UNESP – Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

CRISTIAN ALEJANDRO BERSEZIO MIRANDA

EFICÁCIA ESTÉTICA, QUALIDADE DE VIDA E EXPRESSÃO DE BIOMARCADORES DE INFLAMAÇÃO E REABSORÇÃO DETERMINADOS PELA TÉCNICA WALKING BLEACH DE CLAREAMENTO COM CONCENTRAÇÃO REDUZIDA DE PEROXIDO : ESTUDO CLINICO RANDOMIZADO

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Orientador: Prof. Dr. Osmir Batista de Oliveira Junior.

Co-Orientador: Prof. Dr. Eduardo Fernández

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DEDICATÓRIA

A minha familia especialmente a minha avó Eliana, minha namorada Paulina e meus amigos por seu apoio incondicional e incansáveis esforços para que eu alcance os meus objetivos.

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Bersezio Miranda CA. Eficácia estética, qualidade de vida e expressão de biomarcadores de inflamação e reabsorção determinados pela técnica walking bleach de clareamento com concentração reduzida de peroxido: estudo clinico randomizado [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2016

Resumo

Tres estudos clinicos, randomizados, controlados, cegos e de medidas repetidas foram realizados para avaliar o efeito da técnica walking bleach de clareamento dental realizada com concentração reduzida de peroxido (PH13). O efeito de PH13 foi comparado ao obtido com peroxido de hidrogênio a 35% utilizado como controle (PH35). O desempenho clinico de PH13 e PH35 foi avaliado em função de: 1) eficácia estética, determinada por avaliação objetiva (espectroscopio) e subjetiva de cor (comparação visual) mensuradas por ΔE e ΔSGU; 2) impacto sobre fatores psicosociais de qualidade de vida, mensurados pelos indices PIDAQ e OHIP Estetico e; 3) expressão de biomarcadores de inflamação e reabsorção (IL-1β e RANK-L) no fluido crevicular. Material e Método: Um total de 50 incisivos superiores escurecidos devido ao tratamento endodontico de um total de 47 voluntários foram selecinados e randomicamente divididos nos grupos PH35 (controle) e PH13 (teste). Todos os dentes receberam 4 sessões de clareamento. A eficácia estética e a expressão dos biomarcadores foram registrados antes (baseline - T0), após cada sessão de clareamento (T1, T2, T3, T4), uma semana (T5), um mês (T6) e tres meses (T7) após o fim do tratamento clareador. Os questionarios de qualidade de vida (PIDAQ e OHIP Estético) foram aplicados no baseline, imediatamente após e fim do tratamento e após 1 mês. Resultados: foram observados aumentos significativos nos níveis de IL-1β e RANK-L, a partir do baseline ($P<0.05$); Não houve diferença em níveis de IL-1β entre mês com o terceiro mês pós-tratamento ($p>0.05$). A eficácia estética (ΔE) foi semelhante para ambas as técnicas após 1 mes de tratamento (16,80 ± 6,07 para PH35 e 14,09 ± 4,83) estes resultados mantiveram-se estáveis após 3 meses de acompanhamento ($p>0.05$). Os fatores psicosociais de qualidade de vida foram estatisticamente impactados por PH35 e PH13 mostrando diferenças signficativas a partir do baseline ($p<0,05$). Conclusão: Ambos agentes clareadores utilizados na tecnica WB (PH35 e PH13), são altamente eficazes para reduzir a discrepancia de cor de dentes escurecidos devido ao tratamento endodontico, impactando positivamente nos fatores psicosociais de qualidade de vida dos voluntários avaliados pelos indices PIDAQ e OHIP Estético. No entanto, também causam aumento na expressão dos marcaroes de inflamação e reabsorção (RANK-L e IL-1β) que persiste até 3 meses pós-tratamento, independente da concentração do peróxido utilizado.

Palavras-chave: Peróxido de hidrogênio, Ligante RANK, Interleucemia-1beta, Ensaios clínicos como assunto

Bersezio Miranda CA. Aesthetic effectiveness, quality of life and expression of biomarkers of inflammation and resorption determined by walking bleach technique with reduced concentration of peroxide: randomized clinical study [Tese de Doutorado]. Araraquara: Faculdade de Odontologia da UNESP; 2016

Abstract

Three clinical studies, randomized, controlled, blind and with repeated measurements were performed to evaluate the effect of bleaching, performed with walking bleach technique with reduced concentration of peroxide (PH13). The PH13 effect was compared to that obtained with 35% hydrogen peroxide used as control (PH35). The clinical performance of PH13 and PH35 was evaluated in terms of: 1) aesthetic efficacy, determined by objective evaluation (spectrophotometer) and subjective evaluation (visual comparison), measured in ΔE and ΔSGU respectively; 2) impact on psychosocial factors of quality of life, measured by questionnaire PIDAQ and OHIP aesthetic; 3) expression of biomarkers of inflammation and resorption (IL-1β and RANK-L) in crevicular fluid. Material and Methods: A total of 50 incisors with endodontic treatment and color change of a total of 47 selected volunteers were randomly divided into PH35 groups (control) and PH13 (test). All teeth received 4 bleaching sessions . The aesthetic effect and the expression of biomarkers were recorded before (baseline - T0), after each whitening session (T1, T2, T3, T4), one week (T5), one month (T6) and three months (T7) after the end of the bleaching treatment. The questionnaires of quality of life (OHIP PIDAQ and Esthetic) were applied at baseline, immediately after of treatment and after 1 month. Results: Significant increases were observed in IL-1β and RANK-L from baseline ($P < 0.05$); There was no difference in IL-1β levels between month with the third month post-treatment ($p > 0.05$). The aesthetic efficacy (ΔE) was similar for both groups after one month of treatment (16.80 ± 6.07 to PH35 and 14.09 ± 4.83) these results remained stable after 3 months of follow-up ($p>0.05$). The psychosocial factors of quality of life were significantly impacted by PH35 and PH13 showing significant difference from baseline ($p<0.05$). Conclusion: Both bleaching agents used in the technique WB (PH35 and PH13) are highly effective in reducing the discrepancy of color of non vital discolored teeth, positive impact on psychosocial factors of quality of life of volunteers evaluated by PIDAQ and OHIP Aesthetic questionnaires . However, also cause increased expression of biomarkers of resorption and inflammation (RANK-L and IL-1β) that persists for 3 months after treatment, independent of the concentration of the used peroxide.

Keywords: Hydrogen peroxide, RANK Ligand, interleukin-1beta, Clinical trials.

SUMÁRIO

1 INTRODUÇÃO

A cor do dente é determinada pelas propriedades da dentina e do esmalte dentário e é modificada pelo efeito combinado de colorações extrínsecas e intrínsecas. [22] As colorações extrínsecas são determinadas pelo consumo de certos alimentos, bebidas, habitos pessoais como mascar tabaco e higiene oral inadequada, enquanto as colorações intrinsica são determinadas pela oxidação do estravassamento hemorrágio pós trauma dental, necrose pulpar, calcificação, formação de dentina reacional e impregnação da dentina por pigmentos derivados de soluções e cimentos odontológicos utilizados durante o tratamento endodontico, bem como devido a oxidação de ions metâlicos de certos materiais odontológicos. [37]

A descoloração ou escurecimento de dentes anteriores tratados endodonticamente é uma ocorrência bastante comum que é imediatamene notada devido ao grande contraste de cor entre o dente desvitalizado e os dentes adjacentes´. Esta discrepancia estética gera grande constrangimento, insatisfação, desconforto psicoemocional e discriminação social [25,39].

A OMS define saúde como "um estado de completo bem-estar físico, mental e social e não somente ausência de afeções e enfermidades" assim o clareamento de dentes tratados endodonticamente que apresentam descoloração e/ou escurecimento deve ser entendido como um procedimento terapeutico de promoção de saúde e não simplesmente como um procedimento cosmético.

O nível de satisfação e a melhoria psicoemocional e da qualidade de vida dos pacientes submetidos ao clareamento de dentes com discrepancia de cor devido tratamento endodontico é alto. Além disso, este procedimento é mais conservadora e acessivel para recuperar a estética dos dentes anteriores do que os tratamentos realizados com coroas ou facetas [37] o que torna esta modalidade de tratamento a técnica de eleição para tratar discrepancias de cor devido tratamento endodontico [18].

A técnica de clareamento mais utilizada nestes casos é a chamada técnicas walking bleach (WB). Cada sessão clinica de clareamento WB é constituida, basicamente, pelo selamento e manutenção do agente clareador no interior da camara pulpar por 7 dias. Os agentes clareadores mais utilizados nestes casos são

o Peróxido de Hidrogênio, Peróxido de Carbamida e Perborato Sódico. Tanto o Peróxido de Carbamida como o Perborato de Sódio atuam de forma indireta pois, primeiro precisam se decompor em Peróxido de Hidrogênio para depois promover o clareamento dental [37] .

Os fatores que determinam a eficácia da técnica WB são: a concentração de peróxido utilizado, o tempo de contato deste peróxido com a estrutura dental escurecida e a a capacidade do agente clareador permear a estrutura dental e alcançar as moléculas cromogênicas que estão afetando a cor do dente[9]. Para se obter resultados mais rápidos e maior eficacia estética são utilizadas altas concentrações de peróxido de hidrogêno e realizadas de tres a cinco sessões clinicas de clareamento.

