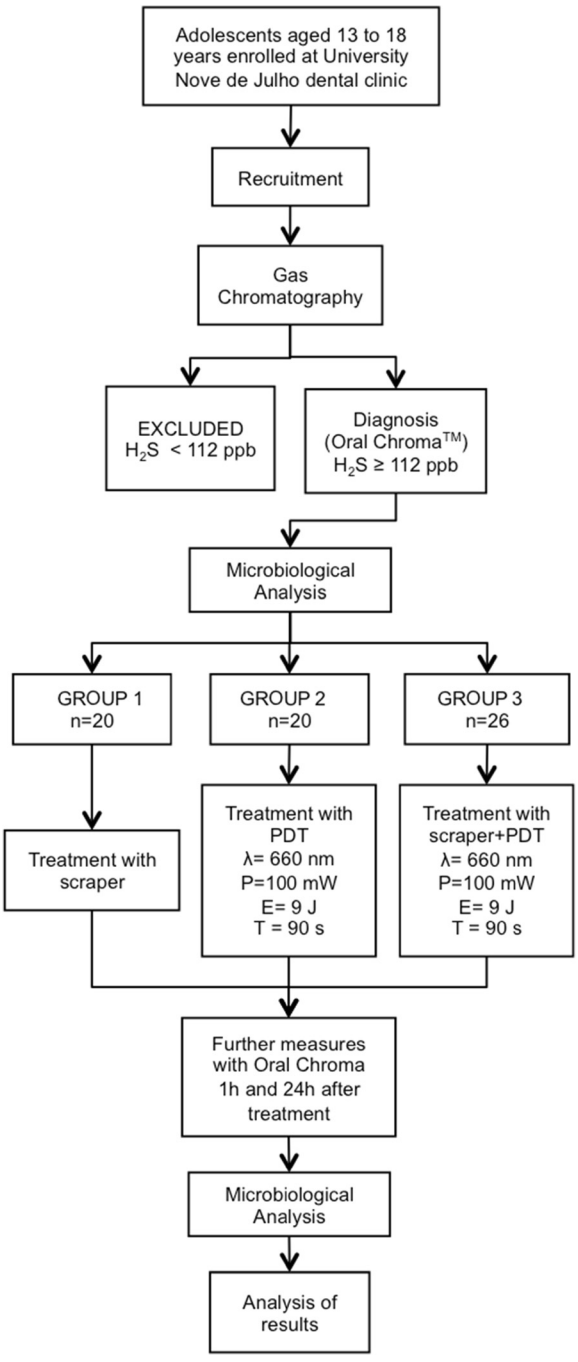

Figure 1 Flowchart of study. P = output power; E = energy and T = time in seconds.

Microbiological analysis

Microbiological analyses of coated tongue will be performed before and after treatment using a 1-μl inoculation loop for the collection of biofilm samples from the dorsum of the tongue. The samples will be transferred to 1.5-ml vials with reduced transport fluid and placed in a vortex mixer (Prolab, São Paulo – Brazil) for approximately 30 seconds for homogenization. Ten-fold serial dilution will be prepared in 180 μl of sterile phosphate buffered saline (Probac, São Paulo – Brazil) and aliquots of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} will be transferred to plates with brain-heart infusion agar (Probac, São Paulo – Brazil). As the main bacteria responsible for the production of VSCs are Gram-negative, the plates will be incubated in anaerobic jar for 72 hours at 37°C, following by the quantification of colony-forming units [10,42].

Halitosis detection

The literature describes a number of methods for measuring halitosis, such as an organoleptic evaluation of the air emanating from the oral cavity [16,26] using a sulfide monitor [16,25,43] or GC [11,43,44]. However, it has been demonstrated that the organoleptic test can be influenced by the olfactory capacity and emotional state of the examiner, as well as climatic conditions [3]. Therefore, the portable OralChroma™ device Abilit Corporation, Chuo-ku, Osaka - Japan) will be employed. This device uses a highly sensitive gas semiconductor sensor.

Table 1 Summary of experimental conditions

Group	Halitosis	Treatment
1	H_2S ≥112 ppb	Tongue scraper
2	H_2S ≥112 ppb	PDT
		E = 9 J T = 90 s
3	SH_2 ≥ 112 ppb	Tongue scraper + PDT
		E = 9 J T = 90 s

H_2S = hydrogen sulfide; ppb = part per billion; PDT = photodynamic therapy; E = energy; T = time in seconds.

