UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE FARMÁCIA, ODONTOLOGIA E ENFERMAGEM
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

JACQUELINE DE SANTIAGO NOJOSA

AVALIAÇÃO DA GLUTATIONA COMO DESSENSIBILIZANTE APÓS CLAREAMENTO DENTÁRIO COM PERÓXIDO DE HIDROGÊNIO: ESTUDOS *IN VITRO* E CLÍNICO RANDOMIZADO

FORTALEZA
2019

JACQUELINE DE SANTIAGO NOJOSA

AVALIAÇÃO DA GLUTATIONA COMO DESSENSIBILIZANTE APÓS CLAREAMENTO DENTÁRIO COM PERÓXIDO DE HIDROGÊNIO: ESTUDOS *IN VITRO* E CLÍNICO RANDOMIZADO

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Doutora em Odontologia. Área de concentração: Clínica Odontológica.

Orientador: Prof. Dr. Juliano Sartori Mendonça

FORTALEZA

2019

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Biblioteca Universitária
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

N66a Nojosa, Jacqueline de Santiago.
Avaliação da glutationa como dessensibilizante após clareamento dentário com peróxido de hidrogênio : estudos in vitro e clínico randomizado / Jacqueline de Santiago Nojosa. – 2019.
95 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Faculdade de Farmácia, Odontologia e Enfermagem, Programa de Pós-Graduação em Odontologia, Fortaleza, 2019.
Orientação: Prof. Dr. Juliano Sartori Mendonça.

1. Clareamento dentário. 2. Agentes dessensibilizantes. 3. Glutationa. 4. Cor. 5. Sensibilidade da dentina. I. Título.

CDD 617.6

JACQUELINE DE SANTIAGO NOJOSA

AVALIAÇÃO DA GLUTATIONA COMO DESSENSIBILIZANTE APÓS CLAREAMENTO DENTÁRIO COM PERÓXIDO DE HIDROGÊNIO: ESTUDOS *IN VITRO* E CLÍNICO RANDOMIZADO

Tese apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Doutora em Odontologia. Área de concentração: Clínica Odontológica.

Aprovada em: ___/___/_____.

BANCA EXAMINADORA

_____
Prof. Dr. Juliano Sartori Mendonça (Orientador)
Universidade Federal do Ceará (UFC)

_____
Prof. Dr. Sérgio Lima Santiago
Universidade Federal do Ceará (UFC)

_____
Profa. Dra. Vanara Florêncio Passos
Universidade Federal do Ceará (UFC)

_____
Prof. Dr. André Luis Dorini
Universidade Federal do Rio Grande do Norte (UFRN)

_____
Prof. Dr. Jiovanne Rabelo Neri
Centro Universitário Christus (Unichristus)

Dedico esta dissertação aos meus pais, Ivan e Imaculada, por estarem sempre ao meu lado e terem me apoiado, incondicionalmente, durante a minha vida acadêmica.

AGRADECIMENTOS

A **Deus**, por iluminar a minha vida e estar sempre presente, transmitindo-me calma nos momentos mais difíceis.

Aos meus pais, **Ivan e Imaculada**, que não mediram esforços durante a minha formação. A realização dos meus sonhos foi possível devido aos inúmeros incentivos que eles me transmitiam diariamente.

Aos meus irmãos, **Alexandre e Ivan Júnior**, pelo companheirismo e pela vontade de superar todas as dificuldades juntos. A vida é um aprendizado constante e agradeço por tê-los ao meu lado.

Às minhas tias **Inês, Socorro, Elizabeth, Ana Lúcia, Deise e Catarina**, por estarem sempre dispostas a ajudar. A minha família não teria a mesma força para viver e superar as dificuldades se não fossem as enriquecedoras mensagens de luz que elas compartilham. Obrigada por priorizarem a união familiar e conseguirem manter o nosso elo permanente.

Ao meu primo **Alan Santiago**, por ter se dedicado na correção desta tese, não medindo esforços para me ajudar. Além de um amigo, é um profissional muito capacitado e dedicado. Obrigada por fazer parte da minha vida!

Aos meus amigos **Felipe Marçal e Gustavo Araújo**, por todo o companheirismo e por tornarem a minha vida mais leve. Espero ter esses grandes amigos sempre por perto.

Ao meu orientador, **Prof. Dr. Juliano Sartori Mendonça**, por ser tão prestativo e atencioso. Levarei comigo aprendizados que vão além dos muros da pós-graduação. Existem qualidades, como paciência, sensibilidade, humildade e respeito, que não são ensinadas com palavras, e sim com atitudes. Com certeza, a convivência me permitiu um grande aprendizado, e tentei absorver um pouco desses ensinamentos. Obrigada por tudo!

Ao **Prof. Dr. Said Gonçalves da Cruz Fonseca**, pela sua disponibilidade em orientar e compartilhar o seu conhecimento científico. Ele foi essencial na formulação dos géis experimentais do estudo.

Ao **Prof. Dr. Sérgio Lima Santiago**, pelos valiosos ensinamentos desde a graduação.

Ao **Prof. Dr. Jiovanne Rabelo Neri**, por ter me orientado na primeira pesquisa que participei no laboratório. Obrigada pela paciência e por ser tão prestativo!

À doutoranda **Talita Arrais Daniel Mendes**, por ter auxiliado na pesquisa clínica. Obrigada pela parceria no desenvolvimento do estudo.

Ao doutorando **Marcelo Victor Sidou Lemos**, por ter contribuído no manuseio do

equipamento durante o teste no Raman.

Ao técnico do Laboratório de Pesquisa da Pós-Graduação, **David Queiroz de Freitas**, por ter me auxiliado no laboratório e estar sempre disponível a ajudar.

Às amigas da pós-graduação **Sarah Guedes, Myrna Arcanjo, Nadine Albuquerque, Daniela Nunes, Lidiane Costa, Ernanda Sales, Weslanny Morais, Nara Rodrigues, Diana Cunha e Helaine Cajado** pelas inúmeras conversas e trocas de experiência. Obrigada por serem pessoas positivas e incentivadoras.

Aos alunos de iniciação científica **Maria Clara, Mayara Sales, Mateus Freire, Thays Cordeiro e Dannylo Macêdo**, que foram importantes na execução do estudo e contribuíram para o desenvolvimento das atividades no laboratório e na clínica.

Aos **participantes do estudo clínico**, que tiveram um papel extremamente importante no desenvolvimento desta tese. Posso afirmar que tive *sorte* na formação desse grupo, pois conseguimos ter uma ótima relação profissional e pessoal. Obrigada pela dedicação de todos!

À minha aluna **Mayara Beserra**, por ter me ajudado na formatação das referências. Obrigada pelo afinco e pela disposição em contribuir nessa etapa.

Às minhas alunas e amigas **Thallita Willi e Ana Caroline Cavalcante**, pela ótima convivência e pelos inúmeros aprendizados que tivemos juntas.

Aos funcionários da coordenação do Programa de Pós-graduação, **Rafael Maia Reis e Joana Karla de Assis Pinheiro**, pela facilitação na resolução de questões burocráticas referentes à pós-graduação.

À Universidade Federal do Ceará, na pessoa do Magnífico Reitor **Prof. Dr. Henry de Holanda Campos**.

À Faculdade de Farmácia, Odontologia e Enfermagem (FFOE), em nome da **Prof.ª Dra. Lidiany Karla Azevedo Rodrigues**.

Ao Programa de Pós-Graduação em Odontologia da FFOE/UFC, em nome do coordenador **Prof. Dr. Vicente de Paulo Aragão Saboia**.

Ao Curso de Odontologia da Universidade Federal do Ceará, na pessoa da Coordenadora **Prof.ª Dra. Ana Karine Macedo Teixeira**.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (**CAPES**), pela concessão da bolsa de estudos.

“Ninguém pode voltar atrás e fazer um novo começo, mas qualquer um pode recomeçar e fazer um novo fim”.

Chico Xavier

RESUMO

O clareamento dentário pode desencadear um processo de sensibilidade dentária ocasionada pela diminuição drástica da glutationa intracelular. Este estudo foi apresentado em dois capítulos, cujos objetivos foram: Capítulo 1) Avaliar *in vitro* o efeito da glutationa no clareamento dentário caseiro com peróxido de hidrogênio a 7,5% sobre as propriedades das superfícies de esmalte e dentina, bem como analisar a eficácia do clareamento; e Capítulo 2) Avaliar o risco absoluto, a intensidade da sensibilidade dentária, a alteração de cor e o grau de satisfação dos participantes após o clareamento caseiro associado à glutationa como um dessensibilizante. No Capítulo 1, os espécimes foram obtidos a partir de sessenta e seis dentes humanos e aleatoriamente divididos em seis grupos: CONTROLE (sem tratamento), CLAREADO (apenas clareamento dentário), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina), CONT-EXP (sem dessensibilizante), GLUTA5 (glutationa a 5%) e GLUTA10 (glutationa a 10%). Os espécimes foram clareados por 14 ou 21 dias, dependendo do teste, com um gel de clareamento caseiro à base de peróxido de hidrogênio a 7,5% durante 1h e aplicação de um agente dessensibilizante por 10 min diariamente. As propriedades das superfícies das amostras foram determinadas por microdureza Knoop (n=5) e pela análise da composição mineral dos substratos (Espectroscopia Raman) (n=1). A alteração da cor dentária (n=5) foi avaliada com um espectrofotômetro portátil antes e após os tratamentos. Para análise estatística da microdureza e da alteração da cor foram realizados os testes ANOVA para medidas repetidas two-way e Holm-Sidak (α=0,05), enquanto os dados do Raman foram analisados por estimativa qualitativa. Todos os grupos submetidos ao clareamento apresentaram redução significativa da microdureza em relação ao CONTROLE. No grupo KF2, não foi possível realizar as medições das indentações. A composição mineral dos grupos com glutationa demonstrou redução na intensidade do pico de fosfato e aumento do carbonato quando comparado ao controle. Os grupos clareados apresentaram ΔE significativamente maior do que o grupo controle ($p<0,05$) e conseguiram estabilizar a cor após dois meses de acompanhamento. Concluiu-se que o uso da glutationa após o clareamento caseiro alterou os picos de fosfato e carbonato, no entanto não modificou a microdureza, nem a eficácia do clareamento ou a estabilidade de cor. No Capítulo 2, sessenta participantes com canino superior direito com cor C2 ou mais escuro foram selecionados e distribuídos aleatoriamente em três grupos (n=20): PLACEBO (sem dessensibilizante), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina) e GLUTA10 (glutationa a 10%). Os dentes foram clareados de 14 a 21 dias, dependendo do grau de

satisfação, com um gel de clareamento à base de peróxido de hidrogênio a 7,5% durante 1h, seguindo-se uma aplicação de um agente dessensibilizante por 10 min diariamente. A sensibilidade dentária foi registrada durante 14 dias de clareamento com uma escala numérica de classificação de quatro pontos (NRS) e uma escala analógica visual (VAS) de 0-10. A alteração da cor foi avaliada nos dentes 11 e 13 pelo método objetivo (Espectrofotômetro Vita Easyshade) no início do estudo, seguindo-se avaliações de acompanhamento (após 1 dia, 15 dias e 2 meses do término do procedimento clareador). O grau de satisfação foi questionado aos participantes após 14 dias de clareamento, de acordo com a escala 0-3. Para análise estatística, o risco absoluto de sensibilidade dentária foi comparado usando teste de independência G. A intensidade de sensibilidade dentária e o grau de satisfação do participante foram analisados usando Kruskal-Wallis. A alteração da cor foi testada usando os testes ANOVA para medidas repetidas two-way e Holm-Sidak (α=0,05). Nenhuma diferença significativa foi observada no risco absoluto (p=0,5703) e na intensidade de sensibilidade dentária entre os grupos (p>0,05). Em todos os grupos houve uma estabilidade de cor até o final do estudo, exceto os grupos placebo e com glutationa a 10% (p<0,05), que, após dois meses de acompanhamento, não estabilizaram os incisivos. Além disso, o grau de satisfação não foi estatisticamente significante entre os grupos (p=0,4101). Clinicamente, a glutationa não reduziu o risco absoluto e a intensidade da sensibilidade dentária, no entanto não interferiu na alteração de cor e no grau de satisfação do participante.

Palavras-chave: Clareamento dentário; Agentes dessensibilizantes; Glutationa; Cor; Teste de microdureza; Análises químicas; Sensibilidade da dentina.

ABSTRACT

Dental bleaching can trigger a process of tooth sensitivity caused by the drastic decrease of intracellular glutathione. This study was presented in two chapters, whose objectives were: Chapter 1) To evaluate *in vitro* the effect of glutathione after at-home dental bleaching with 7.5% hydrogen peroxide on the enamel and dentin surfaces properties, as well as to assess the tooth bleaching effectiveness; and Chapter 2) To evaluate absolute risk, intensity of tooth sensitivity, color change, and degree of participant satisfaction after at-home bleaching performed associated with glutathione as a desensitizer. In the Chapter 1, the specimens were obtained from sixty-six human teeth and randomly divided into six groups: CONTROL (without treatment), BLEACH (only dental bleaching), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina), CONT-EXP (no desensitizing), GLUTA5 (5% glutathione), and GLUTA10 (10% glutathione). Specimens were bleached for 14-21 days, depending on the test, with a 7.5% hydrogen peroxide-based experimental bleaching gel for 1 h, and a desensitizing agent was applied on the dental substrate per day for 10 min. Specimens surfaces properties were determined by Knoop microhardness (n=5), and analyzed the mineral composition of the substrates (Raman spectroscopy) (n=1). Color change (n=5) was evaluated with a portable spectrophotometer before and after treatments. For statistical analysis of microhardness and color change was performed the two-way repeated measures ANOVA and Holm-Sidak test (α=0.05), while the Raman data was analyzed by qualitative estimation. All bleached groups showed a significant reduction of microhardness in relation to the control. In the commercial group, it was not possible to perform indentation measurement. The mineral composition of GLUTA5 and GLUTA10 groups demonstrated a reduction in the intensity of the phosphate peak and an increase of the carbonate when compared to the control. The bleached groups showed a significantly higher ΔE than did the control group ($p<0.05$) and had the color stabilized after two months of follow-up. The conclusion was that the use of glutathione after at-home tooth bleaching altered phosphate and carbonate peaks, there was no change neither in the microhardness, nor in the bleaching efficacy or in the color stability. In the Chapter 2, sixty participants with right maxillary canine of color C2 or darker were selected and randomly distributed in three groups (n=20): PLACEBO (no desensitizing), KF2 (5% potassium nitrate and 2% sodium fluoride), and GLUTA10 (10% glutathione). Teeth were bleached for 14-21 days, depending on the degree of satisfaction, for 1h with a 7.5% hydrogen peroxide-based experimental bleaching gel, and a desensitizing agent was applied on teeth per day for 10 min.

Tooth sensitivity was recorded for 14 days of bleaching with a four-point numeric rating scale (NRS) and a 0-10 visual analog scale (VAS). The color change was evaluated in the teeth 11 and 13 by objective method (Vita Easyshade Spectrophotometer) at baseline and follow-up evaluations (after 1 day, 15 days, and 2 months of the bleaching procedure completion). The degree of satisfaction was questioned after 14 days of bleaching according to a 0-3 scale. For statistical analysis, the absolute risk of tooth sensitivity was compared using the G-test of independence. Intensity of tooth sensitivity and degree of participant satisfaction were analyzed using Kruskal-Wallis. The color change was tested using the two-way repeated measures ANOVA and the Holm-Sidak test (α=0.05). No significant difference was observed in the risks ($p=0.5703$) and intensity of tooth sensitivity between groups ($p>0.05$). The groups showed a color stability up to the end of the study, except the incisors in placebo and 10% glutathione groups ($p<0.05$), that had not stabilized after two months of follow-up. Also, the degree of satisfaction was not significantly different between the groups ($p=0.4101$). Clinically, the glutathione was not efficient to reduce tooth sensitivity after at-home dental bleaching with 7.5% hydrogen peroxide; however, glutathione did not interfere in the color change and the degree of participant satisfaction.

KEYWORDS: Tooth bleaching; Desensitizing agents; Glutathione; Color; Hardness test; Chemical analyses; Dentin sensitivity.

SUMÁRIO

1 INTRODUÇÃO 14

2 PROPOSIÇÃO 18

3 CAPÍTULOS 20

3.1 Capítulo 1 22

3.2 Capítulo 2 50

4 CONCLUSÃO GERAL 77

REFERÊNCIAS 79

APÊNDICE A – TERMO DE CESSÃO DE DENTES 83

APÊNDICE B – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO 84

APÊNDICE C – FICHA CLÍNICA 86

APÊNDICE D – ESCALA NUMÉRICA DE CLASSIFICAÇÃO DE CINCO PONTOS 87

APÊNDICE E – ESCALA VISUAL ANALÓGICA (EVA) 88

ANEXO – PARECER DO COMITÊ DE ÉTICA EM PESQUISA 91

Introdução

1 INTRODUÇÃO

O clareamento dentário é um procedimento estético conservador com bons resultados clínicos (GRAZIOLI *et al.*, 2018). O mecanismo do clareamento consiste em uma reação de oxidação complexa em que espécies de oxigênio reativo, devido ao seu baixo peso molecular, conseguem infiltrar-se através do esmalte e da dentina (HAYWOOD; HEYMANN, 1989; GREGUS; KLAASSEN, 1995; SULIEMAN, 2003; MINOUX; SERFATY, 2008). Os tecidos duros da estrutura dentária são permeáveis aos agentes clareadores (MC EVOY, 1989; BITTENCOURT et al., 2010) que reagem com os pigmentos e promovem a abertura de seus anéis de carbono, transformando-os em cadeias intermediárias mais claras (HAYWOOD; HEYMANN,1989; JOINER, 2006). Então, o peróxido de hidrogênio clareia os dentes, destruindo os cromóforos orgânicos intrínsecos e modificando-os em moléculas mais translúcidas que refletem menos luz (BOWLES; UGWUNERI, 1987; FUSS; SZAJKIS; TAGGER, 1989; JOINER, 2006).

Apesar de ser considerado um tratamento não invasivo, as moléculas de peróxido podem atuar sobre substratos dentários e comprometer sua morfologia e sua estrutura dentária (KWON; WERTZ, 2015), dependendo da concentração do agente clareador (AZRAK *et al.*, 2010; GOLDBERG; GROOTVELD; LYNCH, 2010). Já foram relatados efeitos negativos, como o aumento da rugosidade na superfície do esmalte e a desmineralização dentária (BOLAY; CAKIR; GURGAN, 2012). Além disso, a sensibilidade dentária é um efeito adverso frequente que ocorre durante e após o clareamento (ALMEIDA *et al.*, 2012; HE *et al.*, 2012; REIS *et al.*, 2011) devido a um processo de irritação pulpar iniciado pela difusão dos componentes de degradação do agente clareador através do esmalte e da dentina (DAHL; PALLESEN, 2003; TAY *et al.,* 2009). Adicionalmente, a irritação pulpar pode estar relacionada à grande quantidade de espécies reativas de oxigênio, o que causa a diminuição da glutationa intracelular, responsável pela defesa contra o dano oxidativo (SCHWEIKL; SPAGNUOLO; SCHMALZ, 2006). A sensibilidade, geralmente, é reversível alguns dias após a realização do tratamento (FUGARO *et al.*, 2004); porém, o desconforto pós-clareamento pode ser reduzido com o uso de agentes dessensibilizantes.