No entanto, tanto a alta concentração como o maior tempo de contato do peróxido com os tecidos dentais tem sido associados a possíveis efeitos biológicos adversos da técnica WB, dos quais, a reabsorção cervical externa (RCE) é o mais grave pois pode levar a perda do elemento dental.

O mecanismo exato pelo qual esta RCE ocorre ainda não foi totalmente elucidado. A hipótese mais aceita é que a RCE é desencadeada por inflamação local e/ou alteração de pH causadas pelo uso e extravazamento de altas concentrações de peroxido para região cervical. [37, 13, 24, 35] Este quadro pode der agravado se uma técnica termocatalitica de clareamento WB foi utilizada devido ao dano térmico adicional causado aos tecidos periodontais.

A prevalência de RCE associada exclusivamente a técnica WB é de 3,9%. Porém em dentes que sofreram traumatismo dental, esta prevalência é tres vezes maior (13, 6% dos casos)[19] .

Em um estudo em animais no ano de 1992, Heller et al. [20] encontraram evidência histológica de RCE até 3 meses após o final do tratamento clareador. Alguns autores sugerem que a utilização de peroxidos selados na camara pulpar (WB) gerá pressões internas que favorecem o extravasamento de especimes reativas de oxigênio (EROs) para a superfície externa do dente, o que provoca resposta inflamatória local e, consequentemente RCE. [8, 37] Destacam também que este extravasamento pode modifica a composiçao da dentina, transformando-a um tecido não mais reconhecido pelo sistema imunologico, o que desencadeia uma reação do tipo corpo estranho, com consequente reabsorção tecidual[28, 37]. Além

disso, o simples extravasamento de EROs pode provocar alteração no pH extra-radicular que poderia aumento da atividade osteoclástica[29, 33, 37].

O processo de RCE envolve uma complexa interação entre células inflamatórias, células reabsortivas especializadas e as estruturas dentais. As células responsáveis pela reabsorção do tecido duro dental são os odontoclastos, células multinucleares cuja morfologia e mecanismo de ação são similares aos osteoclastos. Atuam como macrófagos específicos e células inflamatórias especializadas em todo tipo de ressorção dentária. São observados sobre a estrutura mineralizadados dentes somente em condições patológicas.

Estudos in vitro relatam que o peróxido de hidrogênio é altamente citotóxico, causando grandes alterações morfológicas em macrófagos. [2] e em outros componentes teciduais, o que corrobora a hipotese de modulação de inflamação local devido ao extravasamento de EROs, o que, certamente, causa alterações na expressão de marcadores associados aos processos de inflamação. Além disso, se esta inflamação for realmente precursora da RCE, será provavel, que um aumento na expressão de marcadores ligados a reabsorção radicular também seja observado no fluido crevicular.

Do ponto de vista imunohistoquimico, A reabsorção dental e, consequentemente, também a RCE, apresentam a mesma dependência do sistema RANK-RANKL-OPG observado na fisiopatologia óssea. Fukushima relatou que as células do ligamento periodontal expressam RANKL durante os processos de reabsorção fisiológica dos dentes decíduos e é nesta mesma etapa que os níveis de expressão de OPG diminuem. [14] Além disso, o fator de necrose tumoral alfa tem uma contribuição no desenvolvimento de osteoclastos. Tanto RANKL como o fator de necrose tumoral alfa inibem a OPG [14, 27]. Em função disso, a analise da expressão destas proteinas antes e após o clareamento dental poderiam indicar o risco biológico de RCE em função de produtos e técnicas..

Além disso, em decorrência da reação inflamatoria são secretadas diversas proteinas regulatórias da função imuno-inflamatória. [32] Dentre estas as citocinas pró-inflamatórias como a interleucina-1β (IL-1β), o fator de necrose fator-α necrose tumoral (TNF-α) e o interferon-γ (IFN-γ) são considerados os principais mediadores das doenças de inflamação crônica incluindo a periodontite [41] . A IL-1β e o IL-TNF-α são indutores potentes das metaloproteinases de matriz (MMPs)

associados ao receptor ativador do fator nuclear kB (RANK-L), ambos produtos envolvidos na destruição da matriz extracelular, da cartilagem do tecido ósseo e provavelmente do técido radicular.

Ainda não foi estabelecida a segurança biologica da tecnica WB em função da detecção de marcadores de destruição óssea como RANKL e citocinas pró-inflamatórias IL-1β, relacionados com a reabsorção radicular, Também não está estabelecido se o uso de concentrações reduzidas de peroxido na técnica WB reduziria o risco de danos biologicos ocorrência os efeitos adversos.

Apesar de ser senso comum que a a técnica walking bleach resulte em alta satisfação dos pacientes este efeito ainda não esta cientificamente dimensionado, em relação ao impacto sobre os fatores psicosocial e a a qualidade de vida dos indivíduos submetidos a técnica WB. Também não se sabe se , estes indicadores psicosociais são diretamente modulados pela eficacia de produtos e técnicas**.**

Outro aspecto ainda não estabelecido é se a técnica WB realizada com concentração reduzida de peróxido apresenta a mesma longevidade de resultados que a técnica tradicional com peróxido de alta concentração.

2 HIPÓTESE E OBJETIVO

O objetivo deste projeto, composto por 4 estudos clinicos randomizados, foi testar a eficácia estética, qualidade de vida e expressão de biomarcadores de inflamação e reabsorção do clareamento realizado pela tecnica walking bleach com concentração reduzida de peroxido.

As hipoteses testadas foram:

1) O uso de concentração reduzida de peroxido de hidrogênio tem menor impacto sobre os fatores psicosociais de qualidade de vida.

2) O uso de concentração reduzida de peroxido de hidrogênio é menos eficaz do que a concentração tradicional mais elevada.

3) O uso de concentração reduzida de peroxido induz menor expressao de biomarcadores de inflamação e de reabsorção óssea/radicular.

4) O uso de concentração reduzida de peroxido de hidrogênio resulta em efeitos psicosociais, estéticos e de expressão de biomarcadores de menor duração.

3 PUBLICAÇÕES

3.1 Publicação 1

Effectiveness and impact of the walking bleach technique on esthetic self-perception and psychosocial factors: a randomized double-blind clinical trial*

Clinical relevance

The non-vital bleaching produces positive immediate impact on aesthetic perception and psychosocial factors.

Abstract

Objective: This trial evaluates the impact of psychosocial and esthetic self-perceptions of patients undergoing non-vital tooth bleaching using the walking bleach technique. We also assessed the clinical effectiveness of bleaching tooth discoloration.

Methods: Fifty volunteers with non-vital tooth discoloration were enrolled. Teeth were randomized into two groups: 35% hydrogen peroxide (n=25) and 37% carbamide peroxide (n=25). Intracameral bleaching was performed over 4 sessions using the walking bleach technique. Tooth color was evaluated at each session to measure total color variation (ΔE). The shade guide was arranged from highest (B1) to lowest (C4) values to assess the color and calculate the color change in the number of shade guide units (ΔSGU). Subjective and objective assessments were compared with the tooth counterpart.Esthetic self-perception and psychosocial factors were assessed before and after treatment.

Results: Color change (ΔE) was 15.48 (±) 5.17 for hydrogen peroxide and 14.02 (±)

* Bersezio C, Martín J, Estay J, Peña F, Rubio M, Vernal R, de Oliveira OB Jr, Fernández E. Effectiveness and impact of the walking bleach technique on esthetic self-perception and psychosocial factors: a randomized double-blind clinical trial. 2017. J Op Dent.. In press.. Used by permission. © Operative Dentistry, Inc. Anexo 1

4.85 for carbamide peroxide. There was no significant difference at any time point (p> .05) except at sessions 3 and 4 (p< .05). Overall, whitened teeth values similar to those of counterpart teeth (p>.05).There was a decrease in OHIP-Esthetics and PIDAQ scores after treatment compared to baseline (p< .05).

Conclusion: The walking bleach technique was highly effective on nonvital teeth and had a positive effect on self-esthetic perception and psychological impact for the patients.

Key words: Non vital Bleaching, Carbamide peroxide, Hydrogen peroxide, Randomized Clinical Trial

Introduction

Tooth color is one of the most important factors in achieving an esthetically pleasing smile in some cultures of the world.[1] It also substantially influences esthetic self-perception and has a psychosocial impact on people.[2] Thus, when a single tooth is darkened, the negative effect may be more pronounced because the color does not match the rest of the teeth.[3] However, there is no information on the impact of intracoronal bleaching on patient self-perception or on psychosocial impact.[4] Some authors have shown that alterations in cosmetic dentistry can cause psychosocial consequences that could have more of an impact on the person than the biological problems caused by caries lesions do.[5]

Intracoronal bleaching is a minimally invasive method of whitening discolored endodontically treated teeth. In most patients, intracoronal whitening requires more than one appointment to achieve a white smile and to repair or match the dark color of a single tooth to the rest of their teeth.[6] Therefore, it should be considered a color rehabilitation treatment.[7] There are only a small number of clinical studies on the intracoronal whitening technique, and most are difficult to compare with each other, as their conclusions are based on subjective records.[8-10] They are also considered to have low precision.