The participant will first rinse with cysteine (Fórmula & Ação, São Paulo – Brazil) for one minute (cysteine 10 mM - 16 mg of cysteine in 100 ml of distilled water - 16% mg). A syringe will be placed in the participant's mouth with the plunger completely inserted. The participant will close his or her mouth, breathe through the nose and remain still with the mouth closed for one minute. The participant will be instructed not to touch the tip of the syringe with his or her tongue. The plunger will then be withdrawn, pushed back in to empty the air into the participant's mouth and will be withdrawn again to fill the syringe with the breath sample. The tip of the syringe will be cleaned to remove saliva and a gas injection needle will be placed on the syringe. The plunger will be adjusted to 0.5 ml and the contents will be injected into the input of the device in a single motion (Figure 2) [15].

Analysis of VSCs

OralChroma™ (Abilit Corporation) was developed in Japan for the individual determination of H_2S, CH_3SH and CH_3SCH_3, allowing for the evaluation of both the intensity of bad breath and its origin [5,11,15]. H_2S originates mainly from bacteria on the dorsum of the tongue. Values greater than 112 ppb indicate halitosis. CH_3SH is found in greater concentration in periodontal pockets. Values up to 26 ppb are considered normal. Periodontal disease typically results in a high CH_3SH:H_2S ratio (>3:1). CH_3SCH_3 may have a periodontal or systemic (intestine, liver or lung) origin and may also be temporarily caused by the ingestion of certain foods and beverages. The distinction between CH_3SCH_3 of an oral or systemic origin can be made through the comparison of the results of the halimetric (OralChroma™) with and without a cysteine challenge. The perception threshold for CH_3SCH_3 is very low (8 ppb). Other non-VSC odors may appear in a peak prior to the theoretical first peak, which is H_2S [15].

To maximize the standardization of the readings, the exam will be carried out in the morning and the participants will be instructed to avoid the ingestion of foods with garlic, onion or strong spices, as well as the consumption of alcohol and the use of an antiseptic mouthwash. On the morning of the exam, more than two hours should have passed since any food intake and the participants are to abstain from coffee, hard candy, chewing gum, oral hygiene products and personal care items containing fragrances (aftershave, deodorant, perfume and creams). Brushing will be performed with water alone [27,45].

Photodynamic therapy

The THERAPY XT-ES™ (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) with a red (660 nm) and infrared (810 nm) laser and a fine tip (for regions of difficult access) will be used. Only the volunteer and operator will be present at the time of PDT and both will be wearing protective eyewear. The active point of the laser will be covered with disposable clear plastic wrap (PVC)

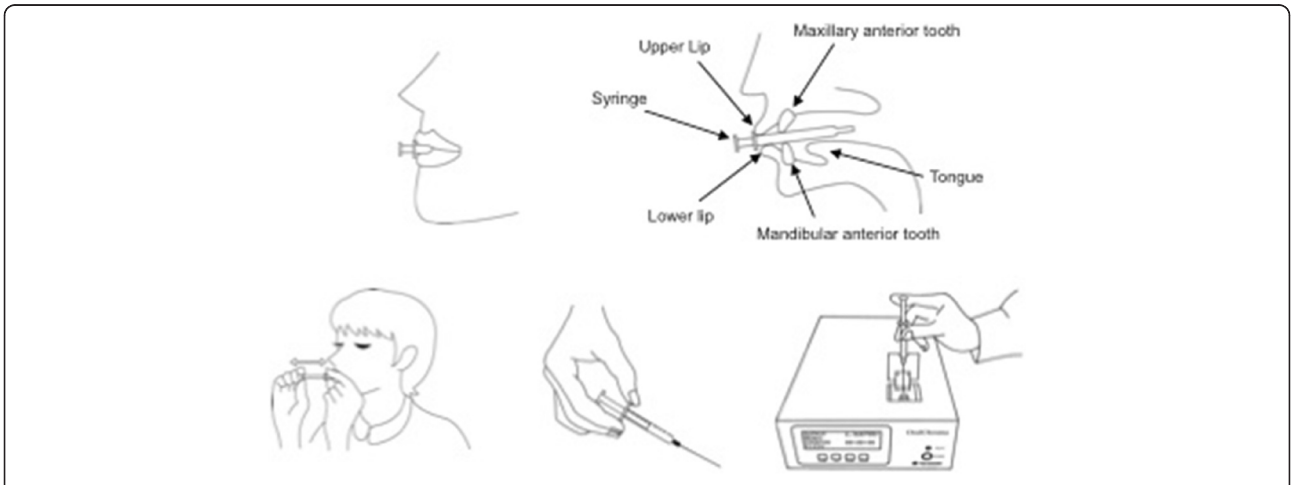

Figure 2 Process for the acquisition of the sample for the halimetric. OralChroma (Abiliti Corporation, Chuo-ku, Osaka – Japan). The OralChroma™ will be connected to the computer with a specific software program that allows the creation of a graph corresponding to the peaks and concentrations of VSCs (0 to 2913 ppb) with considerable precision after eight minutes. The results are stored in the program, as well as in the device itself, and can be retrieved at any time for comparisons of the readings before, during and after treatment.

for hygiene purposes and to avoid cross-contamination. The operator will use the appropriate clothing.