A diminuição satisfatória da sensibilidade durante o tratamento clareador pode ser realizada com agentes dessensibilizantes à base de oxalato de potássio, fluoreto de sódio e nitrato de potássio (ARMÊNIO *et al.*, 2008; TAY *et al.*, 2009). O mecanismo de ação desses agentes pode ser por meio de ação física, vendando os túbulos dentinários; por ação neural,

bloqueando o estímulo nervoso; ou por ambos os tipos de mecanismos (BASTING *et al.*, 2012).

Dentre as substâncias dessensibilizantes que agem por vedamento dos túbulos dentinários, pode-se destacar o fluoreto de sódio, o qual interage com os íons de cálcio e fosfato, formando fluoreto de cálcio, que é mais solúvel e instável, promovendo uma obliteração temporária dos túbulos. Além disso, forma-se um reservatório de fluoreto de cálcio na superfície dentária, favorecendo a remineralização e a formação de fluorapatita, que é mais estável e menos solúvel (ARMÊNIO, 2008).

Em relação aos dessensibilizantes que atuam no bloqueio da transmissão de estímulos sensoriais, um dos mais utilizados é o nitrato de potássio. Sua ação consiste no aumento da concentração de potássio extracelular, que promove a despolarização das membranas das fibras nervosas, impedindo a condução dos impulsos causadores de dor e, consequentemente, reduzindo a sensibilidade (SANTOS *et al.*, 2010).

Por fim, existem os dessensibilizantes que agem por ambos os tipos de mecanismo: vedamento dos túbulos dentinários e bloqueio do estímulo nervoso. O oxalato de potássio interage com o cálcio presente na estrutura dentária, resultando em um processo de cristalização, com a formação de cristais insolúveis, como o oxalato de cálcio, além de impedir a condução dos impulsos nervosos (RICO, 1992).

Outra forma de tentar minimizar a sensibilidade dentária após o clareamento seria através do uso de substâncias antioxidantes, como a glutationa, a fim de evitar a redução drástica da glutationa endógena ocasionada pelo estresse oxidativo celular. A glutationa é um tripeptídeo (g-L-glutamil-L-cisteinil-glicina) que atua em muitos processos biológicos importantes, como síntese de proteínas, metabolismo, proteção celular (DENEKE; FANBURG, 1989) e defesa contra danos oxidativos (KOSOWER; KOSOWER, 1978). No organismo humano, está presente nas formas reduzida (GSH) e oxidada (GSSG) (DENEKE; FANBURG, 1989), sendo GSH a forma responsável por impedir a paralisação do ciclo metabólico da glutationa após um estresse oxidativo (MEISTER; ANDERSON, 1983).

Na presença de reações radicalares prejudiciais *in vivo*, as enzimas atuam como agente de defesa e evitam ou modificam essas reações através da ação de agentes inibidores ou antioxidantes, como a enzima glutationa peroxidase (GSH-Px) (MANNERVIK, 1985; ROVER JÚNIOR; HÖEHR; VELLASCO, 2001). Os mecanismos catalíticos de funcionamento da GSH-Px envolvem o substrato peróxido de hidrogênio (CARSOL *et al.*, 1996; CARSOL *et al.*, 1997; LEHMANN *et al.*, 1998), que tem seu nível controlado no organismo por meio de atividade enzimática contra o ataque de espécies radicalares (MEISTER; ANDERSON, 1983).

Além do peróxido de hidrogênio, GSH-Px também atua sobre a glutationa, utilizando-a como substrato na presença de espécies radicalares (CARSOL *et al.*, 1996; LEHMANN *et al.*, 1998).

Na Odontologia, a ação de GSH-Px pode ser desencadeada durante o procedimento clareador, devido à degradação do peróxido de hidrogênio e à liberação de espécies de oxigênio reativo, tendo como objetivo reduzir o estresse oxidativo. Nesse processo são consumidos os substratos peróxido de hidrogênio e glutationa, com uma redução da sua quantidade intracelular. Dessa forma, a diminuição drástica da glutationa endógena pode estar relacionada à irritação pulpar (SCHWEIKL; SPAGNUOLO; SCHMALZ, 2006) e à possível sensibilidade pós-clareamento.

Apesar da glutationa ser uma substância antioxidante com eficácia comprovada, não existem estudos que confirmem sua ação como agente dessensibilizante. Sua atuação poderia ocorrer por meio da redução de espécies de oxigênio reativo, liberadas durante o clareamento, e da manutenção dos níveis de glutationa intracelular. Nesse contexto, um estudo *in vitro* que avaliasse o efeito da glutationa na composição estrutural dos substratos dentários e analisasse sua possível interferência no clareamento dentário, com uma subsequente avaliação clínica da glutationa como dessensibilizante, é de fundamental importância para a comprovação da sua ação na redução da sensibilidade durante o clareamento dentário.

Proposição

2 PROPOSIÇÃO

Esta tese de doutorado será apresentada em capítulos, tendo como objetivos:

Capítulo 1: Testar *in vitro* o efeito da glutationa após clareamento dentário caseiro com peróxido de hidrogênio a 7,5% sobre a propriedade de dureza e a composição do esmalte, bem como analisar a eficácia do clareamento.

Capítulo 2: Avaliar o risco absoluto de sensibilidade dentária (desfecho primário) do clareamento caseiro realizado com peróxido de hidrogênio a 7,5% associado à glutationa como dessensibilizante. Além disso, verificar a intensidade da sensibilidade dentária, a eficácia do clareamento e o grau de satisfação dos participantes como desfechos secundários.

Capítulos

3. CAPÍTULOS

A presente tese está baseada no artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Por se tratar de pesquisas envolvendo seres humanos, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Universidade Federal do Ceará, através da submissão no site da plataforma Brasil, tendo sido aprovado (Anexo). Dessa forma, esta tese é composta por 2 capítulos que serão submetidos ao periódico citado abaixo:

- Capítulo 1
  Título: The effect of glutathione after at-home dental bleaching on enamel and dentin: An *in vitro* study
  Autores: NOJOSA JS, FONSECA SGC, MENDONÇA JS
  Periódico: Operative Dentistry*

- Capítulo 2
  Título: Clinical evaluation of the efficacy of glutathione in reducing tooth sensitivity after at-home bleaching with hydrogen peroxide-based gel
  Autores: NOJOSA JS, MENDES TAD, FONSECA SGC, MENDONÇA JS
  Periódico: Operative Dentistry*

Normas da revista

* https://www.jopdent.com/authors/authors.php

Capítulo 1

3.1 Capítulo 1

The effect of glutathione after at-home dental bleaching on enamel and dentin: An *in vitro* study

Short Title: The effect of glutathione on dental bleaching

Jacqueline de Santiago Nojosa, DDS, MS
Postgraduate Program in Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará - Fortaleza, Ceará, Brazil
R. Monsenhor Furtado s/n, 60430-655, Fortaleza, Ceará, Brazil

Said Gonçalves da Cruz Fonseca, DDS, MS, PhD
Department of Pharmacy, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará - Fortaleza, Ceará, Brazil
R. Capitão Francisco Pedro, 1210, 60430-372, Fortaleza, Ceará, Brazil

Juliano Sartori Mendonça, DDS, MS, PhD *(Corresponding Author)*
Department of Restorative Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil
R. Monsenhor Furtado s/n, 60430-655, Fortaleza, Ceará, Brazil
Tel.: +55 85 3366 8232
E-mail: mendoncajs@hotmail.com

The effect of glutathione after at-home dental bleaching on enamel and dentin: An *in vitro* study

CLINICAL RELEVANCE

This *in vitro* study investigated the effect of glutathione on dental substrates as a previous test for latter utilization in post-bleaching sensitivity clinical trials. Glutathione after at-home dental bleaching with 7.5% hydrogen peroxide did not change the microhardness caused by the bleaching, however the mineral composition analysis showed an alteration in phosphate and carbonate. And glutathione gels did not interfere with bleaching efficacy and color stability.

ABSTRACT

Objectives: This study aimed at evaluating *in vitro* the effect of glutathione after at-home dental bleaching on the enamel and dentin surfaces properties and on tooth bleaching effectiveness.

Material and Methods: Specimens were obtained from sixty-six human teeth and randomly divided into six groups: CONTROL (without treatment), BLEACH (only dental bleaching), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina), CONT-EXP (no desensitizing), GLUTA5 (5% glutathione), and GLUTA10 (10% glutathione). Specimens were bleached for 14-21 days, depending on the test, with a 7.5% hydrogen peroxide-based bleaching gel for 1 h, and a desensitizing agent was applied on the dental substrate per day for 10 min. Specimens surfaces properties were determined by Knoop microhardness (n=5), and mineral composition (Raman spectroscopy) (n=1). Color change (n=5) was evaluated with a portable spectrophotometer before and after treatments. For statistical analysis of microhardness and color change was performed the two-way repeated measures ANOVA and Holm-Sidak test (α=0.05), while the Raman data was analyzed by qualitative estimation.

Results: All bleached groups showed a significant reduction of microhardness in relation to the control. In the KF2 group, it was not possible to perform indentation measurement. The mineral composition of GLUTA5 and GLUTA10 groups demonstrated a reduction in the intensity of the phosphate peak and an increase of the carbonate when compared to the control. The bleached groups showed a significantly higher ΔE than did the CONTROL group ($p<0.05$) and stabilized the color after two months of follow-up.

Conclusion: Although the use of glutathione after at-home dental bleaching with 7.5% hydrogen peroxide altered phosphate and carbonate peaks, there was no change neither in the microhardness, nor in the bleaching efficacy or in the color stability.

KEYWORDS: Tooth bleaching; Glutathione; Color; Hardness test; Chemical analyses.

INTRODUCTION

Dental bleaching is an aesthetic procedure with good clinical results.[1] Tooth color perception is related to the association of light scattering and adsorption properties of the enamel and dentin, with the properties of dentin playing a major role in determining the general tooth color.[2,3] Bleaching mechanism consists of a complex oxidation reaction in which species of reactive oxygen, due to its low molecular weight, are able to infiltrate through the enamel and dentin.[4-7] Hard tissues of the dental structure are permeable to the bleaching agents[8,9] that react with the pigments and promote the opening of their carbon rings, transforming them into lighter intermediate chains.[4,10] Then, hydrogen peroxide clears the teeth, destroying the intrinsic organic chromophores and modifying them into more translucent molecules that reflect less light.[10-14]

Although tooth bleaching is considered a noninvasive treatment, peroxide molecules can act on dental substrates and compromise their morphology and dental structure,[15] depending of the peroxide concentration.[16,17] In other studies negative side effects have been demonstrated, such as enamel surface alteration,[16] increased surface roughness, and dental demineralization.[18] Also, tooth sensitivity is a frequent adverse effect that occurs after bleaching,[19-21] and is due to a pulp irritation process initiated by bleaching agent degradation components diffusion through enamel and dentin.[22,23] In addition, pulpal irritation may be related to the large amount of reactive oxygen species, which causes the decrease of the intracellular glutathione, responsible for the defenses against oxidative damage.[24]

In this sense, a new agent to minimize pulpal damage after dental bleaching through the use of an antioxidant substance is proposed: glutathione could hinder the drastic reduction of endogenous glutathione caused by cellular oxidative stress during bleaching. This agent is a tripeptide (g-L-glutamyl-L-cysteinyl-glycine) that partakes in many important biological processes, such as protein synthesis, metabolism, cellular protection,[25] and defense against oxidative damage.[26] In the p,resence of injurious radical reactions *in vivo*, the enzymes act as a defense agent and avoid or modify these reactions through the performance of inhibitory agents or antioxidants, such as the enzyme glutathione peroxidase (GSH-Px). In Dentistry, the GSH-Px action is aimed at reducing oxidative stress produced during the bleaching procedure. In this process, hydrogen peroxide and glutathione are consumed, which causes a reduction of their intracellular quantity. Thus, the drastic decrease in endogenous glutathione may be related to pulpal irritation[24] and to the possible post-bleaching sensitivity.

Regarding the dental substrates alteration, studies comparing the effect of desensitizing agents on the potential of bleaching gels and on the enamel structure are restricted.[27-29] Although glutathione is an antioxidant substance with proven efficacy, there are no studies demonstrating its action on dental substrates. Therefore, new *in vitro* studies are needed to evaluate the use of commercial desensitizing agents during bleaching, in parallel with glutathione, which is the new agent suggested in this study. This assessment of dental substrate changes is essential prior to clinical trials testing glutathione as a dental desensitizer. Hence, this study aimed at evaluating *in vitro* the effect of glutathione after at-home dental bleaching on the enamel and dentin surfaces properties and tooth bleaching effectiveness. The hypotheses tested were: 1) glutathione associated with at-home bleaching with 7.5% hydrogen peroxide will be similar in the microhardness when compared to the bleaching groups; and 2) glutathione after bleaching will not reduce the effectiveness of bleaching in relation to control group.

MATERIAL AND METHODS

Experimental design

After approval by Research Ethics Committee (protocol number 2.607.107) of the Federal University of Ceará - UFC (Ceará, Brazil), specimens were obtained in slice and block forms from 66 human third molars. Specimens in slice form underwent the microhardness test (n=5), while in block form were employed to analyze the enamel mineral composition of the enamel with Raman spectroscopy (n=1) and the color change after dental bleaching (n=5). The specimens were randomly divided into six groups: CONTROL (without treatment), BLEACH (only dental bleaching), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina, Brazil, batch number: 131217) (5% potassium nitrate and 2% sodium fluoride), CONT-EXP (no desensitizing), GLUTA5 (5% glutathione), and GLUTA10 (10% glutathione) (Table 1). In all groups, except in the CONTROL group, a bleaching gel based on 7.5% hydrogen peroxide was used for 1 h at room temperature, in a period of 14 days in microhardness and enamel's mineral composition tests, and 21 days in evaluation of color change. The specimens were immersed in artificial saliva between treatments.

Formulation of gels

The experimental gels were manipulated with two hypodermic syringes coupled to each other. For the production of the no desensitizing gel, the first syringe contained sterile

distilled water, and the second contained hietelose, menthol, vitamin E, and saccharin. Glutathione-based gels had the same composition as the above mentioned plus the inclusion of glutathione in the second syringe at concentrations of 5 and 10%. All gels were handled at the time of the first application, with a sufficient amount for the entire treatment.

Preparation of specimens for microhardness test

Five human third molars were fixed with sticky wax (Kota Ind. E Com. Ltda, São Paulo, SP, Brazil) in the center of an acrylic plate (40 x 40 x 5 mm), cross-sectioned at the crown's middle third (Model 12205, Extec®, Enfield, CT, USA), and coupled to a metallographic cutter (Isomet®, Buehler Ltd., Lake Bluff, IL, USA) under low rotation and abundant cooling. Subsequently, longitudinal cuts were made with the purpose of dividing teeth into 6 slices containing each one enamel and dentin (Fig. 1).

Thirty dental fragments were obtained and distributed among the groups. Each group presented 5 specimens, each one coming from a dental fragment. The specimens were embedded (PRE 30Mi, Arotec Indústria e Comércio SA, Cotia, SP, Brazil) in colorless self-curing acrylic resin (JET, Clássico, Campo Limpo Paulista, SP, Brazil), with enamel and dentin facing upwards. In order to obtain polished and standardized surfaces, enamel and dentin fragments were sanded using silicon carbide with sequential granulations of 320, 600, and 1.200, in a polishing machine under refrigeration (AutoMet® 250, Buehler, Lake Bluff, IL, USA), with rotation of 250 rpm. After each sanding granulation was substituted, the specimens were washed in running water and placed for 5 minutes on ultrasound with a frequency of 40 kHz and power of 81 W (Ultrasonic Cleaner, USC 1400, ASonic, Ljubljana, Slovenia). After the use of a 1.200 granulation sandpaper, the specimens were polished with a felt disk and a 1 μm granulation diamond paste, and then immersed for 20 minutes in distilled water on the ultrasound. Specimens were stored for 24 hours in distilled water at 37 °C prior to the treatments and application of the bleaching agent.

Microhardness test

Microhardness testing using a micro-meter (Future Tech 9000 FM, Future-Tech Corp., Kawasaki, Kanagawa, Japan) coupled to a FM-ARS® software and a Knoop type penetrator was carried out for polished enamel and dentin specimens for 14 days at baseline and after the application of the treatments. Five specimens from each group had indentations in longitudinal section of enamel and dentin. An elongated pyramid shaped indenter was used with

a 25 gf static load applied for 5 seconds in the enamel[30] and a 10 gf load for 5 seconds in the dentin.[31] In total six enamel indentations and six dentin indentations with a distance of 100 µm between them were performed from the enamel-dentin interface. Three indentations were performed in each substrate before the application of the treatments (baseline KHN), and three final indentations were performed 24 hours after the end of bleaching (final KHN). It was possible to carry out a direct comparison of the same dental structure before and after the application of the treatments in obtaining the percentage of surface hardness change (%SMC) in enamel and dentin. It was calculated using the formula:

$$\%SMC = \frac{[final\ KHN - baseline\ KHN\ ]\ x\ 100}{baseline\ KHN}$$

Where *baseline KHN* is the average initial microhardness, and *final KHN* is the average final microhardness.

Preparation of specimens for analysis with Raman spectroscopy of the enamel's mineral composition and evaluation of color change

Thirty-six healthy human molar teeth were fixed with sticky wax (Kota Ind. E Com. Ltda, São Paulo, SP, Brazil) in the center of an acrylic plate (40 x 40 x 5 mm). On the buccal surfaces of the teeth, perpendicular serial cuts using a double-faced diamond disk (Model 12205, Extec®, Enfield, CT, USA) coupled to a metallographic cutter (Isomet®, Buehler Ltd., Lake Bluff, IL, USA) were made to obtain enamel blocks (4 x 4 x 2 mm) for enamel's mineral composition analysis (n=1) and evaluation of color change (n=5). Subsequent to the planification of the blocks in a polishing machine under refrigeration (Aropol 2V, Arotec Indústria e Comércio SA, Cotia, SP, Brazil), specimens were fixed with sticky wax in the center of an acrylic plate to stabilize the block and ease its manipulation during tests (Fig. 2).