The most commonly used chemical agents include hydrogen peroxide (>35%) and carbamide peroxide (>37%).[6] Even though these agents are popular, no

clinical studies have used objective methodologies that are highly reproducible or that explain the effectiveness of these agents in the whitening treatment of non-vital teeth.

The null hypothesis of this paper is that there will be no difference in treatment effectiveness or in the esthetic perception and psychosocial impact of patients treated with 35% hydrogen peroxide or 37% carbamide peroxide.

The main objective of this study is to evaluate the psychosocial impact and esthetic self-perceptions of patients undergoing non-vital tooth bleaching with 35% hydrogen peroxide and 37% carbamide peroxide gels using the walking bleach technique and to assess the clinical effectiveness of bleaching for discoloration.

Materials and methods

This randomized clinical study was approved by the Ethics Committee of the Faculty of Dentistry of the University of Chile (2016/04) and was performed according to the Consolidated Standards of Reporting Trials Statement [11] and the Declaration of Helsinki [12] (1975; revised 2000).
Fifty volunteers with at least one non-vital tooth that had a color change A3 or darker, according to the Vita Classical scale (Vita Zahnfabrik, Bad Säckingen, Germany), were enrolled. Before the study began, patients signed an informed consent form that was approved by the local Ethics Committee. Patients received prophylaxis with a brush and pumice paste, and oral hygiene instructions to standardize the volunteers' plaque conditions.

Study design
This trial was a randomized double-blind (patient and evaluator) study. Randomization of study groups occurred using Excel 2013 software (Microsoft, Redmond, Washington, United State). Patients were recruited via flyers within the local Faculty of Dentistry and through social networks like Facebook or Twitter.

Sample size

The sample size was determined using GPower 3.1 [13] software, with a 5% level of significance, 90% statistical power and a dropout of 25%, based in a previous studies. This study corresponds to a therapeutic equivalence type, where a color variation of ΔE tones in the range of 7-10 or more, based on the original color, was considered significant. This gives a sample size of 20, and to compensate the drop-out rate reported in previous studies, we used a sample size of 25 per group.

Entry Criteria

A total of 74 patients were examined to assess whether they met the entry criteria for this study (Figure 1). Inclusion criteria was as follows: patients over the age of 18 years; with one or more non-vital teeth discolored; whose restoration did not include the vestibular surface; with the root canal in good condition; without apical lesions; with no previous tooth whitening experiences; and a tooth pitch A2 or higher, according to the Vita Classical scale. Exclusion criteria was as follows: pregnant or breastfeeding; patients with enamel hypoplasia; teeth stained by tetracycline or fluorosis; in orthodontic treatment with fixed devices; patients with cancer; or with patients with periodontal pathologies. Volunteers with clinically- or radiographically-identified caries, periapical lesions, external or internal tooth resorption and/or periodontal disease were excluded, and these patients were referred for treatment to specialty clinics.

Patients agreed to use the bleaching agents and were randomized into two groups, each with 25 patients, as follows: HP35, teeth bleached with 35% hydrogen peroxide (Opalescence Endo - Ultradent, South Jordan, Utah, United State); and HP13, teeth bleached with 37% carbamide peroxide (Whiteness Superendo, FGM, Joinville, Santa Catarina, Brazil). whith decomposed in hidrogen peroxide 13% (HP13). The bleaching agents were applied according to the manufacturer's instructions, over four sessions using an ambulatory (walking bleach) technique. There was one week between each session.

Preparation session: The root canal was prepared with absolute isolation, and an endodontic seal clearance of 3 mm from the edge of the enamel cement . A 2-mm mechanical seal was placed using a glass ionomer reinforced with composite resin

(Riva light cure, SDI, Bayswater, Victoria, Australia), and it was cured for 60 seconds at a distance of 1 cm with a 1200 mW intensity lamp (Raddi Cal, SDI, Bayswater, Victoria, Australia). A radiographic control seal of the root canal was then performed. Once the proper seal was confirmed, clinical and radiographical intracameral bleaching was performed.

Four whitening sessions: Application of the whitening agent was performed according to the manufacturer's instructions. The correct amount of bleaching gel was placed into the pulpar cavity with presence of mild moisture (using the Walking Bleach technique), which allowed a close and optimum cavity seal. Cavity closure was performed using a temporary cement (Fermin, Detax, Baden-Württemberg, Germany) until the next session in 7 days. This procedure was repeated at each of the next 4 sessions. The same amount of gel was used and changed for both products and the same number of time.

Final session: After washing the cavity access well with water, a temporary restoration was placed for 7 days until the final restoration with composite resin was made. Patients were cautioned not to eat or drink foods that can dye, such as coffee, tea or red wine, during the study period. They were given these directions in writing and contact information for any questions or adverse events was provided.

Color evaluation

Objective evaluation

Two calibrated evaluators (Kappa= .85) were used to measure tooth color for the baseline, immediately after each of the four sessions, at one week and at one month after the last session. Color evaluation was obtained from a 6-mm area located in the middle third of the labial surface of the left and right central incisors. To standardize this evaluation, an impression of the maxillary arch was taken to make a guide using high-putty silicone (Zetaplus, Zhermack, Badia Polesine, Rovigo, Italy). A window was created on the labial surface in the middle third of the central incisor using a device with well-formed borders and a 3-mm radius corresponding to the reflectance of the spectrophotometer (Vita EasyShade Compact, VITA Zahnfabrik, Bad Säckingen, Germany) that has good reliability.[14]

The shade was determined using the obtained parameters L*, a* and b*. Color alteration after each session was given by the differences between the values obtained at the session and the baseline (ΔE). ΔE was calculated using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The evaluators considered the color of the tooth counterpart (treated tooth: upper hight central incisor; tooth counterpart: left central incisor), and the same area (middle) of each tooth was evaluated to compare color matching.

Subjective evaluation

For the subjective evaluation, the 16 tabs of the shade guide (Vita Classic, Vita Zahnfabrik) were arranged from the highest (B1) to the lowest (C4) value to assess the color. Two calibrated evaluators (Kappa= .85) recorded the shade of the upper central left and right incisors at baseline and at the same time points as for the objective evaluation. The investigators checked the color in the middle third area of the labial surface on the anterior central incisor, according to the American Dental Association guidelines.[15] The color changes from the beginning of the active phase through the individual recall times were calculated using the change in the number of shade guide units (ΔSGU). The color of the counterpart tooth was also recorded subjectively and compared to that of the treated to tooth.

Oral Health Impact Profile questionnaire

Satisfaction was measured using the Oral Health Impact Profile (OHIP-Esthetics) questionnaire validated in Chilean Spanish.[16] The questionnaire was administered by a research operator at baseline, one week and one month after bleaching.

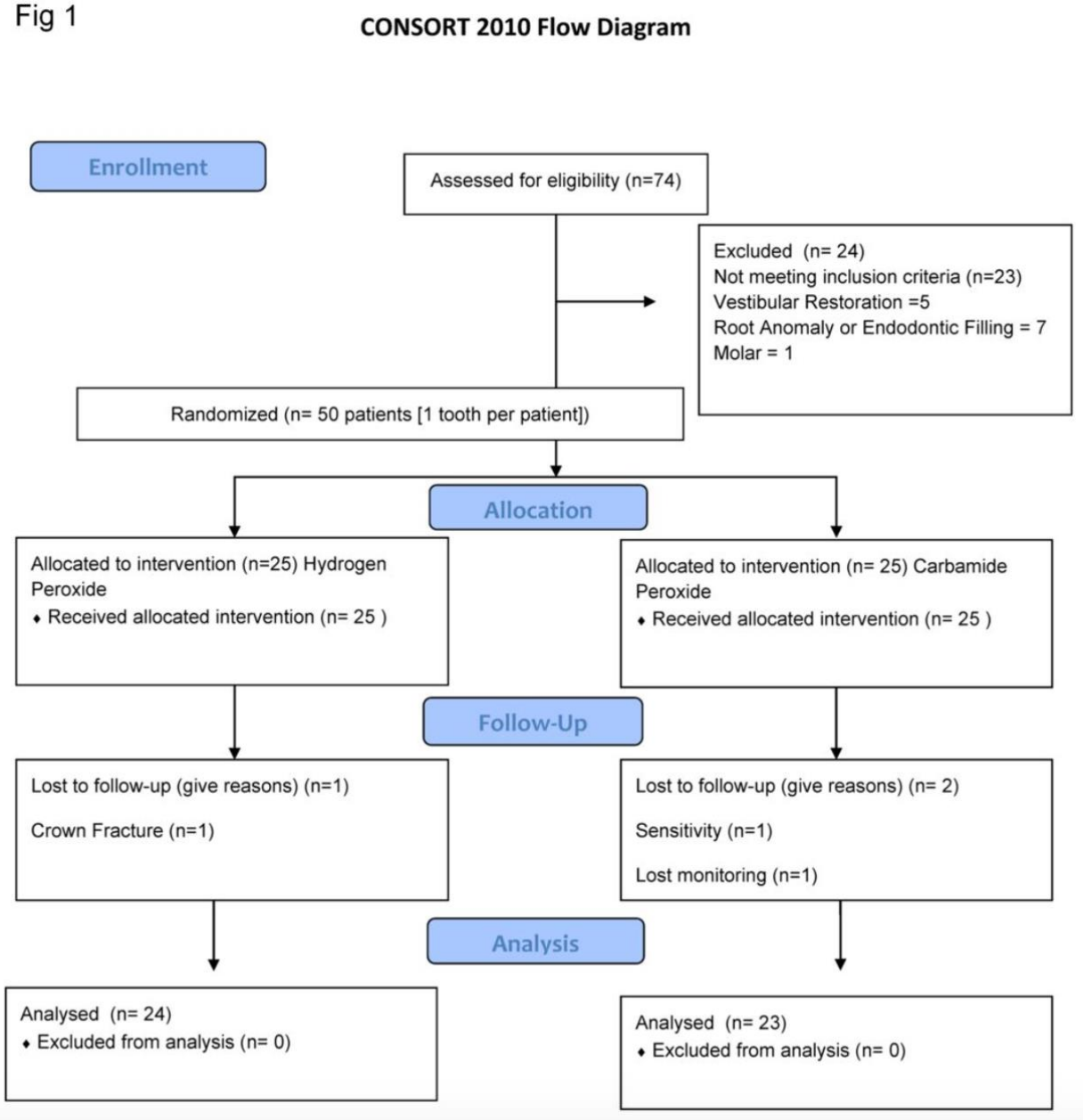
The Psychosocial Impact of Dental Esthetics questionnaire