A single session of PDT will be performed with the Chimiolux™ methylene blue photosensitizer (DMC ABC Medical and Dental Equipment, São Paulo, Brazil) at a concentration of 0.005% (165 μm) applied to the middle and posterior thirds of the dorsum of the tongue. After five minutes of pre-irradiation time for incubation, the excess will be removed with an aspirator to maintain the surface moist with the photosensitizer alone (without the use of water). A total of six points will be irradiated (Figure 3). Based on studies developed for the treatment of periodontal disease with PDT [46-52] and a previous pilot study [53], the device will be calibrated with a wavelength of 660 nm, power output of 100 mW, fluency of 320 J/cm^2, irradiance of 3537 mW/cm^2 and an energy dose of 9 joules for 90 seconds per point in groups 2 and 3. The punctual application method will be used with the conventional tip in contact with the tongue (Table 2).

Tongue scraping intervention

A Halicare™ tongue scraper (Odomed, São Paulo, Brazil) will be used for the removal of biofilm. The participant will be instructed to divide the tongue into two parts and scrape each side 10 times (Figure 4).

Calculation of sample size

The error was established as $err = |\bar{x_1} - \bar{x_2}|$, in which $\bar{x_1}$ and $\bar{x_2}$ are the means of groups 1 and 2. Assuming both samples as having the same size ($n_1 = n_2$), the sample size is obtained from the following equation:

$$n_1 = n_2 = \frac{err}{Z\sqrt{\sigma_1^2 + \sigma_2^2}} \quad (1)$$

in which σ_1^2 and σ_1^2 are the variances in groups 1 and 2, respectively. As more than two groups will be studied, the decision was made to employ the largest error found in the literature [54] to estimate the sample size. Assuming all groups as having normal or approximately normal distribution, and that the sample will be large enough for a significance level of α = 0.05, the Z value was determined to be 1.96. However, for the sample size, the test power was established as 1-β = 0.80. In case the hypothesis of normality in the samples was rejected, the sample size was corrected by 5%. Based on the experimental groups in the study by Tsai *et al.* [54] it was determined that each group should contain 26 participants (n = 26).

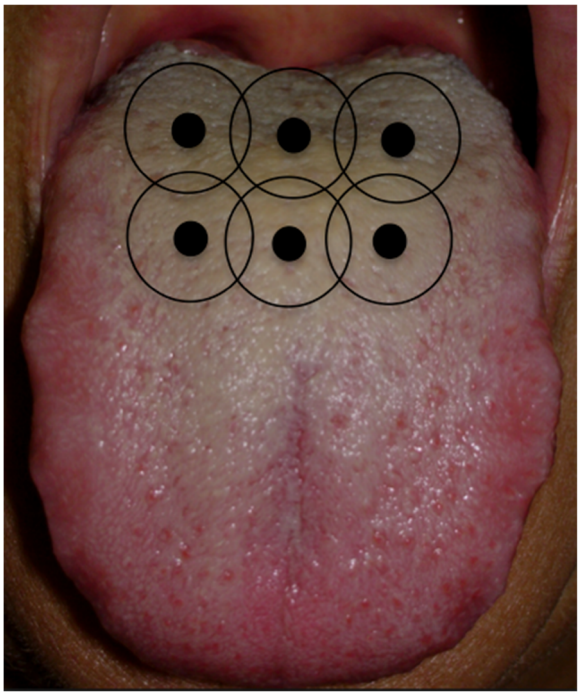

Figure 3 Points of photodynamic therapy application.

Table 2 Parameters of laser

Parameter	Red laser
Center wavelength (nm)	660
Spectral bandwidth (FWHM) (nm)	5
Operating mode	Continuous wave
Average radiant power (mW)	100
Polarization	Random
Aperture diameter (cm)	0.094
Irradiance at aperture (mW/cm^2)	3537
Beam profile	Multimode
Beam spot size at target (cm^2)	0.02827
Irradiance at target (mW/cm^2)	3537
Exposure duration (s)	90/120
Radiant exposure (J/cm^2)	320/428
Radiant energy (J)	9/12
Number of points irradiated	9
Area irradiated (cm^2)	0.254
Application technique	Contact
Number and frequency of treatment sessions	1 session
Total radiant energy (J)	81/108

Outcome measures

Only participants with H_2S ≥112 ppb, CH_3SH ≤26 ppb and CH_3SCH_3 ≤ 8 ppb will be part of the research, which limits participation to individuals with halitosis caused by coated tongue alone. Immediately after treatment, a second halimetry test will be performed and the results will be analyzed.