Analysis with Raman spectroscopy of enamel's mineral composition

Specimens were submitted to a Raman Spectrometer (XploRA ONE, Horiba, Kyoto, Japan) after 24 hours of a 14-day bleaching treatment. This analysis aimed at verifying the enamel's mineral structure and at evaluating with Raman spectra the molecular vibrations of phosphate (PO_4, 961 cm^{-1}) and carbonate (CO_3, 1063 cm^{-1}) in the specimens. The spectrum was obtained from an argon laser with wavelength at 638 nm and power incident at 3.2 mW. The Raman spectrum was obtained in a 700 to 1050 cm^{-1} range with 10 seconds of analysis and

a total of 3 scans applied at only one point on the enamel surface. The concentrations of PO_4 and CO_3 were determined to observe the behavior of the enamel after the treatment (Table 1). The data of spectra were compiled in OriginPro 9.0 software and evaluated by qualitative estimation in relation to the loss or gain of mineral content in the different treated groups.

Assessment of color change after tooth bleaching

Tooth color of the enamel blocks was evaluated with a portable spectrophotometer (Vita Easyshade, Vident, Brea, CA, USA) prior to the bleaching procedure. The treatments were realized for 21 days and the evaluation was obtained again during the follow-up (after 1 day, 15 days, and 2 months of the bleaching completion). A silicone guide was made with a 3 mm circular opening radius, in order to standardize the measurement site. The measurement was obtained after three replicates in each enamel block, using the spectrophotometer to quantify the magnitude of the colorimetric difference or ΔE:

$$\Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$$

Where L* represents the luminosity, a* means the red-green chromaticity, and b* is related to the yellow-blue chromaticity of the blocks after the bleaching procedure in relation to the initial parameters. All values were tabulated for statistical analysis.

Statistical analysis

For statistical analysis of microhardness and color change after tooth bleaching, the data were submitted to normality and homogeneity tests. In the comparison between initial and final values of the microhardness in enamel and dentin, the two-way repeated measures ANOVA and Holm-Sidak test were performed. The data of percentage of surface microhardness change in both substrates were accomplished according to the ANOVA and Tukey tests. The color change data in ΔE of different groups was scrutinized with the two-way repeated measures ANOVA (groups *vs.* assessment time) and the Holm-Sidak test. In all statistical exams, the significance level was set at 5% ($\alpha = 0.05$).

Results

Microhardness test

No statistic significant differences were observed between the groups in relation to the baseline microhardness ($p > 0.05$). After the treatments in enamel and dentin, absolute

microhardness values showed no significant difference between the groups with an ANOVA test ($p > 0.05$), except the one that was not bleached. In the KF2 group, it was not possible to perform indent measurement, and consequently these data were not included in the statistical analysis. All groups on both substrates that were bleached showed a significant reduction of final microhardness ($p < 0.05$); however, the CONT-EXP and GLUTA10 groups whose dentin was treated also had no significant difference in the percentage of surface hardness loss when compared to the group without bleaching (Table 2).

Analysis with Raman spectroscopy of enamel's mineral composition

Fig. 3 shows Raman spectra of all groups after 14 days of at-home tooth bleaching, with immersion in artificial saliva between treatments. The analysis of the molecular vibrations presented in 961 cm^{-1} a reduction in the spectrum related to phosphate in all bleaching groups, with the largest reduction in GLUTA10 group. There was an increase in the 1063 cm^{-1} spectrum of the carbonate in groups GLUTA5 and GLUTA10, with a greater intensity in the high concentration of glutathione.

Assessment of color change after tooth bleaching

The color evaluation with spectrophotometer was achieved in baseline and in the follow-up after 1 day, 15 days, and 2 months of the at-home dental bleaching finalization. The bleached groups showed a significantly higher ΔE than did the CONTROL group ($p < 0.05$). No significant difference in ΔE was observed between bleached groups in the same period (Table 3; $p > 0.05$). When a group is compared with itself in different times, all of them stabilized the color after two months of follow-up (Table 3).

Discussion

Several studies showed the possible effects that peroxides can have on dental tissues. They reported that bleaching gels can modify dental tissue, such as enamel microhardness[32-34] and chemical composition.[35] However, there is no consensus in the literature about the clinical relevance of these modifications in enamel and dentin.[36] Thus, this study was necessary to evaluate the possible changes in both substrates after dental bleaching associated with the use of desensitizing agents. The first hypothesis tested in this study was that the glutathione associated with an at-home 7.5% hydrogen peroxide bleaching will result in

microhardness, surface analysis, and mineral composition similar to those of the bleaching groups; and it was accepted.

In the present study, dental bleaching caused a significant reduction in enamel and dentin microhardness of about 62.06 and 62.14, respectively. Other studies reported a loss of microhardness which increased with the application time.[33,37] Also, the protective effect of saliva was reported, which was able to avoid deleterious effects on the mechanical properties of the enamel, after a hydrogen peroxide high-concentration bleaching. Then, the saliva has the ability to buffer, dilute, and supply calcium and phosphate to dental remineralization[38] and to maintain the substrates hardness. In this study, the immersion of the blocks in artificial saliva during intervals of the bleaching application did not prevent the loss of dental hardness. On the other hand, a study *in vitro* using a high concentration of hydrogen peroxide related that the hardness of both enamel and dentin were not affected by the bleaching. The same study concluded that the adverse effects on enamel and/or dentin reflect not the bleach itself, but the pH of the formulation.[35] Then, one of the reasons for the different results between the studies may be related to the pH of the bleaching agents.

In the literature, a wide variation of pH has been found in different available bleaching gels, and it has been demonstrated that gels with an acidic pH cause more demineralization and reduction of enamel surface microhardness than those with a higher pH.[33,39] Studies have reported that a neutral pH hydrogen peroxide solution applied in the specimens did not demineralize dental enamel.[40-42] Even so, some changes in the enamel by decreasing its surface microhardness were related to some bleaching agents with a pH about neutral.[33,36] In this study, the 7.5% hydrogen peroxide gel was manipulated with pH adjustment in 7.0; however, due to the instability of the substance, the gel was at pH 5.4 a few hours after production. A commercial bleaching gel with 7.5% hydrogen peroxide was reported to display a 5.5 pH.[43] This instability of hydrogen peroxide may be related to the components used in its manufacture. The carrier used in the production of these gels is generally composed of carbopol (an acidic polymer), which requires the addition of a base for forming the gel netting. As hydrogen peroxide is unstable in alkaline pH, upon being incorporated into the gel, the pH of the formulation triggers its decomposition, releasing acid in the medium and leaving the preparation slightly acidic.[44] Thereby, the maintenance of hydrogen peroxide gel in neutral medium is complex, presenting a pH below the critical value for the dental enamel.[45] The gel with the concentration reported, due to the low pH, probably enabled the demineralization and the reduction of enamel hardness in this study.

Another possible explanation for the divergence of results in the literature may be related to the different gel concentrations, the types of bleaching agents, and the time of exposure. Some papers have not reported change in dental substrates associated with tooth bleaching when employing a carbamide peroxide concentration ranging from 10%[46-48] to 15%.[49] However, other studies showed that the same concentrations of carbamide peroxide led to a decrease in enamel microhardness.[50-52] Comparing two types of bleaching agents with different concentrations, a study demonstrated that there was no change in microhardness regardless of composition and concentration of the gels containing 10% and 35% carbamide peroxide, and 3.6% and 10% hydrogen peroxide.[53] Those authors judged that more expressive changes could be restricted to high concentration peroxide gel. The explanation for this may be the prolonged oxidation process, which increases the effects on the enamel surface.[54,55]

After the treatments in enamel and dentin, no significant difference in the absolute microhardness values between the bleached groups was observed. We used a 5% potassium nitrate and 2% sodium fluoride based gel before the dental bleaching, which is the most effective way of this application.[56] A paper evaluated *in vitro* the use for 4 min of a 3% potassium nitrate and 0.11% fluoride based desensitizer gel, after in-office dental bleaching, and found out a reduction in enamel microhardness.[57] However, it was not possible to perform the final evaluation in KF2 group since the images were dark and uneven, which made it difficult to visualize and measure the indentations. It is suggested that this alteration is related to the action of fluorine as a dentinal tubules obliterator, promoting the reduction of its diameter. This effect is possible due to the penetration of 25 μm[58] and 50-200 μm fluoride ions,[59] respectively, in enamel and dentin, resulting in the formation of calcium fluorides. Then, the deposition of crystals could make it difficult to obtain a regular surface for the analysis of the indentations.

Glutathione associated with bleaching came up due to its antioxidant action, for the purpose of reducing reactive oxygen species released during bleaching and maintaining intracellular glutathione levels. This drastic avoidable reduction of endogenous glutathione caused by cellular oxidative stress has already been reported as one of the causes of pulpal damage.[24] In the present study, the GLUTA5 and GLUTA10 groups showed a significant reduction of the final microhardness in relation to CONTROL; however, the GLUTA10 group whose specimens had the dentin treated also had no significant difference in the percentage of surface hardness when we bring to comparison the group without bleaching. Thus, in a future clinical study, a 10% concentration glutathione was suggested, because, despite having reduced

the microhardness of the substrates, it was able to be statistically similar to the group without bleaching.

The mineral structure of the enamel in this study was evaluated by Raman spectroscopy, which has as one of its important advantages not needing a complete planification of the specimens before the analysis. With this method, the aprismatic enamel is preserved and allows a close assessment of reality without an enamel surface modification.[60] The literature concerning the same method we employed here gives attention to the readings of the specimens before and after the treatments, but often they occur in only one point of the specimen, without the standardization of the two analysis in the same locality. The ideal is to perform them at the same point since the dental tissue is not uniform. In our study, the comparison was performed between all groups, using the specimen without bleaching as a control. There was no impairment in our analyzes in relation to the studies reported since in both cases the readings were performed at different points of the enamel. In order to assert that there was no change in the phosphate and carbonate concentrations after the treatments, it would be necessary to carry out the readings at the same point. Thus, the data allow a qualitative estimation of the loss or gain of mineral content in the different treated groups.

In our study, phosphate and carbonate spectra in all experimental groups were examined. Previous studies have used the total area under the band or the width at half the maximum peak value for specimens comparison.[61,62] On the other hand, another paper[63] showed that there was no difference in the results analyzed using maximum peak intensity, area under curve or half-width values, due to the peak shape is highly symmetrical. Similar to what we did, previous studies of the Raman spectra in the quantification of enamel molecules used the maximum peak of intensity given in arbitrary units.[64-66] Then, we observed a decrease in the intensity of the phosphate spectrum in the bleached groups when compared to the group without bleaching; and an increase in the carbonate spectrum in the glutathione groups relative to the other groups. Alterations in the enamel's mineral content promoted by peroxides were also detected by other studies using Raman.[67,68] In the literature, most investigations of bleaching with the same test have reported the effects on the phosphate molecule.[60] A study evaluated the mineral loss of bleached human teeth with 10% carbamide peroxide and deemed that mineral content had a slight decrease after bleaching treatment. Another study[39] came to conclusion that the concentration of phosphate and carbonate did not change after the bleaching agents role in at-home technique. One possible explanation for these results is that they kept the specimens from several individuals in natural saliva, which better simulated the actual oral environment

and also minimized demineralization, inducing a variable related to the diverse composition of saliva.

Regarding carbonate content, a study reported that bleaching gels applied daily for several weeks caused a loss of carbonate, in contrast to in-office bleaching agents that are applied in fewer sessions and have increased the content of the molecule studied.[60] In addition, other researchers[69] demonstrated that carbonate decreased its concentration with acidic hydrogen peroxide. When neutral hydrogen peroxide was used, there were no changes in molecular composition, but acidic bleaching agents caused a decrease in the molecules. However, a paper[68] reported that changes in the enamel surface were directly proportional to the hydrogen peroxide concentration. The dissolution of the enamel in an acid occurs as a result of the interaction of hydrogen ions and hydroxyapatite. Hence, quantification of the phosphate group in hydroxyapatite is a good indicator of the degree of mineralization of the enamel.[70] However, carbonate ions are able to replace hydroxyl or phosphate ions, by filling vacancies between the crystallites, since the enamel molecules are prone to substitutional defects.[71] Thereby, this study confirmed that the application of bleaching and desensitizing agents, depending on the type of treatment performed, contributed to the modification of the phosphate and carbonate contents; nonetheless it was not possible to determine if these alterations could cause negative effects to the dental substrate.

In the present study, the presence of glutathione in the dental bleaching did not reduce the bleaching efficacy in relation to control group, being accepted the second hypothesis. All bleaching groups during three evaluations after the treatments showed an equivalent and significant tooth color enhancement. Corroborating it, a clinical trial concluded that the presence of desensitizing agents did not interfere with the color change.[72] Another paper, whose applications were similar to the ones in this study, evaluated the use of a desensitizing agent containing 2% sodium fluoride prior to bleaching with 35% hydrogen peroxide, which also showed no interference in ΔE.[73] Regarding the color stabilization, the literature reports variations in the color stability of bleached teeth, with 18 months considered the mean stabilization time.[74,75]

From the data of this *in vitro* study, it was evaluate the effect on the enamel and on the dentin of desensitizing agents associated with dental bleaching. The decision to observe the dentin substrate also arose from the difficult in precising the contact area of the bleaching gel during at-home bleaching. In addition, some patients may have gingival recession and exposure of the cervical or incisal dentin, which could make the dentin susceptible to the performance of

the bleaching gel. In all tests, the results were favorable to the two types of desensitizing agents; however, 10% glutathione had the advantage of also presenting the percentage of surface hardness change similar to the group without bleaching. On the other hand, this substance promoted the levels modification of phosphate and carbonate. Then, the results of the laboratory research cannot be directly generalized to the clinical condition, since it is impossible to replicate in the laboratory the physiological conditions of the complex pulp-dentin. Thus, clinical studies on vital human teeth are required to evaluate the efficacy of desensitizing agents during tooth bleaching with 7.5% hydrogen peroxide as well as pulp responses after the respective treatments.

CONCLUSION

The evaluation *in vitro* of the use of glutathione after an at-home dental bleaching with 7.5% hydrogen peroxide indicated a reduction in the intensity of the phosphate peak and an increase of the carbonate, however there was no change neither in the microhardness, nor in the effectiveness and stability of dental bleaching.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Federal University of Ceará. The approval code for this study is 2.607.107.

Conflict of Interest

The authors of this manuscript certify no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

1. Grazioli G, Valente LL, Isolan CP, Pinheiro HA, Duarte CG & Münchow EA (2018) Bleaching and enamel surface interactions resulting from the use of highly-concentrated bleaching gels *Archives of Oral Biology* **87** 157-162.

2. Ten Bosch JJ & Coops JC (1995) Tooth color and reflectance as related to light scattering and enamel hardness *Journal of Dental Research* **74** 374-380.

3. Muia PJ (1985) *The four dimensional tooth color system* Quintessence Publishing Company.

4. Haywood VB & Heymann HO (1989) Nightguard vital bleaching *Quintessence International* **20 (3)** 173-176.

5. Gregus Z & Klaassen CD (1995) *Mechanisms of toxicity* McGraw-Hill Health Professions Division.

6. Sulieman M, Addy M, & Rees JS (2003) Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching *Journal of Dentistry* **31** 415-422.

7. Minoux M & Serfaty R (2008) Vital tooth bleaching: biologic adverse effects – a review *Quintessence International* **39 (8)** 645-659.

8. MC EVOY SA (1989) Chemical agents for removing intrinsic stains from vital teeth. II. Current techniques and their clinical application *Quintessence International* **20 (6)** 379-384.

9. Bittencourt ME, Trentin MS, Linden MS, De Oliveira Lima Arsati YB, França FM, Flório FM & Basting RT (2010) Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations *The Journal of the American Dental Association* **141 (3)** 300-306.

10. Joiner A (2006) The bleaching of teeth: a review of the literature *Journal of Dentistry* **34 (7)** 412–419.

11. Bowles WH & Ugwuneri Z (1987) Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures *Journal of Endodontics* **13 (8)** 375–377.

12. Fuss Z, Szajkis S & Tagger M (1989) Tubular permeability to calcium hydroxide and to bleaching agents *Journal of Endodontics* **15 (8)** 362–364.

13. McCaslin AJ, Haywood VB, Potter BJ, Dickinson GL & Russell CM (1999) Assessing dentin color changes from nightguard vital bleaching *Journal of the American Dental Association* **130** 1485–1490.

14. Joiner A, Thakker G & Cooper Y (2004) Evaluation of a 6% hydrogen peroxide tooth whitening gel on enamel and dentine microhardness in vitro *Journal of Dentistry* **32 Suppl 1** 27–34.

15. Kwon SR & Wertz PW (2015) Review of the mechanism of tooth whitening *Journal of Esthetic Restorative Dentistry* **27 (5)** 240–257.

16. Azrak B, Callaway A, Kurth P & Willershausen B (2010) Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens *Journal of Esthetic Restorative Dentistry* **22 (6)** 391–399.

17. Goldberg M, Grootveld M & Lynch E (2010) Undesirable and adverse effects of toothwhitening products: A review *Clinical Oral Investigations* **14 (1)** 1–10.

18. Bolay S, Cakir FY & Gurgan S (2012) Effects of toothbrushing with fluoride abrasive and whitening dentifrices on both unbleached and bleached human enamel surface in terms of roughness and hardness: An in vitro study *Journal of Contemporary Dental Practice* **13 (5)** 584–589.

19. Almeida LC, Riehl H, Santos PH, Sundfeld ML & Briso AL (2012) Clinical evaluation of the effectiveness of different bleaching therapies in vital teeth *International Journal of Periodontics and Restorative Dentistry* **32 (3)** 303–309.

20. He LB, Shao MY, Tan K, Xu X & Li JY (2012) The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: A systematic review and metaanalysis *Journal of Dentistry* **40 (8)** 644–653.

21. Reis A, Dalanhol AP, Cunha TS, Kossatz S & Loguercio AD (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching *Operative Dentistry* **36 (1)** 12–17.

22. Dahl JE & Pallesen U (2003) Tooth bleaching − a critical review of the biological aspects *Critical Reviews in Oral Biology & Medicine* **14 (4)** 292–304.

23. Tay LY, Kose C, Loguercio AD & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching *The Journal of the American Dental Association* **140 (10)** 1245-1251.

24. Schweikl H, Spagnuolo G & Schmalz G (2006) Genetic and cellular toxicology of dental resin monomers *Journal of Dental Research* **85 (10)** 870-7.

25. Deneke SM & Fanburg BL (1989) Regulation of cellular glutathione *American Journal of Physiology* **257 (4)** L163-L173.

26. Kosower NS & Kosower EM (1978) The glutathione status of cells *International Review of Cytology* **54** 109-160.

27. Sasaki RT, Catelan A, Bertoldo ES, Venancio PC, Groppo FC, Ambrosano GM, Marchi GM, Lima DA & Aguiar FH (2015) Effect of 7.5% hydrogen peroxide containing remineralizing agents on hardness, color change, roughness and micromorphology of human enamel *American Journal of Dentistry* **28 (5)** 261-267.

28. Pinheiro HB & Cardoso PE (2011) Influence of five home whitening gels and a remineralizing gel on the enamel and dentin ultrastructure and hardness *American Journal of Dentistry* **24 (3)** 131-137.