The Psychosocial Impact of Dental Esthetics questionnaire (PIDAQ) consisted of 23 items that were grouped into 4 components using factor analysis: 1) dental self-confidence, 2) social impact, 3) psychosocial impact, and 4) esthetic concern.[17]

Statistical analysis

After verifying the normality of data distribution and the homogeneity of the variance-covariance matrix, the treatment efficacy was evaluated with respect to color alteration (ΔE and ΔSGU) and analyzed using the Wilcoxon test for within-group comparisons, and the Mann-Whitney test for between-group comparisons. The statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA) with α = 0.05.For comparison of OHIP-Esthetics and PIDAQ questionnaires scores, the Wilcoxon test was used. [18]

Fig 1



Results

The participant characteristics are shown in Table 1. There were no statistically significant differences between the participant's characteristics in the diferrent groups (Mann Whitney test p> .05). Results for ΔSGU differed over time, as shown in Table 2, resulting in was more effective in effectiveness in group 1 (p< .05). However, at the final measurement one month post-bleaching, values were not significantly different (p= .59).

Table 1. Participant baseline characteristics

Baseline features	Groups	
	1=Hydrogen Peroxide	2=Carbamide Peroxide
Age (years; mean ± SD)	30.88 ± 11.58	30.83 ± 11.25
Minimum age (years)	19	20
Maximum age (years)	65	65
Male (%)	50	39.13
Trauma (%)	58.33	39.13
Caries (%)	41.67	60.87
SGU Baseline median (min; max) Vita Classic	15 (5; 16)	12 (7; 16)
L* (mean ± SD)	73.55 ± 8.54	75.91 ± 6.81
a* (mean ± SD)	4.48 ± 3.45	4.85 ± 3.39
b* (mean ± SD)	29.14 ± 3.87	31.79 ±6. 60

SD = standard deviation

The color change determined using ΔL, Δa and Δb is shown in Table 3. The ΔL was different at all measurement times, with values higher than Group 1 (p<.05). The values of Δa and Δb were similar for both groups (p> .05). The ΔE color difference is shown in Table 4. The effectiveness was similar to baseline (p>.05) at all time points except that a significant difference from baseline (p<

.05) was observed for both groups at sessions 3 and 4. These two groups showed high effectiveness, with an average change of color of 14 units.

Table 2. Comparison of ΔSGU values at different times using the Vita Classic scale

Assessment points	Color change by ΔSGU		
	HP35	HP13	Mann-Whitney test
Baseline vs. 1-wk bleaching	3.5 (0:10)	3 (0:9)	**.04**
Baseline vs. 2-wk bleaching	8 (0:14)	4 (0:11)	**.04**
Baseline vs. 3-wk bleaching	9.5 (0:14)	7 (0:13)	**.02**
Baseline vs. 4-wk bleaching	11 (1:15)	7 (1:14)	**.02**
Baseline vs. 1-wk after bleaching (before restoration)	10 (-1:14)	7 (1:13)	**.04**
Baseline vs. 1-wk after bleaching (after restoration)	9.5 (1:14)	7 (2:13)	**.04**
Baseline vs. 1-mth after bleaching	8.5 (1:13)	7 (1:13)	.59

Median (min:max)

Table 5 shows the subjective comparison of the treated teeth and their counterparts using Vita Classical SGU. There were no statistically significant differences (p>.05) between treated teeth and controls in the first week. However, in the monthly monitoring of group 2 there were differences in color between treated teeth and counterpart teeth (p<.05). Table 6 shows the objective comparison of the treated teeth and their counterparts from the L, a and b measured by spectrophotometer. The L values from the one week post-bleaching are not statistically significant (p>.05), and the values from the month post-bleaching were statistically significantly different (p <.05) from the L values of the homologous teeth

Regarding the a values in group 1 the comparison of one week after restoration and one month after restoration was statistically significant (p <.05). In group 2 there were no statistically significant differences (p> .05)

Regarding the b values, there was a statistically significant difference in group 2 in the recall of the week, and there was no statistically significant difference after one month ($p > .05$)

Table 3. Color change by ΔL, Δa and Δb (mean and standard deviation) at all time points

Assessment times	Color change by ΔL			Color change by Δa			Color change by Δb		
	Hydrogen Peroxide	Carbamide Peroxide	Mann-Whitney	Hydrogen Peroxide	Carbamide Peroxide	Mann-Whitney	Hydrogen Peroxide	Carbamide Peroxide	Mann-Whitney
Baseline vs. 1-wk bleaching	8.13 ± 4.73	4.33 ± 4.31	**.006**	-2.88 ± 2.14	-2.63 ± 2.32	.709	-1.22 ± 3.82	-3.18 ± 4.18	.100
Baseline vs. 2-wk bleaching	10.26 ± 7.06	5.98 ± 4.70	**.019**	-4.20 ± 2.82	-3.96 ± 2.97	.782	-3.11 ± 4.70	-5.70 ± 5.97	.105
Baseline vs. 3-wk bleaching	11.78 ± 6.55	7.98 ± 4.26	**.023**	-5.03 ± 2.72	-4.51 ± 2.69	.516	-5.68 ± 5.95	-5.38 ± 5.96	.865
Baseline vs. 4-wk bleaching	13.07 ± 7.43	8.64 ± 3.53	**.013**	-5.70 ± 2.99	-5.20 ± 2.85	.561	-6.18 ± 5.93	-6.63 ± 5.85	.796
Baseline vs. 1 week after bleaching (before restoration)	11.54 ± 8.23	7.64 ± 3.93	**.045**	-5.52 ± 3.58	-5.45 ± 2.88	.939	-6.60 ± 5.95	-8.00 ± 7.43	.480
Baseline vs. 1 week after bleaching (after restoration)	10.85 ± 7.20	7.25 ± 4.53	**.047**	-6.32 ± 3.04	-5.67 ± 2.75	.452	-7.24 ± 5.99	-7.92 ± 7.75	.737
Baseline vs. 1 month after bleaching	7.75 ± 6.03	4.41 ± 4.63	**.039**	-6.53 ± 2.92	-6.07 ± 2.50	.569	-9.52 ± 5.49	-9.97 ± 6.21	.796

Table 4. Color change expressed in units (ΔE; mean and standard deviation) at all time points.

Assessment times	Color change by ΔE		
	HP35	HP13	Mann-Whitney
Baseline vs. 1-wk bleaching	9.67 ± 4.79	7.40 ± 4.64	.11
Baseline vs. 2-wk bleaching	13.24 ± 5.94	11.04 ± 5.19	.18
Baseline vs. 3-wk bleaching	15.69 ± 5.79	12.17 ± 4.93	**.03**
Baseline vs. 4-wk bleaching	17.19 ± 6.55	13.31 ± 4.67	**.02**
Baseline vs. 1 week after bleaching (before restoration)	16.44 ± 7.10	14.36 ± 4.74	.25
Baseline vs. 1 week after bleaching (after restoration)	16.22 ± 6.46	14.45 ± 4.88	.30
Baseline vs. 1 month after bleaching	15.48 ± 5.17	14.02 ± 4.85	.32

Table 5. Comparison of SGU values at different times using the Vita Classic scale (Median (min:max))

Assessment times	Color change by SGU	
	G1	G2
Homologou Tooth	5 (1:9)	3 (2:10)
Baseline	15 (5:16) [a]	12 (7:16) [a]
1-wk bleaching	10.5 (2:16) [a]	9 (5:16) [a]
2-wk bleaching	5 (1:15)	7 (1:15) [a]
3-wk bleaching	4 (1:15)	5 (2:15) [a]
4-wk bleaching	2 (1:12) [a]	5 (1:15)
1 week after bleaching (before restoration)	4.5 (1:13)	5 (1:15)
1 week after bleaching (after restoration)	3.5 (1:12)	4 (1:14)
1 month after bleaching	5 (1:13)	6 (1:14) [a]

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus Homologou tooth.