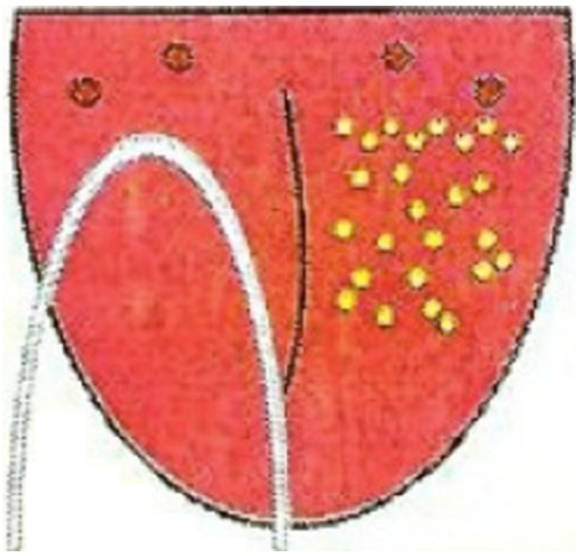

Figure 4 Diagram of tongue scraper use.

Hypothesis

Our null hypothesis is that there will be no change in halitosis following the use of PDT. Our experimental hypothesis is that there will be a reduction in halitosis following the use of PDT alone or in combination with a tongue scraper.

Organization and statistical analysis of data

The Shapiro-Wilk test will be used to determine the distribution of the halimetricdata. If the data presents with normal distribution, analysis of variance (ANOVA) followed by the Tukey test will be used to evaluate the correlation between each of the proposed treatments and halitosis. The paired *t*-test will be used to compare the data before and after each treatment and determine whether the treatments reduced the degree of halitosis. The Kruskal-Wallis test followed by the Student-Newman-Keuls test will be used for data with non-normal distribution, and the Wilcoxon test will be used to analyze the data before and after each treatment. Microbiological data presents with log-normal distribution and will therefore be analyzed using the methods described for data with normal distribution. A significance level of $\alpha = 0.05$ will be used.

Discussion

The main objective of the proposed study is to evaluate the effect of PDT with and without the use of a tongue scraper for the treatment of halitosis in adolescents. This objective has two aspects: the evaluation of VSC levels before and after treatment through a quantitative analysis of H_2S using GC, and a microbiological analysis of the effect of PDT on coated tongue. The findings are expected to provide convincing evidence that PDT is more effective for the treatment of halitosis.

In the literature, the treatment of halitosis is performed using a tongue scraper with or without a mouthwash [2], which leads to a small, long-term reduction in the amount of bacteria on the tongue [38]. Thus, daily oral hygiene is needed to maintain a low level of bacterial proliferation. As the penetration of light and spreading of the photosensitizer do not seem to be affected by the posterior papillae of the tongue, treatment with PDT is promising and may achieve satisfactory results, especially when combined with conventional treatment.

Trial status

The authors are currently recruiting participants. It begun on March of 2014 and we pretend to go until August of 2015.

Abbreviations

CH_3SCH_3: Dimethyl sulfide; CH_3SH: Methanethiol; GC: Gas chromatography; H_2S: Hydrogen sulfide; PDT: Photodynamic therapy; VSC: Volatile sulfur compounds.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RGL participated in the conception and design of the study, data collection and drafting of the present manuscript. CHLG helped draft the manuscript and participated in the data collection. AMD performed the statistical analysis. RAP contributed to the design of the study. MESOS, CMF, KPSF and RAMF critically reviewed the manuscript for intellectual content. SKB conceived the study and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

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ANEXO 6 – Comprovante de submissão

☆ **jbr@iop.org** 10 de dezembro de 2014 07:14

Para: Rubia Garcia Lopes <rubia.lopes.rl@gmail.com>, Carolina ceth Soares, Olinda Tarzia, Deana Deana, Ocultar Detalhes
Renato Prates Prof <pratesra@gmail.com>, cristianepatologia@uninove.br, Kristianne Porta Santos Fernandes, e mais 2...

Your submission to J. Breath Res.: JBR-100192

Dear Ms Lopes,

Re: "Photodynamic Therapy Effect on Halitosis Treatment in Adolescents- Controlled Clinical Trial" by Lopes, Rubia; Soares, Carolina; Tarzia, Olinda; Deana, A; Prates, Renato; França, Cristiane; Fernandes, Kristianne; Ferrari, Raquel; Bussadori, Sandra
Article reference: JBR-100192

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Yours sincerely

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