29. Borges BC, Borges JS, Melo CD, Pinheiro IV, Santos AJ, Braz R & Montes MA (2011) Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel *Operative Dentistry* **36 (5)** 521-528.

30. Petta TM, Gomes YSBL, Esteves RA, Faial KCF, Couto RSDA & Silva CM (2017) Chemical Composition and Microhardness of Human Enamel Treated with Fluoridated Whintening Agents. A Study in Situ *The Open Dentistry Journal* **31 (11)** 34-40.

31. Bridi EC, Do Amaral FL, França FM, Turssi CP & Basting RT (2016) Inhibition of demineralization around the enamel-dentin/restoration interface after dentin

pretreatment with TiF4 and self-etching adhesive systems *Clinical Oral Investigation* **20 (4)** 857-863.

32. Cakir FY, Korkmaz Y, Firat E, Oztas SS & Gurgan S (2011) Chemical analysis of enamel and dentin following the application of three different at-home bleaching systems *Operative Dentistry* **36 (5)** 529-536.

33. Magalhães JG, Marimoto AR, Torres CR, Pagani C, Teixeira SC & Barcellos DC (2012) Microhardness change of enamel due to bleaching with in-office bleaching gels of different acidity *Acta Odontologica Scandinavica* **70 (2)** 122-126.

34. Cavalli V, Arrais CA, Giannini M & Ambrosano GM (2004) High-concentrated carbamide peroxide bleaching agents effects on enamel surface *Journal of Oral Rehabilitation* **31(2)** 155-159.

35. Sulieman M, Addy M, Macdonald E & Rees JS (2004) A safety study *in vitro* for the effects of an in-office bleaching system on the integrity of enamel and dentine *Journal of Dentistry* **32 (7)** 581-590.

36. Crastechini E, Borges AB & Torres C (2018) Effect of Remineralizing Gels on Microhardness, Color and Wear Susceptibility of Bleached Enamel *Operative Dentistry* [Epub ahead of print].

37. Lewinstein I, Fuhrer N, Churaru N & Cardash H (2004) Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin *Journal of Prosthetic Dentistry* **92 (4)** 337-342.

38. Diaz-Arnold AM & Marek CA (2002) The impact of saliva on patient care: A literature review *Journal of Prosthetic Dentistry* **88 (3)** 337-343.

39. Sa Y, Wang Z, Ma X, Lei C, Liang S, Sun L, Jiang T & Wang Y (2012) Investigation of three home-applied bleaching agents on enamel structure and mechanical properties: an in situ study *Journal of Biomedical Optics* **17 (3)** 035002.

40. Shannon H, Spencer P, Gross K & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24 (1)** 39–44.

41. Pretty IA, Edgar WM & Higham SM (2005) The effect of bleaching on enamel susceptibility to acid erosion and demineralisation *British Dental Journal* **198 (5)** 285–290.

42. Li Q, Xu BT, Li R, Yu H & Wang YN (2010) Quantitative evaluation of colour regression and mineral content change of bleached teeth *Journal of Dentistry* **38 (3)** 253–260.

43. Ferreira IA, Lopes GC, Vieira LCC & Araujo E (2006) Effect of hydrogen-peroxide-based home bleaching agents on enamel hardness *Brazilian Journal of Oral Sciences* **5 (18)** 1090-1093.

44. Christensen H, Sehested K & Corfitzen H (1982) Reactions of Hydroxyl Radicals with Hydrogen Peroxide at Ambient and Elevated Temperatures *The journal of physical chemistry* **86 (9)** 1588-1590.

45. Featherstone JD, ten Cate JM, Shariati M & Arends J (1983) Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles *Caries Research* **17 (5)** 385-391.

46. Murchison DF, Charlton DG & Moore BK (1992) Carbamide peroxide bleaching: Effects on enamel surface hardness and bonding *Operative Dentistry* **17 (5)** 181–185.

47. Shannon H, Spencer P, Gross K & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24 (1)** 39–44.

48. Potocnik I, Kosec L & Gaspersic D (2000) Effect of 10% carbamide peroxide bleaching gel on enamel microhardness, microstructure, and mineral content *Journal of Endodontics* **26 (4)** 203–206.

49. Unlu N, Cobankara FK, Altinoz C & Ozer F (2004) Effect of home bleaching agents on the microhardness of human enamel and dentin *Journal of Oral Rehabilitation* **31 (1)** 57–61.

50. Rodrigues JA, Basting RT, Serra MC & Rodrígues Júnior AL (2001) Effects of 10% carbamide peroxide bleaching materials on enamel microhardness *American Journal of Dentistry* **14 (2)** 67–71.

51. Lewinstein I, Fuhrer N, Churaru N & Cardash H (2004) Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin *Journal of Prosthetic Dentistry* **92 (4)** 337–342.

52. Basting RT, Rodrigues AL Jr & Serra MC (2005) The effect of 10% carbamide peroxide, carbopol and/or glycerin on enamel and dentin microhardness *Operative Dentistry* **30 (5)** 608–616.

53. Abouassi T, Wolkewitz M & Hahn P (2011) Effect of carbamide peroxide and hydrogen peroxide on enamel surface: An *in vitro* study *Clinical Oral Investigations* **15 (5)** 673-680.

54. Oskoee PA, Navimipour EJ, Oskoee SS & Moosavi N (2010) Effect of 10% sodium ascorbate on bleached bovine enamel surface morphology and microhardness *Open Dentistry Journal* **4** 207-210.

55. Bodanezi A, de Bittencourt ME, Bodanezi RV, Zottis T, Munhoz EA & Carlini B Jr (2011) Surface modifications on aesthetically restored teeth following home bleaching with 16% peroxide carbamide *European Journal of Dentistry* **5 (2)** 157-162.

56. Tay LY, Kose C, Loguercio AD & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching *The Journal of the American Dental Association* **140 (10)** 1245-1251.

57. Kutuk ZB, Ergin E, Cakir FY & Gurgan S (2018) Effects of in-office bleaching agent combined with different desensitizing agents on enamel *Journal of Applied Oral Science* **27** 1-10.

58. Horst JA, Ellenikiotis H & Milgrom PL (2016) UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications and Consent *Journal of the California Dental Association* **44 (1)** 16–28.

59. Lou YL, Botelho MG & Darvell BW (2011) Reaction of silver diamine [corrected] fluoride with hydroxyapatite and protein *Journal of Dentistry* **39 (9)** 612–618.

60. Vargas-Koudriavtsev T & Herrera-Sancho ÓA (2017) Effect of tooth-bleaching on the carbonate concentration in dental enamel by Raman spectroscopy *Journal of Clinical and Experimental Dentistry* **9 (1)** e101-e106.

61. Tramini P, Pelissier B, Valcarcel J, Bonnet B & Maury L (2000) A Raman spectroscopic investigation of dentin and enamel structures modified by lactic acid *Caries Research* **34 (3)** 233–240.

62. Camerlingo C, Lepore M, Gaeta GM, Riccio R, Riccio C, De Rosa A & De Rosa M (2004) Er:YAG laser treatments on dentine surface: micro-Raman spectroscopy and SEM analysis *Journal of Dentistry* **32 (5)** 399-405.

63. Gilchrist F, Santini A, Harley K & Deery C (2007) The use of micro-Raman spectroscopy to diferenciate between sound and eroded primary Enamel *International Journal* of *Paediatric Dentistry* **17 (4)** 274-280.

64. Tsuda H & Arends J (1997) Raman spectroscopy in dental research: a short review of recent studies *Advances in Dental Research* **11 (4)** 539-547.

65. Santini A, Pulham CR, Rajab A & Ibbetson R (2008) The effect of a 10% carbamide peroxide bleaching agent on the phosphate concentration of tooth enamel assessed by Raman spectroscopy *Dental Traumatology* **24 (2)** 220-223.

66. Götz H, Duschner H, White DJ & Klukowska MA (2007) Effects of elevated hydrogen peroxide ‘strip’ bleaching on surface and subsurface enamel including subsurface histomorphology, micro-chemical composition and fluorescence changes *Journal of Dentistry* **35 (6)** 457-466.

67. Berger SB, Cavalli V, Martin AA, Soares LE, Arruda MA, Brancalion ML & Giannini M (2010) Effects of combined use of light irradiation and 35% hydrogen peroxide for dental bleaching on human enamel mineral content *Photomed and Laser Surgery* **28 (4)** 533-538.

68. Bistey T, Nagy IP, Simo A & Hegedus C (2007) In vitro FT-IR study of the effects of hydrogen peroxide on superficial tooth enamel *Journal of Dentistry* **35 (4)** 325-330.

69. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T & Wang Y (2011) Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide *Journal of Dentistry* **39 (10)** 686-692.

70. Santini A, Pulham CR, Rajab A & Ibbetson R (2008) The effect of a 10% carbamide peroxide bleaching agent on the phosphate concentration of tooth enamel assessed by Raman spectroscopy *Dental Traumatology* **24(2)** 220-223.

71. Pan Y & Fleet ME (2002) Compositions of the apatite-group minerals: substitution mechanisms and controlling factors *Reviews In Mineralogy & Geochemistry* **(48)** 13-49.

72. Maran BM, Vochikovski L, De Andrade Hortkoff DR, Stanislawczuk R, Loguercio AD & Reis A (2018) Tooth sensitivity with a desensitizing-containing at-home bleaching gel-a randomized triple-blind clinical trial *Journal of Dentistry* **72** 64-70.

73. do Carmo Públio J, D'Arce MB, Ambrosano GM, Aguiar FH, Lovadino JR, Paulillo LA, & Lima DA (2015) Efficacy of tooth bleaching with the prior application of a desensitizing agent *Journal of Investigative and Clinical Dentistry* **6 (2)** 133-140.

74. Auschill TM, Schneider-Del Savio T, Hellwig E & Arweiler NB (2012) Randomized clinical trial of the efficacy, tolerability, and long-term color stability of two bleaching techiniques: 18-month follow-up *Quintessence International* **43 (8)** 683–694.

75. Wang Y, Gao J, Jiang T, Liang S, Zhou Y & Matis BA (2015) Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment— A systematic review and meta-analysis *Journal of Dentistry* **43 (8)** 913–923.

FIGURES

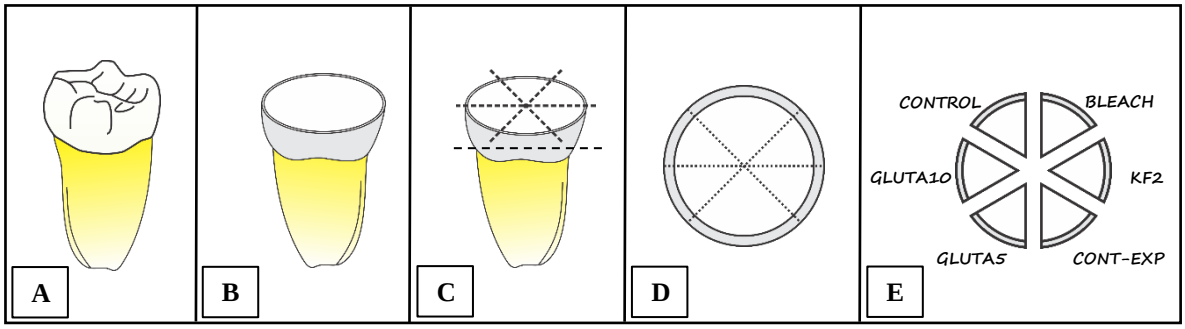


Fig. 1. Schematic drawing representing the sequence of cuts necessary to obtain the specimens in slice form: A) Third molar tooth; B) Tooth after cross section at the height of the middle third of the dental crown; C) Representation of the longitudinal cuts to obtain the specimens; D) Specimens obtained after cutting; and D) Specimens divided by group.

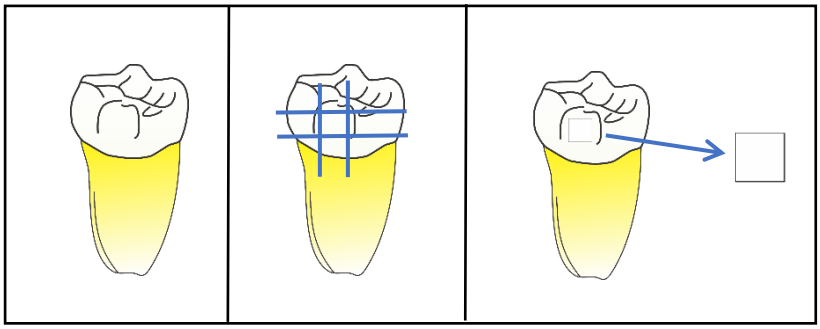


Fig. 2. Schematic drawing representing the sequence of cuts necessary to obtain the specimens in block form: A) Third molar tooth; B) Serial cuts perpendicular to each other; and C) Representation of the specimen.

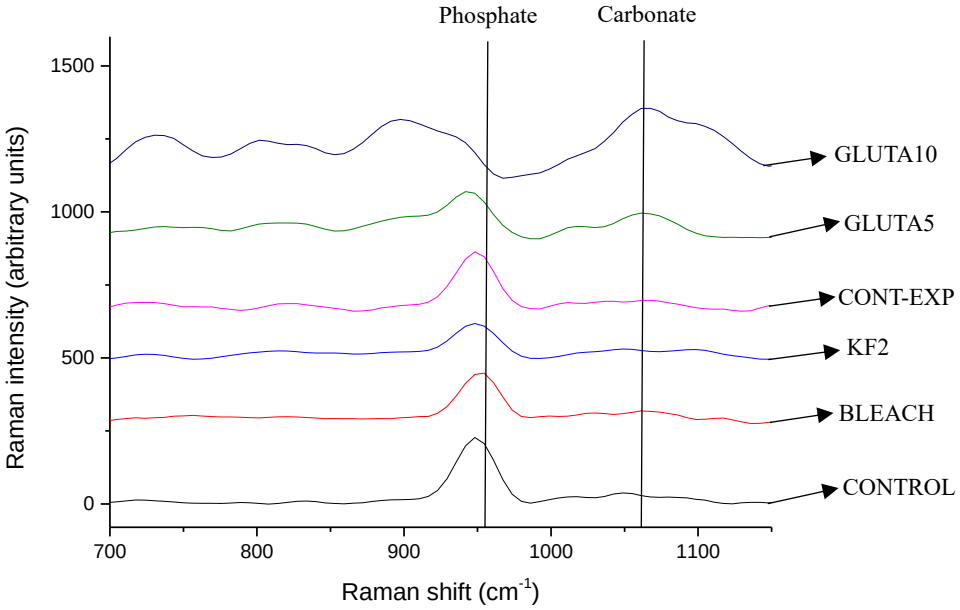


Fig. 3. Spectra of all groups with argon laser (638nm) compiled in OriginPro 9.0 software.

TABLES

Table 1

Description of experimental groups.

Groups	Description: Application of desensitizing substance and bleaching gel
CONTROL	Without dental bleaching and without application of desensitizing gel
BLEACH	Dental bleaching with 7.5% hydrogen peroxide for 1 h, without application of desensitizing gel
KF2	Application of 5% potassium nitrate and 2% sodium fluoride for 10 min and dental bleaching with 7.5% hydrogen peroxide for 1 h
CONT-EXP	Dental bleaching with 7.5% hydrogen peroxide for 1 h and application of gel without active ingredient for 10 min
GLUTA5	Dental bleaching with 7.5% hydrogen peroxide for 1 h and application of gel with 5% glutathione for 10 min
GLUTA10	Dental bleaching with 7.5% hydrogen peroxide for 1 h and application of gel with 10% glutathione for 10 min

Table 2

Means ± standard deviation of baseline and final microhardness (KHN) and percentage of surface microhardness change (%SMC) in enamel and dentin.

Groups (n=5)	Dental substrate	Microhardness*		%SMC**
		Baseline	Final	
CONTROL	Enamel	278,31 ± 29,28 ^{A,a}	263,08 ± 39,76 ^{B,a}	3.85 ± 22.44 ^{Y}
BLEACH		277,43 ± 45,08 ^{A,a}	104,14 ± 36,20 ^{A,b}	62.06 ± 12.60 ^{X}
KF2		291,29 ± 31,20 ^{A,a}	-	-
CONT-EXP		257,22 ± 45,15 ^{A,a}	119,27 ± 19,99 ^{A,b}	51.33 ± 17.47 ^{X}
GLUTA5		307,79 ± 21,78 ^{A,a}	117,22 ± 15,36 ^{A,b}	61.81 ± 5.30 ^{X}
GLUTA10		268,57 ± 36,75 ^{A,a}	95,95 ± 29,96 ^{A,b}	64.65 ± 8.36 ^{X}
CONTROL	Dentin	36,39 ± 7,28 ^{A,a}	31,03 ± 2,73 ^{B,a}	11.66 ± 20.70 ^{Y}
BLEACH		34,10 ± 8,99 ^{A,a}	12,80 ± 3,89 ^{A,b}	62.14 ± 9.66 ^{X}
KF2		31,89 ± 5,68 ^{A,a}	-	-
CONT-EXP		32,96 ± 6,47 ^{A,a}	21,02 ± 9,88 ^{A,b}	34.97 ± 22.27 ^{XY}
GLUTA5		31,84 ± 2,49 ^{A,a}	17,13 ± 3,78 ^{A,b}	46.49 ± 9.36 ^{X}
GLUTA10		30,53 ± 6,06 ^{A,a}	18,75 ± 4,57 ^{A,b}	38.71 ± 9.61 ^{XY}

* Different superscript upper case letters (columns) and different superscript lower case letters (rows) indicate statistically significant differences according to the two-way repeated measures ANOVA and Holm-Sidak test, $\alpha = 0.05$.

** Groups followed by the same letters in columns do not present significant differences according to the ANOVA and Tukey test, $\alpha = 0.05$.

Table 3

Means ± standard deviation demonstrated ΔE of enamel blocks at different times for experimental groups (*).

Groups (n=5)	Color evaluation time after dental bleaching		
	1 day	15 days	2 months
CONTROL	7.74 ± 2.73 ^{A,a}	7.97 ± 5.08 ^{A,a}	8.21 ± 3.06 ^{A,a}
BLEACH	16.72 ± 3.72 ^{B,a}	13.42 ± 5.00 ^{B,a}	15.32 ± 4.0 ^{B,a}
FK2	16.21 ± 3.56 ^{B,a}	17.79 ± 5.18 ^{B,a}	16.86 ± 4.39 ^{B,a}
CONT-EXP	14.88 ± 3.40 ^{B,a}	15.42 ± 2.97 ^{B,a}	16.64 ± 4.73 ^{B,a}
GLUTA5	14.69 ± 4.43 ^{B,a}	15.81 ± 3.01 ^{B,a}	15.54 ± 2.74 ^{B,a}
GLUTA10	14.56 ± 3.17 ^{B,a}	14.40 ± 4.29 ^{B,a}	16.82 ± 4.89 ^{B,a}

(*) Different superscript upper case letters (columns) and different superscript lower case letters (rows) indicate statistically significant differences (two-way repeated measures ANOVA and Holm-Sidak test, $\alpha = 0.05$).