Table 6. Color by L, a and b (mean and standard deviation) at all time points and the color of the Homologou Tooth.

Assessment times	Color change by L		Color change by a		Color change by b	
	G1	G2	G1	G2	G1	G2
Homologou Tooth	84.39 ± 2,62	84.77 ± 4.91	-0.91 ± 0.55	-1.16 ± 0.82	20.10 ± 3.47	19.43 ± 3.21
Baseline	73.55 ± 8.54 [a]	75.91 ± 6.81 [a]	4.48 ± 3.45 [a]	4.85 ± 3.39 [a]	29.14 ± 3.87 [a]	31.79 ± 6.60 [a]
1-wk bleaching	81.68 ± 7.20	80.24 ± 7.51 [a]	1.60 ± 3.57 [a]	2.22 ± 4.02 [a]	27.93 ± 4.54 [a]	28.61 ± 5.51 [a]
2-wk bleaching	83.81 ± 6.47	81.89 ± 6.95	0.28 ± 3.38	0.89 ± 4.35	26.03 ± 4.31 [a]	26.10 ± 5.49 [a]
3-wk bleaching	85.34 ± 5.64	83.89 ± 6.55	-0.55 ± 3.24	0.34 ± 3.98	23.46 ± 5.10 [a]	26.41 ± 5.80 [a]
4-wk bleaching	86.63 ± 5.80	84.56 ± 6.30	-1.23 ± 3.01	-0.36 ± 3.73	22.96 ± 5.08 [a]	25.17 ± 5.30 [a]
1 week after bleaching (before restoration)	85.10 ± 7.08	83.55 ± 5.96	-1.04 ± 3.42	-0.60 ± 3.92	22.54 ± 4.83	23.79 ± 5.99 [a]
1 week after bleaching (after restoration)	84.41 ± 5.64	83.16 ± 7.32	-1.84 ± 2.51 [a]	-0.83 ± 3.77	21.90 ± 4.83	23.87 ± 6.08 [a]
1 month after bleaching	81.31 ± 5.73 [a]	80.33 ± 5.38 [a]	-2.05 ± 2.33 [a]	-1.22 ± 3.03	19.62 ± 4.84	21.83 ± 5.29

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus Homologou Tooth.

Oral Health Impact Profile (OHIP-Esthetics) Table 7

There was a statistically significant difference in the OHIP-Esthetics score for the baseline compared with the sessions and one month post-treatment ($p < .05$; Wilcoxon test). The factors of functional limitation and psychological disability were statistically significant compared to the baseline values ($p < .05$). In group 1, the psychological discomfort and handicap factors were statistically significant at one week and one month compared to baseline ($p < .05$), and the handicap factor at the one-month assessment was statistically significant in group 2 ($p < .05$).

The Psychosocial Impact of Dental Esthetics questionnaire Table 8

The PIDAQ score was significantly different at baseline compared with 1 week and 1 month post-treatment ($p<.05$; Wilcoxon test), except for the psychological assessment and total PIDAQ sum for group 2 at 1 month post-treatment. When the one-week and one-month post-treatment time points are compared, the only statistically significant differences were found in the field of self-confidence ($p<.05$) for group 1 and the esthetic concern factor for both groups ($p<.05$).

Table 7. Effect of intracoronary bleaching in the esthetic self-perception evaluated with the OHIP questionnaire

	Baseline			1 week after bleaching			1 month after bleaching		
Dimension	PH35	PH13	Mann Whitney	PH35	PH13	Mann Whitney	PH35	PH13	Mann Whitney
Functional limitation	5 (2:8)	5 (2:8)	.754	3 (0:6)[a]	4 (1:6)[a]	.357	2.5(0:7)[a]	4 (0:8)[a]	.435
Physical pain	3.5 (0:6)	3 (0:5)	.366	3 (1:6)	3(1:6)	.575	2.5 (0:6)	2(0:8)	.829
Psychological discomfort	5 (2:6)	5 (0:7)	.670	4 (0:6)[a]	3 (2:7)	.974	4 (0:6)[a]	4 (0:8)	.754
Physical disability	1.5 (0:6)	2 (0:5)	.710	1.5 (0.4)	1 (0:6)	.893	1 (0:5)	0 (0:6)	.672
Psychological disability	2.5 (0:6)	3 (0:8)	.257	2 (0:6)[a]	2 (0.7)[a]	.905	1.5 (0:5)[a]	2 (0:6)[a]	.533
Social disability	0 (0:5)	1 (0:6)	.854	0 (0:4)[a]	0 (0:6)	.921	0 (0:6)	0 (0:4)	.950
Handicap	1 (0:6)	1(0:6)	.718	0 (0:5)[a]	0(0:6)	.690	0 (0:6)[a]	0 (0:4)[a]	.933
Sum	18 (5:38)	19 (5:42)	.873	13 (3:33)[a]	15 (4:41)[a]	.529	14(2 :31)[a]	13 (5:41)[a]	.983

[a]Statistically significant difference (Wilcoxon test, $p<0.05$) versus baseline

Table 8. PIDAQ results at different time points

	Baseline			1 week after bleaching			1 month after bleaching		
Dimension	H35	H13	Mann Whitney	H35	H13	Mann Whitney	H35	H13	Mann Whitney
Dental Self-Confidence	16.5 (6:26)	14 (10:26)	.386	21 (11:30)[a]	23 (12:30)[a]	.653	19 (10:30)[ab]	22 (6:29)[a]	.708
Social Impact	24 (8:40)	23 (8:34)	.693	17.5 (8:31)[a]	16 (8:26)[a]	.315	15.5 (8:31)[a]	17 (8:33)[a]	.764
Psychological Impact	19.5 (8:26)	17 (6:24)	.347	13.5 (6:24)[a]	15 (6:21)[a]	.949	14 (6:24)[a]	14 (6:25)	.991
Esthetic Concern	9.5(3:14)	9 (3:14)	.604	8 (3:12)[a]	5 (3:12)[a]	.094	5.5 (3:12)[ab]	6 (3:12)[ab]	.320
Sum	71 (38:98)	66 (39:81)	.365	63.5 (40:79)[a]	57 (39:74)[a]	.287	54 (39:79)[a]	58 (40:94)	.733

[a]Statistically significant difference (Wilcoxon test, $p<0.05$) versus baseline
[b]Statistically significant difference (Wilcoxon test, $p<0.05$) versus 1 week after bleaching

Discussion

This randomized clinical study showed that the effectiveness of two bleaching agents (35% hydrogen peroxide and 37% carbamide peroxide) can be measured objectively and subjectively, and both agents can be applied using the walking bleach technique for bleaching non-vital intracoronal teeth. Both products showed high effectiveness, and the results were similar and highly reproducible one month post-whitening. Our results showed that the treatment had a positive influence on esthetic self-perception and psychosocial impact at one month post-treatment, after improvement in the color of only one tooth in most volunteers in this trial. Therefore, the null hypothesis is accepted, as the two gels were widely effective according to objective and subjective measurements, and they had similar positive effects on the esthetic perception and psychosocial impact of patients in this clinical trial.

Several studies have showed that bleaching can be considered effective when there is a change of at least 5 ΔE units.[19] Our results showed that in four sessions using the walking bleach technique and up to one month post-treatment, there was a change of 15.48 ± 5.17 of ΔE for group 1 and 14.02 ± 4.85 of ΔE for group 2, which was highly effective. The first session achieved a considerable change of approximately 50% of the final result. There was no difference between the agents in the final results ($p>.05$), although there was a trend for color to recur at one month post-treatment. Statistical significance in ΔE was found only at the second and third sessions during treatment, even though ΔL was different at all times, with higher values for hydrogen peroxide. However, ΔE was similar in both groups, which suggests that there is a different chemical mechanism for both gels in the respective groups; hydrogen peroxide gel has a lower molecular weight and a quicker and more direct action, so it probably spreads faster compared than carbamide peroxide.[20] Carbamide peroxide degrades at a lower concentration of hydrogen peroxide,[21] which results in a slower process and no difference in effectiveness one month after bleaching. Subjective evaluation of the change in coloration also indicates a highly effective treatment. When comparing both products using subjective measurement, a statistically significant difference was found at all time points until one week post-treatment, while differences in the

objective measurement were only found at the second and third sessions of whitening. Luminosity can explain the differences (Table 3) because it is a parameter with more power to determine color that the human eye.[19] In recall of the month there was a slight rebound of color, which can be explained by the rehydration of the tooth subjected to high concentrations of peroxide for several sessions. This may suggest that clinicians should aim to "over-bleach" by at least a tone to compensate for this rebound.

Few clinical studies have evaluated the effectiveness of non-vital bleaching, and the most commonly used bleaching agent is sodium perborate.[7] An in vitro study conducted by Lim concluded that 35% carbamide peroxide and 35% hydrogen peroxide are more effective tan sodium perborate.[22] In this study, the color of each tooth was evaluated using the Vita Lumin shade guide; after 7 days, there was change of 8 ΔSGU, and the color changed by 2 additional units after 14 days for carbamide and hydrogen peroxide.[22]

Regarding comparing subjective and objective evaluations of tooth color with an untreated counterpart, we can say that the color of the treated tooth resembled that of the counterpart according to the subjective evaluation; however, it is not possible to perfectly match the color of all teeth. This represents a difficulty of the technique, and perhaps the clinician should apply a “custom technique” for each patient based on the tones that should be whitened.

The same situation occurs in the objective assessment of the L values, which are similar after one week but are different at one month because of the rebound in luminosity. In group 1, there was apparently an influence of color restoration on values “a” which remained for a month, whereas in group 2 there was no difference between the treated tooth and the tooth counterpart after one month. As for values "b," there was no difference between the treated tooth hand the counterpart tooth after one month.

It is likely that stronger action of agents generates color change in a darker tooth.[23] The research team was not surprised at the high degree of patient satisfaction achieved with a bleaching procedure in this trial,[24] even though, for various reasons, we were unable to completely match tooth color to the neighboring teeth

using our non-vital tooth-whitening method. Findings on the perception of esthetics and psychosocial impact were positive and significant up to one month post-whitening. Maintaining this positive effect over time should be confirmed in patients undergoing bleaching of vital teeth[25] and correlated with the maintenance of the effectiveness by a bleaching procedure.

The OHIP-Esthetics survey also showed a positive effect on patients' esthetic self-perception. This result is supported by other studies [22,25] that evaluated the influence on esthetic self-perception of patients undergoing extra-coronary vital whitening. Our data confirm that quality of life is complex, important and poorly reported, and there are multiple factors that influence it. However, dental esthetics is important, even if the influence of patients' perception and the impact of psychological and social factors is unknown. the impact of perception on psychological and social factors is unknown. There were no differences in OHIP-esthetics scores between groups, but there were differences in the changes over time in each group. This resulted in a more pronounced and positive effect for group 1, which may be explained by the more rapid effect on the modification of color brightness, as shown in Table 3. This should be further investigated to assess the correlation between self-perception and esthetic changes in the brightness of the color.

While the PIDAQ is not designed for patients who have undergone bleaching, it is usually used for orthodontic patients to determine their esthetic expectations, but it fails to evaluate which areas are is expected to be modified to solve a particular esthetic problem. The PIDAQ has been shown to decrease the negative impact of cosmetic dentistry for a patient and to decrease the values in the field of social impact, psychological impact and esthetic concerns. The psychosocial impact was similar in both groups ($p>0.05$). We found that there was a large impact reported by the PIDAQ data for all relevant factors, but a minimal intervention on a tooth has an important effect on people who undergo intracameral bleaching.[25]

Conclusion

Both 35% hydrogen peroxide and 37% carbamide peroxide are highly effective for the walking bleach technique in non-vital teeth and achieve a high degree of color matching with the counterpart teeth. Each gel resulted in a positive impact on patients' esthetic self-perception and psychosocial self-perception after intracoronal whitening.

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3.2 Publicação 2

Effect on IL-1β and RANK-L using a walking bleaching non-vital technique*

ABSTRACT

INTRODUCTION: Bleaching non-vital teeth, has been associated with resorption of hard tissues (root resorption), our hypothesis is that the technique "walking bleaching" would generate an increasing biomarkers IL-1β and RANKL. This randomized double-blind clinical study was aimed to evaluate the *in vivo* effect of hydrogen peroxide 35% and carbamide peroxide 37% using an intracameral bleaching technique (i.e.,"walking bleaching") for 4 sessions with the following periodontal markers: receptor activator of nuclear factor kappa B-ligand (RANKL) and interleukin-1β (IL-1β). METHODS: 47 volunteers participated with discoloration of non-vital teeth and the endodontic treatment in good condition. 50 teeth were randomly divided into two study groups according to product G1=hydrogen peroxide 35% (n=25) and G2=carbamide peroxide 37%(n=25). The intracameral bleaching was performed with a walking bleaching technique consisting of 4 sessions. Gingival crevicular fluid samples were taken in order to quantify the RANKL and IL-1β levels by ELISA. Samples were obtained from six periodontal sites for each bleached tooth: 3 vestibular and 3 palatine (mesial, middle, and distal), in 7 opportunities: baseline, after 4 sessions of intracameral bleaching, one week, and one month after treatment. Tooth color variations were

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analyzed in each session by assigning a numerical value in order to calculate changes in unit scales (ΔSGU). RESULTS: Significant increments in the RANKL and IL-1β levels were detected in each evaluated time compared with baseline ($p<0.05$); however, no differences were detected between G1 and G1. The color changes were 5 for G1 and 4 for G2. CONCLUSIONS: Intracameral bleaching induced an increment in the RANKL and IL-1β levels in periodontal tissues.

Key words: tooth bleaching, biomarkers, clinical studies/trials, endodontics, Root caries/resorption, bone loss.

Introduction

The intracoronal bleaching technique has traditionally been used to solve aesthetic problems of non-vital teeth discoloration, since it is a minimally invasive, fast, and effective treatment. In general, gels of sodium perborate, carbamide peroxide, and hydrogen peroxide are used to discolored teeth bleaching, and always the active agent is Hydrogen peroxide to chemical redox reactions. The literature has extensively reported that the high oxidative power of hydrogen peroxide can alter the histological and morphological properties of tooth structure (1-3), and one of the adverse effects of intracoronary bleaching have been reported is the external root resorption, which is increasingly rare but still apparent when occurs quite frequently the tooth loss is obtained (4).

One possible explanation is that adverse reactions due to the low molecular weight oh H_2O_2 would be able to diffuse through the tooth structure and stay in periodontal tissues (5). An extra-root effect produced by free radical peroxide has produced periodontal cell cytotoxicity and increased extra-radicular pH (6). Recent studies showed an increase in the activity of inflammatory cytokines and

metalloproteinases by bleaching techniques (1, 7). These studies suggest that this could also have an effect on levels of markers of bone destruction, considering that they would have spread to the periodontal tissues. The most important marker of destruction that have been extensively studied is receptor activator of nuclear factor kappa B-ligand (RANKL), which also is associated with regulating the process of root resorption (8,9).

The aim of this study was to evaluate the *in vivo* effect of hydrogen peroxide and 35% carbamide peroxide 37% in an intracameral bleaching technique of "walking bleaching" (4 sessions of treatment) on periodontal markers RANKL and Interleukin 1β. The null hypothesis is that the two gels do not produce an alteration in the basal levels of RANKL and IL-1β.

Material and Methods

47 volunteers were selected with at least one non-vital teeth discoloration. Prior tothe start of the study, patients signed an informed consent form. They received a prophylaxis brush and a slurry of pumice and water. In addition, oral higiene instructions were given to standardize the oral conditions of each volunteer and control basal periodontal health status.

Study Design

The trial was randomized and double blind (patient and evaluator). Randomization to the study group was performed using Excel 2010 (Microsoft, Seattle, USA) software. Advertising was held to invite participation in the dental school and through social networks like Facebook or Twitter.

Sample size

To determine the size of the sample, GPower 3.1(10) software was used, 8 considering a significance level of 5% statistical power of 80% and 10% abandoned.

A total of 74 patients were examined to assess whether they met the criteria for inclusion and exclusion. Patients included were over 18 years of age with one or more non-vital teeth, the restoration does not cover the vestibular face of tooth, endodontic treatment is in good condition, without apical lesion, with no previous experience of tooth bleaching, and tooth shade A2 or greater according to the Vita Classical scale. Patients were excluded if they were pregnant or lactating, with enamel hypoplasia, with teeth stained by tetracycline or fluorosis, in orthodontic treatment with fixed appliances, with cancer, or with periodontal diseases. In addition to those volunteers to be examined clinically and radiographically present caries, periapical lesions, external or internal dental resorption and / or periodontal disease, as may be informed and referred to specialists for treatment.

47 patients, of whom 46 completed treatment and attended all controls were selected. Two study groups were formed using randomization for whether the bleaching agent used, each with teeth n = 25, G1 = teeth bleached with Hydrogen Peroxide 35% (Opalescence Endo - Ultradent, USA). G2 = bleached teeth with carbamide peroxide 37% (Whiteness Superendo, FGM, Brazil).

Bleaching procedure

The application of the bleaching agents was performed according to the manufacturer's instructions in four sessions with an outpatient technique (Walking Bleaching); each session was separated by one week.

Session Preparation: To prepare the root canal with absolute isolation, were removed 3 mm seal the endodontic from cement-enamel limit like reference. The final sealing was made with 2 mm with glass ionomer resin with composite resin (Riva Light Cure, SDI, Australia) and light-cured for 40 seconds (Cal Radii, SDI, Australia). By radiographic control was corroborated the final seal.