Capítulo 2

3.2 Capítulo 2

Clinical evaluation of the efficacy of glutathione in tooth sensitivity after at-home bleaching with hydrogen peroxide-based gel

Short Title: Evaluation of the glutathione as desensitizing agent after at-home bleaching

Jacqueline de Santiago Nojosa, DDS, MS
Postgraduate Program in Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará - Fortaleza, Ceará, Brazil
R. Monsenhor Furtado s/n, 60430-655, Fortaleza, Ceará, Brazil

Talita Arrais Daniel Mendes, DDS, MS
Postgraduate Program in Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará - Fortaleza, Ceará, Brazil
R. Monsenhor Furtado s/n, 60430-655, Fortaleza, Ceará, Brazil

Said Gonçalves da Cruz Fonseca, DDS, MS, PhD
Department of Pharmacy, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará - Fortaleza, Ceará, Brazil
R. Capitão Francisco Pedro, 1210, 60430-372, Fortaleza, Ceará, Brazil

Juliano Sartori Mendonça, DDS, MS, PhD *(Corresponding Author)*
Department of Restorative Dentistry, Faculty of Pharmacy Dentistry and Nursing, Federal University of Ceará, Fortaleza, Ceará, Brazil
R. Monsenhor Furtado s/n, 60430-655, Fortaleza, Ceará, Brazil
Tel.: +55 85 3366 8232
E-mail: mendoncajs@hotmail.com

Clinical evaluation of the efficacy of glutathione in tooth sensitivity after at-home bleaching with hydrogen peroxide-based gel

CLINICAL RELEVANCE

The results of this randomized, parallel, double-blinded, placebo-controlled clinical trial demonstrated that the glutathione was not efficient to reduce tooth sensitivity after at-home dental bleaching with 7.5% hydrogen peroxide; however, glutathione did not interfere in the color change and the degree of participant satisfaction.

ABSTRACT

Objectives: This study aimed at evaluating absolute risk, intensity of tooth sensitivity, color change, and degree of participant satisfaction after at-home bleaching performed with 7.5% hydrogen peroxide associated with glutathione as a desensitizer.

Material and Methods: Sixty participants with right maxillary canine of color C2 or darker were selected and randomly distributed in three groups (n=20): PLACEBO (no desensitizing), KF2 (5% potassium nitrate and 2% sodium fluoride), and GLUTA10 (10% glutathione). Teeth were bleached for 14-21 days, depending on the degree of satisfaction, for 1 h with a 7.5% hydrogen peroxide-based experimental bleaching gel, and a desensitizing agent was applied on teeth per day for 10 min. Tooth sensitivity was recorded for 14 days of bleaching with a four-point numeric rating scale (NRS) and a 0-10 visual analog scale (VAS). The color change was evaluated in the teeth 11 and 13 by objective method (Vita Easyshade Spectrophotometer, Vident, Brea, CA, USA) at baseline, and follow-up evaluations (after 1 day, 15 days and 2 months of the bleaching procedure completion). The degree of satisfaction was questioned after 14 days of bleaching according to a 0-3 scale. For statistical analysis, the absolute risk of tooth sensitivity was compared using the G-test of independence. Intensity of tooth sensitivity and degree of participant satisfaction were analyzed using Kruskal-Wallis. The color change was tested using the two-way repeated measures ANOVA and the Holm-Sidak test ($\alpha = 0.05$).

Results: No significant difference was observed in the risks ($p = 0.5703$) and intensity of tooth sensitivity among groups ($p > 0.05$). The groups showed a color stability up to the end of the study, except the incisors in PLACEBO and GLUTA10 groups ($p < 0.05$), that had not stabilized after two months of follow-up. Also, the degree of satisfaction was not significantly different between the groups ($p = 0.4101$).

Conclusion: The use of glutathione after at-home dental bleaching with 7.5% hydrogen peroxide did not reduce the absolute risk and the intensity of postoperative tooth sensitivity.

Furthermore, glutathione did not interfere in the color change and the degree of participant satisfaction.

KEYWORDS: Tooth bleaching agents; Desensitizing agents; Dentin sensitivity; Glutathione.

INTRODUCTION

Dental bleaching is a conservative esthetic procedure with good clinical results,[1,2] but it has some adverse effects. The most common of these effects is tooth sensitivity,[3] which occurs due to a pulp irritation process initiated by bleaching agent degradation components diffusion through enamel and dentin.[3,4] In addition, pulpal irritation may be related to the large amount of reactive oxygen species, which causes the decrease of the intracellular glutathione, responsible for the defense against oxidative damage.[5] Sensitivity is usually reversible a few days after the treatment. However, post-bleaching discomfort can be reduced with the use of desensitizing agents.[6]

Sensitivity reduction during bleaching treatment can be performed with desensitizing agents based on potassium oxalate, sodium fluoride, and potassium nitrate, which demonstrate satisfactory results.[3,7] These agents action mechanism can be: a) by means of physical action, sealing the dentinal tubules; b) by neural action, blocking the nervous stimulus; or c) by both.[8]

Another way to minimize pulpal damage after dental bleaching could be through the use of antioxidant substances, such as glutathione, in order to avoid the drastic reduction of endogenous glutathione caused by cellular oxidative stress. Glutathione is a tripeptide (g-L-glutamyl-L-cysteinyl-glycine) that acts in many important biological processes, such as protein synthesis, metabolism, cellular protection,[9] and defense against oxidative damage.[10] In the human body, it is present in reduced (GSH) and oxidized (GSSG) forms,[9] where GSH is the form responsible for preventing the paralysis of the metabolic cycle of glutathione after oxidative stress.[11]

In the presence of injurious radical reactions *in vivo*, the enzymes act as a defense agent and avoid or modify these reactions through the performance of inhibitory agents or antioxidants, such as the enzyme glutathione peroxidase (GSH-Px).[12,13] The GSH-Px operation catalytic mechanisms encompass the substrate hydrogen peroxide,[14,15,16] which has its level controlled in the body by means of enzymatic activity against the attack of radical species.[11] In addition to hydrogen peroxide, GSH-Px also acts on glutathione, using it as a substrate in the presence of radical species.[14,16]

In Dentistry, the GSH-Px action is aimed at reducing oxidative stress produced during the bleaching procedure. This enzyme is activated by the degradation of hydrogen peroxide and the following release of reactive oxygen species. In this process, hydrogen peroxide and glutathione are consumed, which causes a reduction of their intracellular quantity.

Thus, the drastic decrease in endogenous glutathione may be related to pulpal irritation[5] and to the possible post-bleaching sensitivity.

Although glutathione is an antioxidant substance with proven efficacy, there are no studies demonstrating its action as a desensitizing agent. Its action occurs through the reduction of reactive oxygen species released during bleaching and the maintenance of intracellular glutathione levels. Therefore, the aim of this double-blind, controlled, and parallel randomized clinical trial was to evaluate the absolute risk of tooth sensitivity (primary outcome) of at-home bleaching performed with 7.5% hydrogen peroxide associated with glutathione as a desensitizer. Also, the intensity of tooth sensitivity, bleaching effectiveness, and degree of participant satisfaction were evaluated as secondary outcomes. The hypothesis was that the use of glutathione after dental bleaching with 7.5% hydrogen peroxide would reduce the absolute risk and the intensity of postoperative tooth sensitivity when compared to a placebo and a 5% potassium nitrate and 2% sodium fluoride based gel. Also, no differences would be expected in the color change and in the degree of participant satisfaction when all groups were compared.

MATERIAL AND METHODS

This study followed the CONSORT (Consolidated Reporting Standards) statement[17] and was approved (protocol number 2.607.107) by Research Ethics Committee of the Federal University of Ceará - UFC (Ceará, Brazil). It was characterized as randomized, parallel, double-blinded, placebo-controlled clinical trial with an equal allocation rate. Its trials were conducted at the Faculty of Pharmacy Dentistry and Nursing - UFC (Ceará, Brazil) between May and November 2018.

Sample size calculation

The sample size was calculated on the website Sealed Envelope (www.sealedenvelope.com) to detect a high significant effect. The mean absolute risk of tooth sensitivity was 83%,[18] using 5% alpha and 80% power. Considering a possible loss of 10%, 20 volunteers were assembled per group.

Inclusion and exclusion criteria

The participants were selected in the dental clinics of the UFC by an evaluator who underwent theoretical and practical training. Before the clinical exams and the procedures were performed, the Free and Informed Consent Form was signed by the ones sorted out.

Those included in this clinical trial were over 18 years old and had good general and oral health. They could not have caries, cervical or incisal dentin exposure, gingival recession, alterations in the enamel or dentin, and restorations in the anterosuperior and inferior teeth. Right maxillary canine (tooth 13) should present the color C2 or darker in a value-oriented shade guide (Vita Classical, Vita Zahnfabrik, Bad Säckingen, Baden-Württemberg, Germany).

Exclusion criteria comprises previous tooth-bleaching procedures, endodontic treatment in anterior teeth, pregnancy or lactation, previous history of tooth sensitivity, use of desensitizing agents or anti-inflammatory in the last six months, allergic to the product, smokers, any type of oral pathology, and parafunctional habits that could cause sensitivity. Participants that discontinued intervention and lost to follow-up were also excluded.

Experimental design

The volunteers were randomly distributed by Sealed Envelope, according to the desensitizer application, in three groups: PLACEBO (no desensitizing), KF2 (Desensibilize KF 2%®, FGM, Joinville, Santa Catarina, Brazil, batch number: 131217) (5% potassium nitrate and 2% sodium fluoride), and GLUTA10 (10% glutathione) for 10 min (Table 1). In all groups 7.5% hydrogen peroxide-based bleaching gel was used for 1 hour daily during a period of 14 to 21 days, depending on the degree of participant satisfaction. The evaluator did not act on the participant's treatment phase and did not know to which group the participant had been assigned.

Preparation of trays, bleaching procedure, and application of desensitizing gel

Before the bleaching procedure, the clinician made the molding of maxillary and mandibular arches of each volunteer with alginate (Cavex ColorChange Alginate Type I, Cavex Holland BV, Haarlem, North Holland, The Netherlands) to obtain study models (Herodent Stone Gypsum Type III, Vigodent SA Industria e Comércio, Rio de Janeiro, RJ, Brazil). The custom trays for bleaching gel application were produced with a soft acetate board of 1 mm thickness (Bio-art Equipamentos Odontológicos Ltda, São Carlos, SP, Brazil) pressed onto the study models in a vacuum plasticizer (Plastvac-P7, Bioart, São Carlos, SP, Brazil). The tray material excess was cut 1 mm away from the buccal and lingual gingival margins. The bleaching tray was tested on each patient; adjustment was made to remove possible areas of interference and to improve the adaptation of the participant's arcades.

Each volunteer received a kit containing 7.5% hydrogen peroxide-based bleaching gel, desensitizing gel, trays, tray holder, toothbrush (Nobre, Fortaleza, CE, Brazil), and toothpaste containing fluoride without active desensitizing agent (Oral-B® Anticáries, Seropédica, RJ, Brazil). They were instructed to use, for a period of 14-21 days, the bleaching gel and the desensitizer according to their respective group. On the fifteenth day after the bleaching procedure beginning, they returned for color evaluation and for the collection of values regarding the tooth sensitivity perception. At that moment, the participants were asked about the degree of satisfaction with the bleaching according to the scale: 0 = unsatisfied, 1 = partially satisfied, 2 = satisfied, and 3 = very satisfied. Those who answered scores 0 or 1 were instructed to continue bleaching for another 1 week and to return 1 day after the procedure completion to perform a new color evaluation. After the treatment completion, tooth coloring was performed after 1 day, 15 days and 2 months of bleaching.

Dental sensitivity evaluation

The dental sensitivity perception was recorded in a table, daily, by the participants during 14 days of bleaching. Two scales were used for evaluation: four-point numeric rating scale (NRS), where 0 = absent, 1 = mild, 2 = moderate, and 3 = strong; and visual analogue scale (VAS), consisting of a horizontal line with 10 cm dimension, where the initial point represents the absence of pain and the final, severe pain. The values obtained from the sensitivity analysis were organized into two categories: percentage of volunteer reporting sensitivity at least once during treatment (absolute risk) and intensity of tooth sensitivity.

Dental color evaluation

All participants underwent dental prophylaxis prior to the color evaluation. The assessment consisted in an objective evaluation using a portable spectrophotometer (Vita Easyshade, Vident, Brea, CA, USA). The measurement area of the dental color was in the middle third of the right maxillary incisor (tooth 11) and of the right maxillary canine (tooth 13) vestibular surface. The dental color was examined at baseline (prior to the dental bleaching), and follow-up evaluations (after 1 day, 15 days and 2 months of the bleaching procedure completion).

Before the appraisal, an addition silicone device (Adsil Putty Soft, Vigodent SA Indústria e Comércio, Rio de Janeiro, RJ, Brazil) was obtained by preliminary impression of the maxillary arch, including teeth 21, 11, 12, 13, and 14. After molding, a circular aperture

was produced, with a radius of 3 mm, in the middle third of the teeth 11 and 13. This silicone device standardized the measurement site to prevent the entrance of ambient light and to orient the spectrophotometer. The objective measure was obtained after three repetitions in each tooth. The spectrophotometer was used to quantify the colorimetric difference magnitude or ΔE:

$$\Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1\backslash 2}$$

Where L* represents luminosity, a* the red-green chromaticity, and b* is related to the blue-yellow chromaticity) of tooth 11 and 13 after the bleaching procedure in relation to the initial parameters.

Statistical analysis of results

The analysis followed the intention-to-treat protocol and involved all participants who were randomly assigned.[17] The statistics considered the worst score obtained in each treatment week. The absolute risk of tooth sensitivity — primary outcome — was compared using the G-test of independence. Secondary outcomes were intensity of tooth sensitivity (NRS and VAS), bleaching effectiveness, and degree of the participant satisfaction. Intensity of tooth sensitivity was analyzed using Kruskal-Wallis, to compare the groups in the same period (first or second week) and Wilcoxon paired test, to evaluate the same group in the two periods. The color change data in ΔE of different groups was analyzed using the two-way repeated measures ANOVA (groups *vs.* assessment time) and the Holm-Sidak test. And the degree of participant satisfaction was evaluated by the Kruskal-Wallis test. In all statistical tests, the significance level was set at 5% ($\alpha = 0.05$).

RESULTS

Characteristics of included participants

A total of 132 participants were examined to check whether they were eligible according to the inclusion and exclusion criteria: 60 remained for the clinical trial. The general distribution of the ages (years mean ± standard deviation) was 22.6 ± 3.5. The male and female genders accounted for 32.2% and 67.8% of the participants, respectively. The baseline characteristics for each group was showed in Table 2. Only two of the individuals did not attend

the recall visits and quitted the treatment. The participants flow diagram in the different phases of the study design is depicted in Fig. 1.

Dental sensitivity

The absolute risk of tooth sensitivity at least once during the dental bleaching was observed, and no significant difference between the groups showed up (p=0.5703). Regarding the intensity (NRS) in the same period, the groups did not differ statistically neither in the first (p = 0.6971) nor in the second week (p = 0.2666), as described in Table 3. When each group was compared with itself in two periods, their results revealed no significant difference: PLACEBO (p = 1.000), KF2 (p = 0.4008), and GLUTA10 (p = 0.4446).

The groups did not differ statistically about the intensity of tooth sensitivity (VAS) in the same timeframe neither in the first (p = 0.4638) nor in the second week (p = 0.2849), as demonstrated in Table 3. The comparison of each group with itself in two moments revealed no significant difference: PLACEBO (p = 0.9547), KF2 (p = 0.8613), and GLUTA10 (p = 0.1089).

Color evaluation

The color evaluation with spectrophotometer was carried out in teeth 11 and 13 after 1 day, 15 days, and 2 months of the dental at-home bleaching finalization. No significant difference of color was observed between groups in the same period (Table 4; $p > 0.05$). When the same group was compared in different times, only incisors treated in PLACEBO and in GLUTA10 groups ($p < 0.05$) did not stabilized after two months of follow-up. The incisors treated in KF2 and the canines in all the groups showed a color stability up to the end of the study.

Degree of participant satisfaction

The degree of satisfaction was analyzed on the fifteenth day after the bleaching procedure beginning. The participants who were unsatisfied and partially satisfied added up to 13, 10, and 10 in PLACEBO, KF2, and GLUTA10 groups, respectively. The evaluation found out no significant difference between the groups (p=0.4101).

DISCUSSION

The absolute risk of tooth sensitivity reported in this study showed no significant difference between the groups. These reports were in agreement with other papers that

compared the use of desensitizing agents during dental bleaching with placebo.[19-21] Tooth sensitivity was not reduced by the application of 10% glutathione gel when compared to a placebo and a 5% potassium nitrate and 2% sodium fluoride based gel; therefore, we were unable to accept the hypothesis.

The sensitivity sensation often occurs during the early stages of treatment and is usually mild to moderate and transient.[22-24] The use of hydrogen peroxides in dental bleaching results in an increased expression of inflammatory mediators, which trigger nociceptive impulses for the perception of pain.[25,26] Subsequently, the gain in vascular permeability and the tissue pressure rise will result in a response of the dental pulp.[27,28] Furthermore, the penetration of hydrogen peroxide causes an oxidative stress and consequent glutathione decrease that also could be reported as a cause to pulpal irritation.[5]

In this study, the replacement of oxidized intracellular glutathione by means of topical application was suggested, but there was no difference to the other groups. It is likely its high molecular weight (307.32 g/mol), when compared to hydrogen peroxide (34.01 g/mol), is the reason why glutathione could not diffuse throughout the enamel and dentin. Additionally, the residual oxygen species remaining in the dentin tubules continued to trigger the cellular oxidation process until its complete dissipation. Thereby, the consumed glutathione recovery was not possible, due to its lower penetration, which makes the gel unfeasible to reach deep dental structures near the pulp, as the hydrogen peroxide did.

Regarding the use of desensitizing agents during tooth bleaching, some studies have reported efficacy if the patient has undergone a treatment with potassium nitrate and/or sodium fluoride. Thereby, the intensity of tooth sensitivity and the efficacy of tooth bleaching was not affected by this procedure.[7,29-32] According to reports in literature, the desensitizing agent used in this study as positive control (KF2) was a potassium nitrate and sodium fluoride based commercial product. However, corroborating our findings, similar results have been noted about the percentage and the level of tooth sensitivity in both desensitizing agents and placebo.[21]

Various factors may be related to the efficacy and to the safety of desensitizing agent in tooth bleaching. The agent concentration (potassium nitrate, 5%; sodium fluoride, 2%) may be one of the justifications for these study results. A previous clinical research compared the use of 3% potassium nitrate and 0.5% potassium nitrate during tooth bleaching. Differently of 3% potassium nitrate-based agent, the application of 0.5% potassium nitrate significantly reduced the number of days participants experienced sensitivity.[30] The possible explanation was

related to a dose-time response and a difference of osmotic gradient between dentinal fluid and potassium nitrate concentration. The higher concentration of 3% potassium nitrate induced more outward seepage of dentinal fluid. Thereby the inward diffusion of 3% potassium slowed sufficiently to cancel its desensitizing effect.[30,33,34] Other study that investigated desensitizing agents reported a reduction in sensitivity when 0.25% sodium fluoride was incorporated in a 0.5% potassium nitrate agent. As the preceding data was not among the hypotheses tested in our trials, further research is required to determine whether this is a significant trend or just random variation between groups.[30]

Sodium fluoride agent, for its part, is rarely evaluated separately. An association of potassium nitrate with sodium fluoride or potassium nitrate alone were both usually applied before tooth bleaching.[7] However, sodium fluoride was usually applied only after patients experienced tooth sensitivity.[7] Although fluoride has been recognized as a desensitizer, few studies have addressed its effects to decrease tooth sensitivity. The first ever investigation compared fluoride with a placebo in a double-blind randomized clinical study using 16% carbamide peroxide. In this case, no difference was found between the groups receiving the placebo and the fluoride treatment in terms of tooth sensitivity experience; however, patients who received the placebo had a higher intensity tooth sensitivity than those who received the fluoride.[7]

According to pain intensity, the measurements were described by medians of numeric rating scale (NRS) and visual analogue scale (VAS). These tools are generally used to evaluate pain sensations that represent subjective data, not assessable by objective methods. In the present study, NRS and VAS pain scores, documented by the participants, unfold no significant difference between the groups. The results indicated also low tooth sensitivity in all groups, which is in agreement with other studies that evaluated at-home bleaching.[20,35-38] In a NRS 0-3 scale, the median was 1 unit, and in VAS with a 0–10 scale the highest median pain intensity was 1.4 unit. Contrary to previous expectations, the age of the patients was not related to the risk and intensity of tooth sensitivity. In present study, the overall mean age was approximately 22.6, which did not imply a high sensitivity. This inference was based on the fact that the thicker dentin structure in older patients would reduce the amount of hydrogen peroxide to reach the pulp and cause the consequent damage.[39] However, in other paper, this correlation between age and bleaching-induced tooth sensitivity was not observed; the thickness of dentin was not more susceptible to bleaching-induced tooth sensitivity than those with higher

dental volume.[40] Therefore, it suggests that the effect of age on dental bleaching should be investigated more deeply in future clinical trials.[39]

Concerning color evaluation, an objective method was used in this study and provided both a systematic and an objective color assessment. Then, the influence of external factors on shade matching, as illumination and human physiological variabilities, was prevented.[20] The bleaching results may depend on the bleaching agent concentration, the agent's ability to oxidize the dentin organic component, the patient's age, the number of times the agent is in contact with the tooth, and this contact duration.[4] In the present study, the presence of the commercial desensitizing agent or glutathione in the dental bleaching did not influence the bleaching efficacy. An equivalent and significant tooth color enhancement was observed in all study groups during three evaluations after dental bleaching. Corroborating it, a clinical trial concluded that the presence of desensitizing agents did not interfere with the color change.[20]

Additionally, the patients' ages can also influence the effectiveness of tooth whitening.[39] The participants engaged in our research were in general young, which present dentinal tissue that facilitates the bleaching gel penetration. Teeth color will be determined by characteristics of dentin that are correlated with age. Then, older patients do not have as effective whitening as younger ones, due to a dentinal tubules occlusion by mineral deposition and consequent peritubular dentin thickening or a lower permeability of the enamel to hydrogen peroxide due to an increase of hydroxyapatite crystals.[41,42] Also the protein component of the permanent teeth is reduced in older patients as a result of the remineralization occurred in the oral environment.[42-44] Thereby, the decrease in the organic content with increasing age could be one of the reasons for less efficacy in dental bleaching in the elderly.[43]

As another characteristic of this study, the selection of participants was done through the exam of canines, because the recruitment is more easily. It is quite difficult to find individuals with incisors darker than C2 who also meet the inclusion criteria.[45-47] However, the color evaluation after bleaching was done both in incisors and in canines to observe the color variation in different teeth, where no significant differences of colors were detected between the groups with similar long-term results at the end of the study. According to the literature, the incisors bleach at a faster rate than the canines, probably because canines are more saturated, with a greater dental mass and a larger amount of extrinsic pigment. Thereby, regardless of the technique employed, baseline tooth color has been shown to a significant effect on overall color

change, where the bleaching effect is higher in darker teeth as canines with greater variation of color when compared to incisors.[32,47]

Regarding the color stabilization, previous study assessed the use of 10% carbamide peroxide for 14 days and concluded that the color stabilization of incisors and canines occurred in the sixth and in the twelfth week, respectively.[48] On the other hand, the literature reports variations in the color stability of bleached teeth, with 18 months considered the mean stabilization time.[49-52] In present study, incisors treated in PLACEBO and GLUTA10 groups did not stabilized after two months, while canines were stabilized in all the groups in the same time. Additionally, another study reported that bleaching produces dental color homogenization at the end of the treatment, where no significant differences of color were observed among teeth.[53] At-home bleaching procedure in the present study lasted for 14-21 days, revealing not to be possible to saturate the color of dental substrate and homogenize both teeth. Then, the color investigation of incisors and canines is necessary to allow a more effective judgment of color changes.

Finally, the participant satisfaction showed no significant difference between the groups at the end of two weeks. The color change clinically perceptible to the naked eye was suggested when ΔE values had been above 3.3;[54,55] however, in present study, the lower ΔE was about 6.39 and even so approximately the half of the individuals opted to keep dental bleaching after that time. It is therefore important to evaluate levels of satisfaction after treatment to analyze whether the experimental gel interfered in the participant's perception of color. In contrast, a study investigated the treatment time required to achieve participant satisfaction with at-home and in-office tooth bleaching procedures and found that for the participant to be completely satisfied with their bleaching is necessary from four up to six weeks of treatment regardless of the bleaching protocol employed.[56] Since tooth bleaching is usually an elective treatment[57] and is sought due to personal desires,[58,59] it is relevant to determine participant satisfaction at the end of the treatment.

From the absence of statistical difference in the risk and intensity of dental sensitivity variables between all the groups, we can infer that one reason for the equivalent result in the variables could be related to a low sensitivity of these patients in the at-home bleaching. These results should be interpreted with attention, since they do not conclude that glutathione is not a desensitizing agent, but only demonstrate its non-efficacy with the 7.5% hydrogen peroxide bleaching protocol adopted in this study. The fact that there is no difference between PLACEBO and KF2 still leads us to question whether the use of this desensitizing

agent can be employed as a gold standard in all bleaching protocols. The results suggest that the choice of this agent should be adopted individually for each patient and not as a general rule. Therefore, other studies involving glutathione should be developed to verify its performance with other techniques and other bleaching agents.

CONCLUSION

The use of glutathione after at-home dental bleaching with 7.5% hydrogen peroxide tested in this study did not reduce the absolute risk and the intensity of postoperative tooth sensitivity when compared to a placebo and a 5% potassium nitrate and 2% sodium fluoride based gel. Furthermore, the color change and the degree of satisfaction were not affected by glutathione.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the Federal University of Ceará. The approval code for this study is 2.607.107.

Conflict of Interest

The authors of this manuscript certify no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

1. Arcari GM, Araújo E, Baratieri LN & Lopes GC (2007) Microtensile bond strength of a nanofilled composite resin to human dentin after nonvital tooth bleaching *The Journal of Adhesive Dentistry* **9 (3)** 333-340.

2. Bittencourt ME, Trentin MS, Linden MS, De Oliveira Lima Arsati YB, França FM, Flório FM & Basting RT (2010) Influence of in situ postbleaching times on shear bond

strength of resin-based composite restorations *The Journal of the American Dental Association* **141 (3)** 300-306.

3. Tay LY, Kose C, Loguercio AD & Reis A (2009) Assessing the effect of a desensitizing agent used before in-office tooth bleaching *The Journal of the American Dental Association* **140 (10)** 1245-1251.

4. Dahl JE & Pallesen U (2003) Tooth bleaching − a critical review of the biological aspects *Critical Reviews in Oral Biology & Medicine* **14 (4)** 292–304.

5. Schweikl H, Spagnuolo G & Schmalz G (2006) Genetic and cellular toxicology of dental resin monomers *Journal of Dental Research* **85 (10)** 870-7.

6. Fugaro JO, Nordahl I, Fugaro OJ, Matis BA & Mjör IA (2004) Pulp reaction of vital bleaching *Operative Dentistry* **29 (4)** 363-368.

7. Armênio RV, Fitarelli F, Armênio MF, Demarco FF, Reis A & Loguercio AD (2008) The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial *The Journal of the American Dental Association* **139 (5)** 592-597.

8. Basting RT, Amaral FL, França FM & Flório FM (2012) Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents *Operative Dentistry* **37 (5)** 464-473.

9. Deneke SM & Fanburg BL (1989) Regulation of cellular glutathione *American Journal of Physiology* **257 (4)** L163-L173.

10. Kosower NS & Kosower EM (1978) The glutathione status of cells *International Review of Cytology* **54** 109-160.

11. Meister A & Anderson ME (1983) Glutathione *Annual Review of Biochemistry* **52** 711-760.

12. Mannervik B (1985) The isoenzymes of glutathione transferase *Advances in Enzymology and Related Areas of Molecular Biology* **57** 357-417.

13. Rover Jr L, Hoehr NF & Vellasco AP (2001) Sistema antioxidante envolvendo o ciclo metabólico da glutationa associado à métodos eletroanalíticos na avaliação do estresse oxidativo *Química Nova* **24 (1)** 112-119.

14. Carsol MA, Pouliquen I, Giamarchi P, Lesgards G, Sergent M & Luu RPT (1996) Determination of glutathione peroxidase kinetic parameters by experimental design *Analusis* **24 (5)** 195-199.

15. Carsol MA, Pouliquen-Sonaglia I, Lesgards G, Marchis-Mouren G, Puigserver A & Santimone M (1997) A new kinetic model for the mode of action of soluble and membrane-immobilized glutathione peroxidase from bovine erythrocytes *European Journal of Biochemistry* **247 (1)** 248-255.

16. Lehmann C, Wollenberger U, Brigelius-Flohé R & Scheller FW (1998) Bioelectrocatalysis by a selenoenzyme *Journal Electroanalytical Chemistry* **455** 259-263.

17. Schulz KF, Altman DG, Moher D & Consort Group (2011) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials *International Journal Surgery* **9 (8)** 672-677.

18. Kossatz S, Martins G, Loguercio AD & Reis A (2012) Tooth sensitivity and bleaching effectiveness associated with use of a calcium-containing in-office bleaching gel *The Journal of the American Dental Association* **143 (12)** 81-87.

19. Loguercio AD, Tay LY, Herrera DR, Bauer J & Reis A (2015) Effectiveness of nano-calcium phosphate paste on sensitivity during and after bleaching: a randomized clinical trial *Brazilian Oral Research* **29** 1-7.

20. Maran BM, Vochikovski L, De Andrade Hortkoff DR, Stanislawczuk R, Loguercio AD & Reis A (2018) Tooth sensitivity with a desensitizing-containing at-home bleaching gel-a randomized triple-blind clinical trial *Journal of Dentistry* **72** 64-70.

21. Kose C, Reis A, Baratieri LN & Loguercio AD (2011) Clinical effects of at-home bleaching along with desensitizing agent application *American Journal of Dentistry* **24 (6)** 379–382.

22. Leonard RH Jr, Haywood VB & Phillips C (1997) Risk factors for developing tooth sensitivity and gingival irritation in nightguard vital bleaching *Quintessence International* **28 (8)** 527–534.

23. Jorgensen MG & Carroll WB (2002) Incidence of tooth sensitivity after home whitening treatment *Journal of the American Dental Association* **133 (8)** 1076–1082.

24. Pohjola RM, Browning WD, Hackman ST, Myers ML & Downey MC (2002) Sensitivity and tooth whitening agents *Journal of Esthetic and Restorative Dentistry* **14 (2)** 85–91.

25. Caviedes-Bucheli J, Gutierrez-Guerra JE, Salazar F, Pichardo D, Moreno GC & Munoz HR (2007) Substance P receptor expression in healthy and inflamed human pulp tissue *International Endodontic Journal* **40 (2)** 106–111.

26. Caviedes-Bucheli J, Ariza-García G, Restrepo-Méndez S, Ríos-Osorio N, Lombana N & Muñoz Hr (2008) The effect of tooth bleaching on substance P expression in human dental pulp *Journal of Endodontics* **34 (12)** 1462–1465.

27. Costa CA, Riehl H, Kina JF, Sacono NT & Hebling J (2010) Human pulp responses to in-office tooth bleaching *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **109 (4)** 59–64.

28. Vaz MM, Lopes LG, Cardoso PC, Souza JB, Batista AC, Costa NL, Torres ÉM & Estrela C (2016) Inflammatory response of human dental pulp to at-home and in-office tooth bleaching *Journal of Applied Oral Science* **24 (5)** 509–517.

29. Haywood VB, Caughman WF, Frazier KB & Myers ML (2001) Tray delivery of potassium nitrate–fluoride to reduce bleaching sensitivity *Quintessence International* **32 (2)** 105–109.

30. Browning WD, Chan DC, Myers ML, Brackett WW, Brackett MG & Pashley DH (2008) Comparison of traditional and low sensitivity whiteners *Operative Dentistry* **33 (4)** 379-385.

31. Gallo JR, Burgess JO, Ripps AH, Bell MJ, Mercante DE & Davidson JM (2009) Evaluation of 30% carbamide peroxide at-home bleaching gels with and without potassium nitrate – a pilot study *Quintessence International* **40 (4)**1–6.

32. Bonafé E, Loguercio AD, Reis A & Kossatz S (2014) Effectiveness of a desensitizing agent before in-office tooth bleaching in restored teeth *Clinical Oral Investigations* **18 (3)** 839–845.

33. Pashley DH & Matthews WG (1993) The effects of outward convective flow on inward diffusion in human dentine in vitro *Archives of Oral Biology* **38 (7)** 577-582.

34. Pashley DH, Agee K, Zhang Y, Smith A, Tavss EA & Gambogi RJ (2002) The effects of outward forced convective flow on inward diffusion of potassium across human dentin *American Journal of Dentistry* **15 (4)** 256-261.

35. Matis BA, Cochran MA, Eckert GJ & Matis JI (2007) In vivo study of two carbamide peroxide gels with different desensitizing agents *Operative Dentistry* **32 (6)** 549–555.

36. Meireles SS, Heckmann SS, Leida FL, Dos Santos Ida S, Della Bona A & Demarco FF (2008) Efficacy and safety of 10% and 16% carbamide peroxide tooth whitening gels: a randomized clinical trial *Operative Dentistry* **33 (6)** 606–612.

37. Tay LY, Kose C, Herrera DR, Reis A & Loguercio AD (2012) Long-term efficacy of in office and at-home bleaching: a 2-year double-blind randomized clinical trial *American Journal of Dentistry* **25 (4)** 199–204.

38. Morgan S, Jum'ah AA & Brunton P (2015) Assessment of efficacy and post-bleaching sensitivity of home bleaching using 10% carbamide peroxide in extended and nonextended bleaching trays *British Dental Journal* **218 (10)** 579–582.

39. Rezende M, Loguercio AD, Kossatz S & Reis A (2016) Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: a multi regression and logistic analysis *Journal of Dentistry* **45** 1–6.

40. Moncada G, Sepúlveda D, Elphick K, Contente M, Estay J, Bahamondes V, Fernandez E, Oliveira OB & Martin J (2013) Effects of light activation, agent concentration, and tooth thickness on dental sensitivity after bleaching *Operative Dentistry* **38** 467–476.

41. Morse DR (1991) Age-related changes of the dental pulp complex and their relationship to systemic aging *Oral Surgery, Oral Medicine, Oral Pathology* **72 (6)** 721–745.

42. He B, Huang S, Zhang C, Jing J, Hao Y, Xiao L, Zhou X (2011) Mineral densities and elemental content in different layers of healthy human enamel with varying teeth age *Archives of Oral Biology* **56 (10)** 997–1004.

43. Gerlach RW & Zhou X (2001) Vital bleaching with whitening strips: summary of clinical research on effectiveness and tolerability *The Journal of Contemporary Dental Practice* **2 (3)** 1–16.

44. Fujikawa H, Matsuyama K, Uchiyama A, Nakashima S & Ujiie T (2008) Influence of salivary macromolecules and fluoride on enamel lesion remineralization in vitro *Caries Research* **42 (1)** 37–45.

45. Wetter NU, Branco EP, Deana AM & Pelino JE (2009) Color differences of canines and incisors in a comparative long-term clinical trial of three bleaching systems *Lasers in Medical Sciences* **24 (6)** 941–947.

46. Polydorou O, Wirsching M, Wokewitz M & Hahn P (2013) Three-month evaluation of vital tooth bleaching using light units-a randomized clinical study *Operative Dentistry* **38 (1)** 21–32.

47. Mena-Serrano AP, Garcia E, Luque-Martinez I, Grande R, Loguercio A & Reis A (2016) A single-blind randomized trial about the effect of hydrogen peroxide concentration on light-activated bleaching *Operative Dentistry* **41 (5)** 455–464.

48. Matis BA, Cochran MA, Eckert G & Carlson TJ (1998) The efficacy and safety of a 10% carbamide peroxide bleaching gel *Quintessence International* **29 (9)** 555–563.

49. Scaminaci Russo D, Viano M, Bambi C, Nieri M & Giachetti L (2010) Color stability of bleached teeth over time: an in vitro study *The European Journal of Esthetic Dentistry* **5 (3)** 300–310.

50. Auschill TM, Schneider-Del Savio T, Hellwig E & Arweiler NB (2012) Randomized clinical trial of the efficacy, tolerability, and long-term color stability of two bleaching techiniques: 18-month follow-up *Quintessence International* **43 (8)** 683–694.

51. Mondelli RF, Azevedo JF, Francisconi AC, Almeida CM & Ishikiriama SK (2012) Comparative clinical study of the effectiveness of different dental bleaching methods - two-year follow-up *Journal of Applied Oral Science* **20 (4)** 435–443.

52. Wang Y, Gao J, Jiang T, Liang S, Zhou Y & Matis BA (2015) Evaluation of the efficacy of potassium nitrate and sodium fluoride as desensitizing agents during tooth bleaching treatment – A systematic review and meta-analysis *Journal of Dentistry* **43 (8)** 913–923.

53. Hasegawa A, Motonomi A, Ikeda I & Kawaguchi S (2000) Color of natural tooth crown in japanese people *Color Research and Application* **25 (1)** 43–48.