4 sessions of Bleaching: The application of the bleaching agent was performed according to the manufacturer's instructions. An amount of bleaching gel was left into the pulpar cavity with presence of mild moisture (Walking Bleach technique). Subsequently, the cavity was sealed with a temporary cement (Fermin, Detax, Germany) to the next session.

Closing session: The access cavity was washed with water, and a temporary sealing was left for 7 days prior to the completion of the final seal.

Patients were advised not to eat or drink foods that contained stain, such as coffee, tea, red wine, etc. during the study period. They were given written instructions and contact information if any questions or problems were to arise.

Color Evaluation

Two calibrated evaluators with 80% agreement (Kappa test) recorded the color of teeth at baseline (baseline), immediately after each bleaching session, and one week and one month post treatment. Color evaluation was in the middle third of the labial surface of tooth bleaching according to ADA recommendations (11). Patients were examined in the same room with the same lighting, for both examiners independently, using the Vita Bleachguide scale (Vita Zahnfabrik, Bad Säckingen, Germany). For every "value", color is assigned a numerical value in

order to calculate the change in units scale (SGU Δ).

Quantification of receptor activator of nuclear factor kappa B-ligand (RANKL) and interleukin-1β (IL-1β) levels in gingival crevicular fluid (GCF)

GCF Sample collection.

After isolating the tooth with a cotton roll, supragingival plaque was removed with a curette Gracey 3/4 without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe, and GCF was collected using absorbent paper strips (Periopaper®; OraFlow Inc., New York, NY, USA), which were placed into the selected periodontal sulci until mild resistance was felt and left in place for 30 seconds. Strips contaminated by saliva or blood were excluded. GCF samples were obtained from six periodontal localizations: 3 vestibular and 3 palatal (mesial, middle, and distal) sites from teeth under bleaching procedures. Samples were taken before the bleaching (baseline) and after each bleaching session as well as one week and one month post bleaching. Following GCF collection, strips were placed in eppendorf vials containing 100 µl of phosphate buffered saline with 0.05% Tween-20 (Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) and centrifuged at 10,000 xg for 5 minutes at 4 °C. The elution procedure was repeated twice, and obtained samples were stored at −80°C until further analysis.

Quantification of RANKL, OPG, and IL-1β

Total proteins were quantified (Bradford®; R&D Systems Inc., Minneapolis, MN, USA), and the RANKL and IL-1β levels were measured by ELISA (Quantikine®; R&D Systems Inc.) following the manufacturer's instructions. Absorbance was measured at 492 nm with a wavelength correction of 630 nm using an automatic

microplate reader (Synergy HT; Bio-Tek Instrument Inc., Winooski, VT, USA). The concentration of each marker in each sample was calculated by a four-parameter logistic equation

Results

A total of 74 volunteers were examined, and 47 patients met the inclusion criteria. 50 teeth were selected from these 47 patients, of which three did not complete the treatment and one tooth was excluded from analysis due to problems with the analysis of samples of the gingival fluid. The final sample consisted of a total of 47non-vital teeth that were bleached; the distribution of the group can be seen in Table 1.

Table 1. Baseline features of the participants

Baseline features	**Groups**	
	Hydrogen Peroxide	**Carbamide Peroxide**
Age (years; means ± SD)	30.57 ± 11.74	30.82 ± 11.25
Minimum age (years)	19	20
Maximum age (years)	65	65
Male (%)	47.83	39.13
Trauma (%)	56.52	39.13
Vita 3d Bleach Median (Min;Max)	12 (7;15)	11 (9;15)

The color change was measured subjectively and expressed in ΔSGU units,

which are shown in Table 2. There was a statistically significant difference ($p < 0.05$) in the third and fourth week, showing more color change in the group with hydrogen peroxide. At one week post bleaching, the color change does not present significant differences.

Table 2. Changes of color by ΔSGU by group in different time frames expressed by median, mínimum, maximum and statistical significance

Assessment points	**Color change**		Mann-Whitney
	G1	**G2**	
Baseline *vs.* 1-wk bleaching	3 (0;5) [a]	1 (0;4)	0.410 [a]
Baseline *vs.* 2-wk bleaching	4 (0;8) [a]	2 (0;5)	0.002 [a]
Baseline *vs.* 3-wk bleaching	5 (0;9) [b]	4 (1;5)	0.007 [a]
Baseline *vs.* 4-wk bleaching	5.5 (0;9)[b]	4 (2;8)	0.019
Baseline *vs.* 1-wk after bleaching (before restoration)	5 (0;9)	4 (2;7)	0.037
Baseline *vs.* 1-wk after bleaching (after restoration)	5 (0;9)	4 (2;7)	0.040
Baseline *vs.* 1-mth after bleaching	5 (0;9)	4 (2;7)	0.027

[a] Statistically significant difference with all time in the group ($p < 0.05$)
[b] Statistically significant difference with between *Baseline vs. 3-wk bleaching* and *Baseline vs. 4-wk bleaching.*

RANKL and IL-1β levels

Six sites per tooth (mesiobuccal, mesiovestibular, distobuccal, mesiopalatino / lingual, mediopalatino / lingual and distopalatino / lingual) were measured. 966 samples in total were taken from each group. 959 samples (7 were excluded) for RANKL for the group with hydrogen peroxide, and all samples were analyzed from the carbamide peroxide group. Table 3 shows the levels of RANKL expressed in pg/ul, considering all the sites and the buccal and palatal sites, respectively. All evaluation times have statistically significant differences between them ($p<0.05$).

For the group with hydrogen peroxide, it was possible to analyze 963 (3 loss) samples for IL-1β, and all samples for the carbamide peroxide group. Table 4 shows the levels IL-1β expressed in pg / ul, considering all the sites and the vestibular and palatal sites, respectively. All evaluation times have statistically significant differences between them ($p<0.05$).

Table 3.- Rankl levels expressed in pg/ul, median (min;max)

Assessment points	Hydrogen Peroxide total	Carbamide Peroxide total	ΔPH	ΔPC	Mann Whitney	Hydrogen Peroxide vestibular	Carbamide Peroxide vestibular	ΔPH	ΔPC	Mann Whitney	Hydrogen Peroxide palatino	Carbamide Peroxide palatino	ΔPH	ΔPC	Mann Whitney
Baseline	12.55 (3.39 ; 30.35)	13.84 (4.42 ; 27.84)				12.85 (3.42 ; 30.35)	14.86 (5.49 ; 27.84)				10.98 (3.39 ; 22.27)	12.44 (4.42 ; 20.98)			
1-wk bleaching	12.58 [a] (3.25 ; 31.39)	14.47 [a] (4.36 ; 27.76)	0.52	0.54	0.728	13.32[a] (3.44 ; 31.39)	15.52 [a] (5.60 ; 27.76)	0.47	0.69	0.059	11.52 [a] (3.25 ; 23.98)	13.21 [a] (4.36 ; 20.45)	0.53	0.37	0.129
2-wk bleaching	14.05 [ab] (3.48 ; 32.38)	15.76 [ab] (4.47 ; 28.64)	1.92[b]	1.71[b]	0.487	15.34 [ab] (4.42 ; 32.38)	17.94 [ab] (6.54 ; 28.64)	2.11[b]	2.09[b]	0.845	12.55 [ab] (3.48 ; 24.86)	14.46 [ab] (4.47 ; 21.98)	1.75[b]	1.44[b]	0.259
3-wk bleaching	16.30 [ab] (4.83 ; 37.29)	17.76 [ab] (6.13 ; 31.27)	4.17[b]	3.40[b]	0.067	18.65 [ab] (6.11 ; 37.29)	20.07 [ab] (8.60 ; 31.27)	4.75[b]	4.13[b]	0. 382	14.65 [ab] (4.83 ; 28.72)	15.42 [ab] (6.13 ; 23.95)	3.84[b]	3.01[b]	0.099
4-wk bleaching	19.25 [ab] (5.53 ; 44.23)	20.18 [ab] (8.31 ; 37.42)	8.02[b]	6.62[b]	0.075	23.04 [ab] (8.42 ; 44.23)	24.34 [ab] (9.23 ; 37.42)	9.26[b]	7.61[b]	0. 182	16.89 [ab] (5.53 ; 33.28)	18.51 [ab] (8.31 ; 27.98)	6.15[b]	5.39[b]	0.451
1-wk after bleaching	22.63 [ab] (6.18 ; 53.42)	24.74 [ab] (9.67 ; 47.26)	12.17 [b]	11.19 [b]	0.233	28.23 [ab] (9.67 ; 53.42)	30.76 [ab] (14.30 ; 47.26)	15.51 [b]	14.96 [b]	0.342	20.16 [ab] (6.18 ; 38.37)	22.21 [ab] (9.67 ; 34.01)	9.34[b]	9.06[b]	0.474
1-mth after bleaching	25.88 [ab] (8.47 ; 60.31)	28.05 [ab] (10.34 ; 53.84)	15.20 [b]	14.57 [b]	0.413	31.36 [ab] (11.21 ; 60.31)	35.41 [ab] (15.17 ; 53.84)	19.99 [b]	19.52 [b]	0.686	21.84 [ab] (8.47 ; 41.42)	24.68 [ab] (10.34 ; 36.42)	11.76 [b]	11.06 [b]	0.514

Δ (Assessment points vs baseline)

[a] statistically significant difference with baseline with the Wilcoxon test ($p < 0.05$).