54. Stober T, Gilde H & Lenz P (2001) Color stability of highly filled composite resin materials for facings *Dental Materials* **17 (1)** 87–94

55. Vichi A, Ferrari M, Davidson CL (2004) Color and opacity variations in three different resin-based composite products after water aging *Dental Materials* **20 (6)** 530–534

56. Bernardon JK, Ferrari P, Baratieri LN & Rauber GB (2015) Comparison of treatment time versus patient satisfaction in at-home and in-office tooth bleaching therapy *The Journal of Prosthetic Dentistry* **114 (6)** 826–830.

57. Tin-Oo MM, Saddki N & Hassan N (2011) Factors influencing patient satisfaction with dental appearance and treatments they desire to improve aesthetics *BMC Oral Health* **11** 6.

58. Montero J, Gómez-Polo C, Santos JA, Portillo M, Lorenzo MC & Albaladejo A (2014) Contributions of dental colour to the physical attractiveness stereotype *Journal of Oral Rehabilitation* **41 (10)** 768–782.

59. Maida CA, Marcus M, Hays RD, Coulter ID, Ramos-Gomez F, Lee SY, McClory PS, Van LV, Wang Y, Shen J, Cai L, Spolsky VW, Crall JJ & Liu H (2015) Child and adolescent perceptions of oral health over the life course *Quality of Life Research* **24 (11)** 2739–2751.

FIGURE

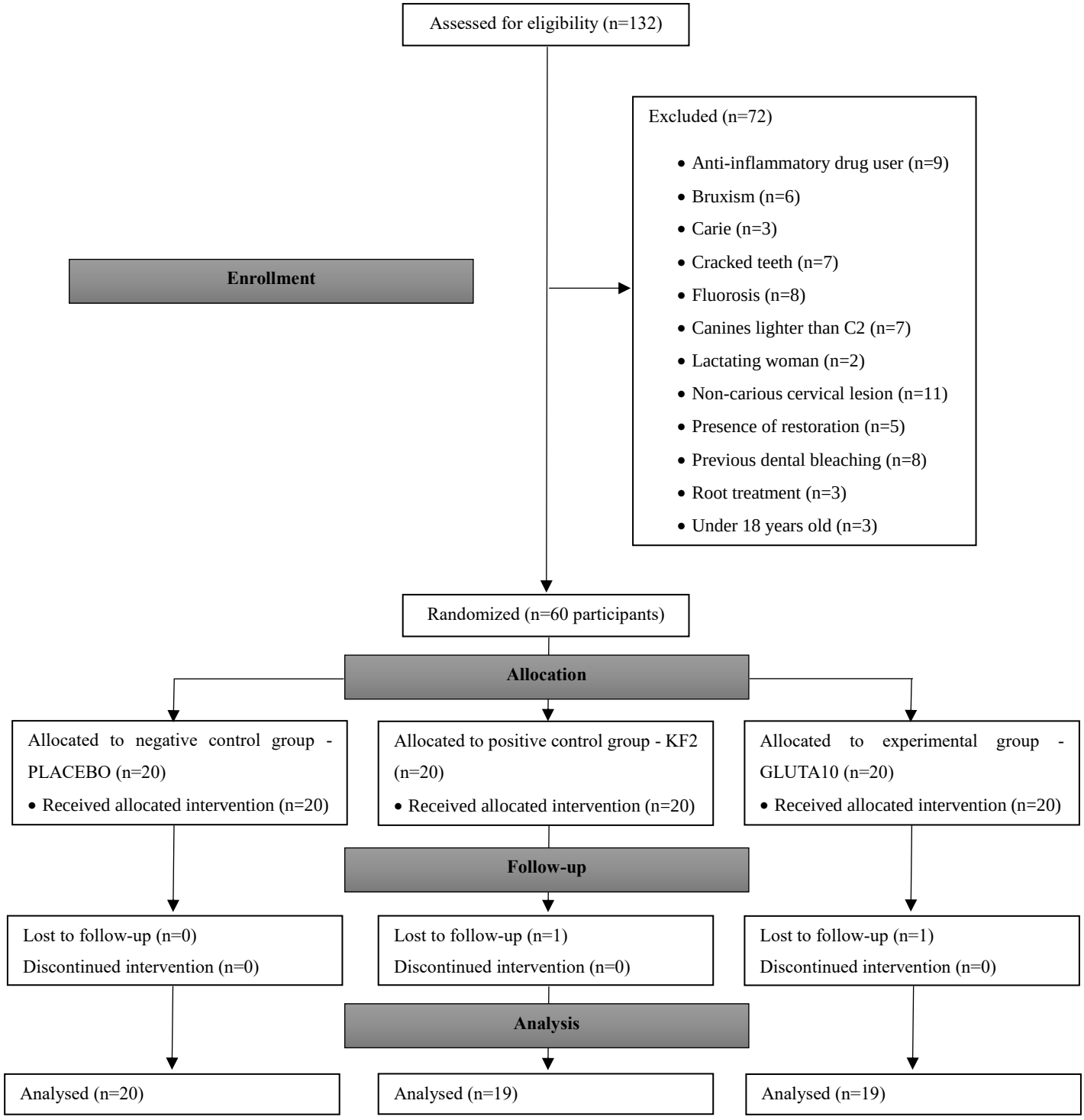


Fig. 1. Flow diagram of the study design phases, including enrollment and allocation criteria.

TABLES

Table 1

Description of Experimental Groups.

Group (n=20)	Description: Application of desensitizing substance and bleaching gel
PLACEBO	Dental bleaching with 7.5% hydrogen peroxide for 1 h and application of gel without desensitizing for 10 min
KF2	Application of 5% potassium nitrate and 2% sodium fluoride for 10 min and dental bleaching with 7.5% hydrogen peroxide for 1 h
GLUTA10	Dental bleaching with 7.5% hydrogen peroxide for 1 h and application of gel with 10% glutathione for 10 min

Table 2

Baseline Characteristics of the Participants.

Characteristics		PLACEBO	KF2	GLUTA10
Age (years; mean ± SD *)		22.2 ± 3.8	22.2 ± 2.2	23.3 ± 4.0
Gender	(male; %)	30%	36.8%	30%
	(female; %)	70%	63.2%	70%

* Abbreviation: SD, standard deviation.

Table 3

Medians (minimum – maximum values) of the tooth sensitivity using two pain scales during two periods.

Period	Pain scale	PLACEBO	KF2	GLUTA10	p-value*
First week	NRS 0-4	1 (0 – 3)	1 (0 – 3)	1 (0 – 2)	0.6971
	VAS 0-10	1.35 (0 – 10)	0.7 (0 – 7)	1.1 (0 – 4.3)	0.4638
Second week	NRS 0-4	1 (0 – 3)	1 (0 – 3)	1 (0 – 2)	0.2666
	VAS 0-10	0.95 (0 – 10)	0.4 (0 – 5)	1.4 (0 – 10)	0.2849

* Kruskal-Wallis test – NRS/VAS

Table 4

Means ± standard deviation demonstrated ΔE of teeth 11 and 13 in different times for experimental groups (*).

Groups	Tooth	Color evaluation time after dental bleaching		
		1 day	15 days	2 months
PLACEBO	11	8.91 ± 4.41 ^{A,a}	7.84 ± 2.73 ^{A,a}	5.21 ± 2.19 ^{A,b}
KF2		6.39 ± 3.80 ^{A,a}	6.23 ± 3.40 ^{A,a}	5.45 ± 3.70 ^{A,a}
GLUTA10		8.44 ± 4.60 ^{A,a}	8.05 ± 3.20 ^{A,a}	4.97 ± 3.04 ^{A,b}
PLACEBO	13	12.46 ± 4.57 ^{X,y}	11.15 ± 5.58 ^{X,y}	9.26 ± 4.07 ^{X,y}
KF2		10.79 ± 4.71 ^{X,y}	11.57 ± 4.87 ^{X,y}	8.89 ± 5.19 ^{X,y}
GLUTA10		11.11 ± 6.29 ^{X,y}	10.81 ± 4.82 ^{X,y}	9.17 ± 4.54 ^{X,y}

(*) Different superscript upper case letters (columns) and different superscript lower case letters (rows) indicate statistically significant differences (two-way repeated measures ANOVA and Holm-Sidak test, $\alpha = 0.05$).

Conclusão geral

4. CONCLUSÃO GERAL

De acordo com os resultados do presente estudo, pode-se concluir que:

1) O estudo *in vitro* avaliando o uso da glutationa após clareamento caseiro com peróxido de hidrogênio a 7,5% mostrou que a microdureza e a análise superficial dos substratos de esmalte e dentina foram semelhantes aos grupos clareados; enquanto a análise da composição mineral apresentou uma redução na intensidade do pico de fosfato e aumento do carbonato quando comparado ao controle. Além disso, não houve interferência na eficácia do clareamento dental nos grupos com aplicação da glutationa.

2) O uso da glutationa após clareamento caseiro com peróxido de hidrogênio a 7,5% não reduziu o risco absoluto e a intensidade da sensibilidade dentária quando comparado ao placebo e ao grupo com aplicação de nitrato de potássio a 5% e fluoreto de sódio a 2%. Ademais, a alteração da cor e o grau de satisfação não foram afetados pela glutationa.

Referências

REFERÊNCIAS

ALMEIDA, L. C.; RIEHL, H.; SANTOS, P. H.; SUNDFELD, M. L.; BRISO, A. L. Clinical evaluation of the effectiveness of different bleaching therapies in vital teeth. **International Journal of Periodontics and Restorative Dentistry**, v. 32, n. 3, p. 303–9, 2012.

ARMÊNIO, R. V.; FITARELLI, F.; ARMÊNIO, M. F.; DEMARCO, F. F.; REIS, A.; LOGUERCIO, A. D. The effect of fluoride gel use on bleaching sensitivity: a double-blind randomized controlled clinical trial. **The Journal of the American Dental Association**, v. 139, n. 5, p. 592-7, 2008.

AZRAK, B.; CALLAWAY, A.; KURTH, P.; WILLERSHAUSEN, B. Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. **Journal of Esthetic Restorative Dentistry,** v. 22, n. 6, p. 391–9, 2010.

BASTING, R. T.; AMARAL, F. L.; FRANÇA, F. M.; FLÓRIO, F. M. Clinical comparative study of the effectiveness of and tooth sensitivity to 10% and 20% carbamide peroxide home-use and 35% and 38% hydrogen peroxide in-office bleaching materials containing desensitizing agents. **Operative Dentistry**, v. 37, n. 5, p. 464-73, 2012.

BITTENCOURT, M. E.; TRENTIN, M. S.; LINDEN, M. S.; DE OLIVEIRA LIMA ARSATI, Y. B.; FRANÇA, F. M.; FLÓRIO, F. M; BASTING, R. T. Influence of in situ postbleaching times on shear bond strength of resin-based composite restorations. **The Journal of the American Dental Association**, v. 141, n. 3, p. 300-6, 2010.

BOLAY, S.; CAKIR, F. Y.; GURGAN, S. Effects of toothbrushing with fluoride abrasive and whitening dentifrices on both unbleached and bleached human enamel surface in terms of roughness and hardness: An in vitro study. **Journal of Contemporary Dental Practice**, v. 13, n. 5, p. 584–9, 2012.

BOWLES, W. H.; UGWUNERI, Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. **Journal of Endodontics,** v. 13, n. 8, p. 375–7, 1987.

CARSOL, M. A.; POULIQUEN, I.; GIAMARCHI, P.; LESGARDS, G.; SERGENT, M.; LUU, R. P. T. Determination of glutathione peroxidase kinetic parameters by experimental design. **Analusis**, v. 24, p. 195-9, 1996.

CARSOL, M. A.; POULIQUEN-SONAGLIA, I.; LESGARDS, G.; MARCHIS-MOUREN, G.; PUIGSERVER, A.; SANTIMONE, M. A new kinetic model for the mode of action of soluble and membrane-immobilized glutathione peroxidase from bovine erythrocytes. **European Journal of Biochemistry**, v. 247, 248-55, 1997.

DAHL, J. E.; PALLESEN, U. Tooth bleaching—a critical review of the biological aspects. **Critical Reviews in Oral Biology & Medicine**, v. 14, p. 292-304, 2003.

DENEKE, S. M.; FANBURG, B. L. Regulation of cellular glutathione. **American Journal of Physiology**, v. 257, n. 4, p. L163-L173, 1989.

FUGARO, J. O.; NORDAHL, I.; FUGARO, O. J.; MATIS, B. A.; MJÖR, I. A. Pulp reaction

of vital bleaching. **Operative Dentistry**, v. 29, n. 4, p. 363-8, 2004.

FUSS, Z.; SZAJKIS, S.; TAGGER, M. Tubular permeability to calcium hydroxide and to bleaching agents. **Journal of Endodontics**, v. 15, n. 8, p. 362–4, 1989.

GRAZIOLI, G.; VALENTE, L. L.; ISOLAN, C. P.; PINHEIRO, H. A.; DUARTE, C. G.; MÜNCHOW, E. A. Bleaching and enamel surface interactions resulting from the use of highly-concentrated bleaching gels. **Archives of Oral Biology**, v. 87, p. 157-62, 2018.

GREGUS, Z.; KLAASSEN, C. D. Mechanisms of toxicity. In: KLAASSEN, C.D. (ed.). Casarett & Doull's Toxicology. The basic science of poisons. 5.ª ed. New York: McGraw-Hill Health Professions Division, 1995.

GOLDBERG, M.; GROOTVELD, M.; LYNCH, E. Undesirable and adverse effects of toothwhitening products: A review. **Clinical Oral Investigations**, n. 14, v. 1, p. 1–10, 2010.

HAYWOOD, V. B.; HEYMANN, H. O. Nightguard vital bleaching. **Quintessence International**, v. 20, n. 3, p. 173-6, 1989.

HE, L. B.; SHAO, M. Y.; TAN, K; XU, X.; LI, J. Y. The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: A systematic review and metaanalysis. **Journal of Dentistry**, v. 40, n. 8, p. 644–53, 2012.

JOINER, A. The bleaching of teeth: a review of the literature. **Journal of Dentistry**, v. 34, n. 7, p. 412-9, 2006.

KOSOWER, N. S.; KOSOWER, E. M. The glutathione status of cells. **International Review of Cytology**, v. 54, p. 109-60, 1978.

KWON, S. R.; WERTZ, P. W. Review of the mechanism of tooth whitening. **Journal of Esthetic Restorative Dentistry**, v. 27, n. 5, p. 240–57, 2015.

LEHMANN, C.; WOLLENBERGER, U.; BRIGELIUS-FLOHÉ, R. ; SCHELLER, F. W. Bioelectrocatalysis by a selenoenzyme. **Journal Electroanalytical Chemistry**, v. 455, p. 259-63, 1998.

MANNERVIK, B. The isoenzymes of glutathione transferase. **Advances in Enzymology and Related Areas of Molecular Biology**, v. 57, p. 357-417, 1985.

MC EVOY, S. A. Chemical agents for removing intrinsic stains from vital teeth. II. Current techniques and their clinical application. **Quintessence International**, v. 20, n. 6, p. 379-84, jun. 1989.

MEISTER, A.; ANDERSON, M. E. Glutathione. **Annual Review of Biochemistry**, v. 52, p. 711-60, 1983.

MINOUX, M., SERFATY, R. Vital tooth bleaching: biologic adverse effects – a review. **Quintessence International**, v. 39, n. 8, p. 645-659, set. 2008.

REIS, A.; DALANHOL, A. P.; CUNHA, T. S.; KOSSATZ, S.; LOGUERCIO, A. D.

Assessment of tooth sensitivity using a desensitizer before light-activated bleaching. **Operative Dentistry**, v. 36, n. 1, p. 12–7, 2011.

RICO, A. J. Hipersensibilidad dentinal. **Acta Clinica Odontologica**, v. 15, n. 28, p. 17-29, jul./dic. 1992.

ROVER JR, L.; HOEHR, N. F.; VELLASCO, A. P. Sistema antioxidante envolvendo o ciclo metabólico da glutationa associado à métodos eletroanalíticos na avaliação do estresse oxidativo. **Química Nova**, v. 24, n. 1, p. 112-9, 2001.

SANTOS, A. P. M.; PEREIRA, M. N.; LEITE, F. P. P.; OLIVEIRA, J. M.; CHAVES, M. G. A. M. Um sintoma preocupante: a hipersensibilidade dentinária. **Revista Brasileira de Odontologia**, v. 67, n. 2, p. 242-6, jul./dez. 2010.

SCHWEIKL, H.; SPAGNUOLO. G.; SCHMALZ G. Genetic and cellular toxicology of dental resin monomers. **Journal of Dental Research**, v. 85, p. 870-7, 2006.

SULIEMAN, M.; ADDY, M.; REES, J. S. Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching. **Journal of Dentistry**, v. 31, p. 415-22, 2003.

TAY, L. Y.; KOSE, C.; LOGUERCIO, A. D.; REIS, A. Assessing the effect of a desensitizing agent used before in-office tooth bleaching. **The Journal of the American Dental Association**, v. 140, n. 10, p. 1245-51, 2009.

Apêndices

APÊNDICE A – TERMO DE CESSÃO DE DENTES

Identificação do Participante:
Nome (Legível): _____

Data de Nascimento: ____/____/____ Local de Nascimento: _____ UF: ____

RG nº: _____ CPF nº: _____

Endereço completo: _____

Cidade: _____ UF: _____CEP: _____

Telefones para contato:_____

E-mail: _____

DECLARAÇÃO

DECLARO ter sido esclarecido(a) sobre quais os motivos que levaram a necessidade de remoção do(s) dente(s)..... (código) – por razões ..... - e concordo que seja(m) utilizado(s) na pesquisa intitulada “Avaliação da glutationa como dessensibilizante após clareamento dentário com peróxido de hidrogênio: estudos *in vitro* e clínico randomizado", de autoria de Jacqueline de Santiago Nojosa, sob orientação do Prof. Dr. Juliano Sartori Mendonça, cujo objetivo é o de avaliar *in vitro* o efeito da glutationa na composição estrutural dos substratos dentários, após clareamento com peróxido de hidrogênio a 7,5%.

DECLARO ainda, ter sido esclarecido(a) pelo pesquisador que minha identidade não será divulgada por qualquer meio e que o material recolhido será utilizado unicamente para a presente pesquisa e o excedente será descartado. O pesquisador se compromete a cumprir as exigências contidas na Resolução 466/12.

Local e data:

Assinatura do participante:

Assinatura do pesquisador responsável:

APÊNDICE B – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado(a) por Jacqueline de Santiago Nojosa como participante da pesquisa clínica odontológica intitulada "AVALIAÇÃO DA GLUTATIONA COMO DESSENSIBILIZANTE APÓS CLAREAMENTO DENTÁRIO COM PERÓXIDO DE HIDROGÊNIO: ESTUDOS *IN VITRO* E CLÍNICO RANDOMIZADO". **Você não deve participar contra a sua vontade. Leia atentamente as informações abaixo e faça qualquer pergunta que desejar, para que todos os procedimentos desta pesquisa sejam esclarecidos.**

1. Nesta pesquisa será realizada uma moldagem da arcada superior e inferior, para confecção de moldeira (material flexível e macio) bem adaptada aos dentes. O paciente receberá uma profilaxia antes do tratamento clareador. Será distribuído a bisnaga com o gel clareador de peróxido de oxigênio a 7,5% e uma tabela para anotação diária da escala de sensibilidade.
2. O estudo irá avaliar o efeito da glutationa na redução da sensibilidade dentária após clareamento com peróxido de hidrogênio a 7,5%. Os materiais utilizados encontram-se disponíveis no mercado e foram previamente estudados, de modo que não causarão risco ao ser humano.
3. Haverá uma consulta para a profilaxia e moldagem, e outra para entrega da moldeira, gel clareador e tabela. Nessa última, será explicado tudo que for necessário para uso do produto e o paciente receberá as instruções por escrito. As consultas demorarão aproximadamente 1 hora. O paciente retornará para acompanhamento, avaliação da cor e da sensibilidade dentária após 15 dias, 1 mês, 6 meses, 1 ano e 2 anos do início do tratamento. Em qualquer tempo e diante de qualquer situação de desconforto, o paciente poderá entrar em contato com os responsáveis da pesquisa para receber orientações e serem providenciadas clínicas cabíveis.
4. Não haverá nenhum custo para o paciente por estas consultas, nem pelo material utilizado.
5. O paciente será diretamente beneficiado(a) por participar deste estudo pelo fato de ter o clareamento realizado e a estética melhorada.
6. Os possíveis riscos estão relacionados à reação de sensibilidade dentária, que é bastante comum no procedimento clareador, e à irritação gengival, que pode ocorrer quando a quantidade de gel clareador é aplicada de forma inadequada. Esta pesquisa buscará minimizar os possíveis danos previsíveis ao paciente no âmbito moral, físico, intelectual, social ou psíquico, a curto ou longo prazo, que podem estar relacionados ao constrangimento intelectual e alteração da autoestima, principalmente pela revelação dos hábitos de saúde bucal; constrangimento social, particularmente se considerada a estigmatização associada à participação em pesquisas; e constrangimento cultural, pela exposição de hábitos relacionados à saúde bucal e geral. No entanto, esses possíveis riscos serão minimizados pelo fato do projeto assegurar confidencialidade, privacidade e proteção da imagem dos participantes, além de garantir o acesso restrito às informações coletadas. Caso o participante necessite de apoio psicológico, o mesmo será devidamente encaminhado a um serviço especializado mais próximo da sua residência.
7. Todas as anotações relativas ao(s) seu(s) dente(s) e à sua pessoa serão mantidas confidenciais, exceto aos responsáveis pela pesquisa, e a divulgação das mencionadas informações serão feitas apenas entre os profissionais estudiosos do assunto. O paciente não será identificado em nenhum relatório ou publicação.
8. O paciente poderá sair deste projeto de pesquisa a qualquer momento e também poderá retirar

o seu consentimento, sem que isso lhe traga qualquer prejuízo. A saída não afetará sua oportunidade de obter tratamento no Curso de Odontologia da Universidade Federal do Ceará (UFC).

9. Questões sobre o projeto e sua participação serão respondidas pelos responsáveis pela pesquisa:

Nome: Doutoranda: Me. Jacqueline de Santiago Nojosa
Instituição: Programa de Pós-graduação em Odontologia-UFC
Endereço: Rua Monsenhor Furtado s/n - Rodolfo Teófilo - CEP: 60430-355 – Fortaleza - Ceara – Brasil
Telefones para contato: (85) 988290337

Nome: Prof. Dr. Juliano Sartori Mendonça
Instituição: Programa de Pós-graduação em Odontologia-UFC
Endereço: Rua Monsenhor Furtado s/n - Rodolfo Teófilo - CEP: 60430-355 – Fortaleza - Ceara – Brasil
Telefones para contato: (85) 3366-8232

ATENÇÃO: Se você tiver alguma consideração ou dúvida, sobre a sua participação na pesquisa, entre em contato com o Comitê de Ética em Pesquisa da UFC/PROPESQ – Rua Coronel Nunes de Melo, 1000 - Rodolfo Teófilo, fone: 3366-8344. (Horário: 08:00-12:00 horas de segunda a sexta-feira).
O CEP/UFC/PROPESQ é a instância da Universidade Federal do Ceará responsável pela avaliação e acompanhamento dos aspectos éticos de todas as pesquisas envolvendo seres humanos.

O abaixo assinado _____,___anos, RG:_____, declara que é de livre e espontânea vontade que está como participante de uma pesquisa. Eu declaro que li cuidadosamente este Termo de Consentimento Livre e Esclarecido e que, após sua leitura, tive a oportunidade de fazer perguntas sobre o seu conteúdo, como também sobre a pesquisa, e recebi explicações que responderam por completo minhas dúvidas. E declaro, ainda, estar recebendo uma via assinada deste termo.

Fortaleza, _____/_____/_____

Nome do participante da pesquisa:_____
Assinatura: _____

Nome do pesquisador:_____
Assinatura: _____

Nome da testemunha (se o voluntário não souber ler): _____
Assinatura: _____

Nome do profissional que aplicou o TCLE:_____
Assinatura: _____

APÊNDICE C – FICHA CLÍNICA

Nome:_____ Data Nascimento: ___/___/___
Sexo: F ( ) M ( ) Naturalidade: _____Profissão:_____
Endereço:_____ Telefone: _____
Sofre de alguma doença: ( ) Não ( ) Sim - Qual(is)_____
Está em tratamento médico atualmente? ( ) Sim ( ) Não
Está fazendo uso de alguma medicação? ( ) Não ( ) Sim - Qual(is) _____
Nome do médico assistente/telefone: _____
Fez uso de algum anti-inflamatório nos últimos 6 meses? ( ) Não ( ) Sim
Possui alguma alergia? ( ) Não ( ) Sim - O que:_____
Já foi operado? ( ) Não ( ) Sim - Qual(is): _____
Teve problemas com a cicatrização? Sim ( ) Não ( )
Teve problemas com a anestesia? Sim ( ) Não ( )
Teve problemas de hemorragia? Sim ( ) Não ( )
Sofre de alguma das seguintes doenças?
Febre Reumática: Sim ( ) Não ( ); Problemas cardíacos: Sim ( ) Não ( )
Problemas renais: Sim ( ) Não ( ); Problemas gástricos: Sim ( ) Não ( )
Problemas respiratórios: Sim ( ) Não ( ); Problemas alérgicos: Sim ( ) Não ( )
Problemas articulares ou reumatismo: Sim ( ) Não ( ); Diabetes: Sim ( ) Não ( )
Gravidez: Sim ( ) Não ( )
Hipertensão arterial: Sim ( ) Não ( );
Hábitos: Fuma ( ) Bebe ( ) Drogas ( ) Outros:_____
Antecedentes familiares:_____
Outras observações importantes:_____
_____
_____
Já recebeu instruções de higiene? Sim ( ) Não ( )
Está satisfeito com a cor dos dentes? Sim ( ) Não ( )
Possui sensibilidade dentária? Sim ( ) Não ( )
Fez uso de algum dessensibilizante nos últimos 6 meses? Sim ( ) Não ( )
Qual creme dental você usa?
Usa: Fio dental: Sim ( ) Não ( ); Bochecho: Sim ( ) Não ( ) Qual? _____
Tem bruxismo: Sim ( ) Não ( )
Tratamento endodôntico: Sim ( ) Não ( )
Já fez clareamento? Sim ( ) Não ( )
Sente alguma dor de dente espontânea? Sim ( ) Não ( )
Cor: Avaliador 1: _____ Avaliador 2: _____ Easyshade:_____

Declaro que as informações acima prestadas são totalmente verdadeiras.

Fortaleza, _____/_____/_____ _____

Assinatura do paciente ou seu responsável Legal

APÊNDICE D – ESCALA NUMÉRICA DE CLASSIFICAÇÃO DE QUATRO PONTOS

INSTRUÇÃO AO PARTICIPANTE DO ESTUDO

De acordo com a legenda, preencher a tabela abaixo com um número que seja representativo à sua sensibilidade dentária em cada dia do procedimento clareador.

LEGENDA
0 = ausente
1 = leve
2 = moderada
3 = forte

1º dia	2º dia	3º dia	4º dia	5º dia
6º dia	7º dia	8º dia	9º dia	10º dia
11º dia	12º dia	13º dia	14º dia	

APÊNDICE E – ESCALA VISUAL ANALÓGICA (EVA)

INSTRUÇÃO AO PARTICIPANTE DO ESTUDO

Marcar na linha horizontal o ponto que representa a sua sensibilidade dentária em cada dia do procedimento clareador, onde o ponto inicial é referente à ausência de dor e o final à dor forte.

1º dia

AUSÊNCIA DE DOR — DOR FORTE

2º dia

AUSÊNCIA DE DOR — DOR FORTE

3º dia

AUSÊNCIA DE DOR — DOR FORTE

4º dia

AUSÊNCIA DE DOR — DOR FORTE

5º dia

AUSÊNCIA DE DOR — DOR FORTE

6º dia

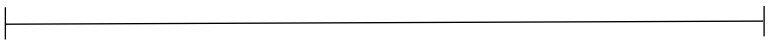
AUSÊNCIA DE DOR — DOR FORTE

7º dia

AUSÊNCIA DE DOR — DOR FORTE

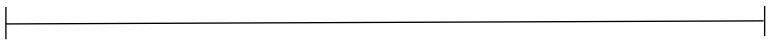
8º dia

AUSÊNCIA DE DOR — DOR FORTE



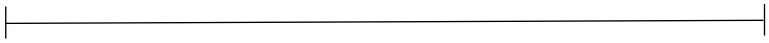
9º dia

AUSÊNCIA DE DOR — DOR FORTE



10º dia

AUSÊNCIA DE DOR — DOR FORTE



11º dia

AUSÊNCIA DE DOR — DOR FORTE

12º dia

AUSÊNCIA DE DOR — DOR FORTE

13º dia

AUSÊNCIA DE DOR — DOR FORTE

14º dia

AUSÊNCIA DE DOR — DOR FORTE

Anexo

ANEXO – PARECER DO COMITÊ DE ÉTICA EM PESQUISA

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DA GLUTATIONA COMO DESSENSIBILIZANTE APÓS CLAREAMENTO DENTÁRIO COM PERÓXIDO DE HIDROGÊNIO: ESTUDOS IN VITRO E CLÍNICO RANDOMIZADO

Pesquisador: Jacqueline de Santiago Nojosa

Área Temática:

Versão: 1

CAAE: 86263018.9.0000.5054

Instituição Proponente: Universidade Federal do Ceará/ PROPESQ

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.607.107

Apresentação do Projeto:

O clareamento dentário pode desencadear um processo de sensibilidade dentária ocasionada pela diminuição drástica da glutationa intracelular. Este estudo tem como objetivo avaliar in vitro e clinicamente o efeito da glutationa após clareamento com peróxido de hidrogênio a 7,5%. No estudo in vitro será avaliado o efeito da glutationa na microdureza, na estrutura do esmalte e da dentina, na alteração de cor após clareamento dentário e na composição mineral do esmalte. Serão testados seis grupos (n=5): controle negativo (com clareamento dentário), controle positivo (sem clareamento dentário), controle do experimento (gel sem princípio ativo e clareamento dentário), dessensibilizante comercial (nitrato de potássio 5% e fluoreto de sódio 2% e clareamento dentário) e dessensibilizantes experimentais (clareamento dentário e aplicação da glutationa 5% e 10%). Em todos os grupos será utilizado gel clareador à base de peróxido de hidrogênio a 7,5%. O ensaio de microdureza será realizada em um microdurômetro acoplado a um penetrador do tipo Knoop, que fornecerá a microdureza em corte longitudinal do esmalte e da dentina, a partir da realização de 3 indentações em cada substrato, antes e após os tratamentos. A análise estrutural no Microscópio Eletrônico de Varredura (MEV) será realizada após a fixação e a desidratação dos espécimes em soluções de etanol com concentrações crescentes. Serão obtidas imagens das áreas representativas das superfícies do esmalte e da dentina, com aumento de até 5.000X. A análise estrutural para o esmalte levará em consideração as alterações na característica morfológica

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br

superficial, como o aumento da rugosidade, presença de fendas ou porosidades. Para a análise estrutural em dentina, será considerado o percentual de túbulos dentinários expostos ou obliterados. A alteração de cor após clareamento dentário será avaliada com um espectrofotômetro portátil, previamente ao procedimento clareador e posteriormente ao tratamento. A medida será obtida após três repetições em cada bloco de esmalte, empregando o espectrofotômetro para quantificar a magnitude da diferença colorimétrica, por meio dos parâmetros CIELab. A análise da composição mineral do esmalte será realizada em um Espectrômetro Raman para avaliar as vibrações moleculares e verificar a estrutura mineral do esmalte, a partir da determinação das concentrações de fosfato (PO4, 961 cm-1) e carbonato (CO3, 1063 cm-1). O ensaio clínico, randomizado e cego será realizado para testar o uso da glutationa como dessensibilizante após clareamento dentário. Os participantes selecionados deverão ter a cor C2 ou mais escura no canino superior (dente 13) e não poderão apresentar história prévia de sensibilidade dentária. Os participantes serão distribuídos aleatoriamente, de acordo com a aplicação do dessensibilizante, em quatro grupos: controle negativo (sem princípio ativo), controle positivo(nitrato de potássio 5% e fluoreto de sódio 2%) e experimentais (glutationa 5% e glutationa 10%), durante 10 min. Será utilizado um gel clareador comercial à base de peróxido de hidrogênio a 7,5%, durante 1 h, por um período de 14 dias. A alteração de cor após clareamento dentário será avaliada por meio da escala de cor Vita Clássica e com um espectrofotômetro portátil, previamente ao procedimento clareador e posteriormente ao tratamento, após 15 dias, 1 mês, 6 meses, 1 ano e 2 anos do início do clareamento. Os pacientes registrarão diariamente se houve sensibilidade dentária em duas escalas: classificação verbal de quatro pontos e a escala visual analógica. Na análise estatística dos ensaios in vitro, os dados serão submetidos aos testes de normalidade e de homogeneidade, seguidos por ANOVA ou teste de Kruskal-Wallis quando os dados forem, respectivamente, paramétricos ou não-paramétricos. Os resultados obtidos pela análise estrutural do esmalte e da dentina no MEV serão submetidos ao teste não-paramétrico de Kruskal-Wallis seguido do pós-teste de Dunn para comparação entre os grupos experimentais em esmalte e em dentina. No ensaio clínico, serão realizados os testes de normalidade e de homogeneidade seguidos por ANOVA two-way para medidas repetidas ou teste de Kruskal-Wallis a depender da indicação. Serão analisados os resultados de sensibilidade dentária, utilizando o teste de Mann-Whitney e Kruskal-Wallis. Em todas as situações, será adotado o nível de significância de 5% (= 0,05).

Objetivo da Pesquisa:

Objetivo Primário: Avaliar in vitro e clinicamente o efeito da glutationa após clareamento com peróxido de hidrogênio a 7,5%.

Objetivo Secundário: Analisar in vitro se a glutationa associada ao uso do peróxido de hidrogênio a 7,5% modificará a microdureza, a estrutura dos substratos dentários e a composição mineral do esmalte;Verificar in vitro e clinicamente se o uso da glutationa interferirá na ação do agente clareador;Avaliar clinicamente se haverá redução da sensibilidade dentária com o uso da glutationa após clareamento com peróxido de hidrogênio a 7,5%.

Avaliação dos Riscos e Benefícios:

Riscos: Os possíveis riscos estão relacionados à reação de sensibilidade dentária, que é bastante comum no procedimento clareador, e irritação gengival, quando a quantidade de gel clareador for aplicada de forma inadequada. Esta pesquisa buscará minimizar os possíveis danos previsíveis ao paciente no âmbito moral, cultural, físico, intelectual, social, psíquico ou espiritual, a curto ou longo prazo, que podem estar relacionados ao constrangimento intelectual e alteração da autoestima, principalmente pela revelação dos hábitos de saúde bucal, constrangimento social, particularmente se considerada a estigmatização associada à participação em pesquisas, e constrangimento cultural, pela exposição de hábitos relacionados à saúde bucal e geral. No entanto, esses possíveis riscos serão minimizados pelo fato do projeto assegurar confidencialidade, privacidade e proteção da imagem dos participantes, além de garantir o acesso restrito às informações coletadas. Caso o participante necessite de apoio psicológico o mesmo será devidamente encaminhado a um serviço especializado mais próximo da sua residência.

Benefícios: O paciente será diretamente beneficiado(a) por participar deste estudo pelo fato de ter o clareamento dentário realizado e a estética melhorada.

Comentários e Considerações sobre a Pesquisa:

De relevância para a área de estética odontológica

Considerações sobre os Termos de apresentação obrigatória:

Todos apresentados.

Conclusões ou Pendências e Lista de Inadequações:

Nenhuma.

UFC - UNIVERSIDADE FEDERAL DO CEARÁ /

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BASICAS_DO_PROJETO_1100805.pdf	26/03/2018 22:29:21		Aceito
Outros	cv_Jacqueline_de_Santiago_Nojosa.pdf	26/03/2018 22:27:53	Jacqueline de Santiago Nojosa	Aceito
Outros	Carta_de_solicitacao.pdf	26/03/2018 22:20:23	Jacqueline de Santiago Nojosa	Aceito
Declaração de Instituição e Infraestrutura	Autorizacao_institucional.pdf	26/03/2018 22:19:43	Jacqueline de Santiago Nojosa	Aceito
Declaração de Pesquisadores	Declaracao_de_concordancia.pdf	26/03/2018 22:15:54	Jacqueline de Santiago Nojosa	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO_DE_CONSENTIMENTO_LIVRE_E_ESCLARECIDO.pdf	26/03/2018 22:14:43	Jacqueline de Santiago Nojosa	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Jacqueline_de_Santiago_Nojosa.pdf	26/03/2018 22:14:15	Jacqueline de Santiago Nojosa	Aceito
Orçamento	ORCAMENTO_FINANCEIRO.pdf	26/03/2018 22:13:17	Jacqueline de Santiago Nojosa	Aceito
Cronograma	CRONOGRAMA.pdf	26/03/2018 22:11:48	Jacqueline de Santiago Nojosa	Aceito
Folha de Rosto	FolhaDeRosto.pdf	26/03/2018 22:11:21	Jacqueline de Santiago Nojosa	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FORTALEZA, 18 de Abril de 2018

Assinado por:
FERNANDO ANTONIO FROTA BEZERRA
(Coordenador)

Endereço: Rua Cel. Nunes de Melo, 1000
Bairro: Rodolfo Teófilo **CEP:** 60.430-275
UF: CE **Município:** FORTALEZA
Telefone: (85)3366-8344 **E-mail:** comepe@ufc.br