[b] statistically significant difference with previous time with the Wilcoxon test ($p < 0.05$).

Table 4.- Il1b levels expressed in pg/ul, median (min;max)

Assessment points	Hydrogen Peroxide total	Carbamide Peroxide total	ΔPH	ΔPC	Mann Whitney	Hydrogen Peroxide vestibular	Carbamide Peroxide vestibular	ΔPH	ΔPC	Mann Whitney	Hydrogen Peroxide palatino	Carbamide Peroxide palatino	ΔPH	ΔPC	Mann Whitney
Baseline	91.13 (20.67 ; 206.01)	100.38 (30.65 ; 279.54)			b	107.32 (23.31 ; 206.01)	112.26 (41.12 ; 279.54)				78.54 (20.67 ; 151.48)	84.32 (30.65 ; 164.22)			
1-wk bleaching	94.98 [a] (16.34 ; 217.53)	106.07 [a] (33.41 ; 287.32)	2.77	2.64	0.228	111.78 [a] (31.28 ; 217.53)	116.85 [a] (44.98 ; 287.32)	3.75	3.79	0.203	80.76 [a] (16.34 ; 159.43)	89.53 [a] (33.41 ; 165.81)	2.14	1.98	0.470
2-wk bleaching	99.34 [ab] (17.54 ; 231.27)	116.87 [ab] (37.54 ; 298.23)	9.78[b]	8.64[b]	0.198	125.43 [ab] (31.28 ; 231.27)	125.24 [ab] (52.65 ; 298.23)	12.61[b]	11.11[b]	0.472	86.73 [ab] (17.54 ; 171.72)	94.42 [ab] (37.54 ; 168.54)	7.00[b]	6.53[b]	0.255
3-wk bleaching	107.98 [ab] (20.14 ; 254.36)	129.47 [ab] (42.36 ; 303.64)	21.53[b]	18.27[b]	**0.049**	145.75 [ab] (39.23 ; 254.36)	145.49 [ab] (65.45 ; 303.54)	28.41[b]	23.13[b]	0.172	95.52 [ab] (20.14 ; 185.47)	102.22 [ab] (42.36 ; 176.35)	16.33[b]	13.89[b]	0.110
4-wk bleaching	125.04 [ab] (25.21 ; 301.16)	144.29 [ab] (48.81 ; 325.11)	40.38[b]	34.23 [b]	0.064	169.93[ab] (53.34 ; 3012.16)	167.43 [ab] (76.23 ; 325.11)	52.39[b]	45.22[b]	0.142	107.32 [ab] (25.21 ; 210.61)	117.83 [ab] (48.81 ; 192.27)	29.51[b]	25.21[b]	0.192
1-wk after bleaching	148.63 [ab] (35.52 ; 371.71)	164.42 [ab] (55.24 ; 352.13)	66.98[b]	62.62[b]	0.124	198.25[ab] (60.32 ; 371.71)	196.52 [ab] (93.49 ; 352.13)	81.19[b]	72.59[b]	0.159	123.46 [ab] (35.52 ; 256.72)	136.43 [ab] (55.24 ; 226.50)	51.34[b]	43.28[b]	0.360
1-mth after bleaching	166.70 [ab] (51.51 ; 436.76)	185.91 [ab] (61.93 ; 391.12)	81.74[b]	79.37[b]	0.082	222.32[ab] (65.42 ; 436.76)	221.64 [ab] (97.43 ; 391.12)	105.11[b]	102.78[b]	0.159	140.21 [ab] (51.51 ; 296.64)	160.45 [ab] (61.93 ; 253.47)	64.90[b]	57.33[b]	0.222

Δ (Assessment points vs baseline)

[a] statistically significant difference with baseline with the Wilcoxon test ($p < 0.05$).

[b] statistically significant difference with previous time with the Wilcoxon test ($p < 0.05$).

Discussion

Our results show that hydrogen peroxide and carbamide peroxide influence the RANK-RANKL-OPG axis by increasing RANK-L and IL-1 β from the first session of intracameral bleaching until the month post bleaching and significantly increasing between those times.

The levels found are not increased as much as an active periodontal disease (12,13). But the peroxide diffuses through the dentinal tubules and generates an effect on periodontal tissues subclinical, and because of the sensitivity of the tests used to determine possible differences RANK balance shaft (14). Peroxide generates an imbalance in the IL-1β levels and RANK-RANKL-OPG axis in concordance with our results, and it is not clear where the effect occurs because the peroxide could generate a trigger to a chronic process, which should be confirmed with follow-up until normal levels of IL-1β and RANK-L are reached. This is demonstrated by the progressive increase in the levels of IL-1β and RANK-L after completion of bleaching up to one month of monitoring. The two compounds hydrogen peroxide and carbamide peroxide had a similar effect, although the resulting concentration of hydrogen peroxide in group 2 is smaller(15), so we can infer that the resulting concentration of peroxide would not be relevant in increasing levels of RANK-L and IL-1b were similar to the 2 groups (Tables 3 and 4).

Another important fact is that there was a correlation between the effectiveness of bleaching and the effect of RANK axis on adjacent periodontal tissues. This could suggest that the redox reaction of degradation of pigments is

independent to the adjacent bone process. Isolating the variables as traumatic origin of endodontics or caries, there was no difference between the levels of IL-1β and RANK-L for the different teeth studied, revealing that traumatized teeth stabilize their levels of RANKL and were increased because of intracameral bleaching (data non showed). There were differences between the vestibular and palatal sites, which agrees with previous studies(12).

The imbalance shaft of IL-1β and RANKL levels is a finding that could be initially explained by a sudden drop in pH, and in turn autocatalized to achieve a stabilization of metalloproteinase and cathepsin, because according to a recent report, proteolytic enzymes, cysteine cathepsins, and MMP are activated in mineralized dentin during degradation tooth-bleaching treatment with 35% H_2O_2 (1).

There are many studies that attempt to explain the phenomenon of external root resorption associated with intracameral bleaching, especially with a thermocatalytic technique infrequently reported in the literature(16-18). Our findings may explain to some extent the beginning of this process, perhaps as fractures associated with anatomical factors that create a point spread faster than peroxide or the inflammatory response with high inter-subject variability explain better root resorption mediated by increase of levels IL-1β and RANK-L markers.

However, increased levels of RANKL are not comparable to those achieved by a patient with active periodontal disease, since these levels could suggest an activation process of bone resorption in most susceptible patients or periodontal risk (13) (diabetics or patients with history of such aggressive periodontitis, etc.).

What could be a precedent for contraindication intracoronal bleaching procedures in these patients 'risk' activation periodontal lesions ; this is certainly a

line of research to develop. Also, this protocol (walking bleaching) could be a technique widely questioned by the data from this trial.

The role of IL-1β, a key cytokine with proinflammatory functions, has not yet been studied in the case of bleaching treatment. Studies have linked increased levels of IL-1β in teeth with apical external resorption associated with orthodontic treatment sequelae (19). In this study, a steady and significant increase in IL-1β levels was post-bleaching at one month compared with baseline levels. This demonstrates the presence of an inflammatory process (20), which will be the explanation of debalance levels of RANKL (21). This inflammatory process would be caused by the diffusion of peroxide through the tooth structure to the periodontium (22). The levels of IL-1β (about 190 pg/ul) are comparable with active sites of periodontitis (12) , although basal levels of patients were already high (about 100 pg / ul). This could be due to a previous process not associated with unclear non-vital teeth in the literature. IL-1β is a potent osteoclast activity initiator (23) and generally stimulates macrophages, monocytes, or between other endothelial cells to produce metalloproteinases, prostaglandins, and other proinflammatory cytokines (24, 25).

Also, it stimulates osteoblasts RANKL production, causing differentiation and maintenance of osteoclasts (26). Among the factors that triggers increased IL-1 beta are non- microbial factors such as tissue injury or the presence of inflammatory molecules among others (24), a situation generated by the peroxide released from the pulp chamber to the periodontium.

The two compounds, hydrogen peroxide and carbamide peroxide, had a similar effect after bleaching in the levels of IL1 β. The carbamide peroxide group had a statistically significant higher level, which could be explained by greater

persistence of the molecule of carbamide peroxide had a slow the chemical degradation reaction(15), so perhaps could be more relevant this phenomenon (remanence), which directly the hydrogen peroxide concentration on the effect of RANK axis on the adjacent periodontal tissues.

The important thing is that there is an increase in markers responsible for the activation of osteoclasts and may be a predisposing factor for the marginal bone resorption and an explanation for the phenomenon of external teeth cervical resorption-mediated odontoclasts.

An important factor to consider is that there is great variability in the anatomy of teeth endodontically treated, and the thickness of the remaining walls are not uniform, so it is expected that in teeth with less dentin thickness, diffusion of peroxide into the periodontal I can have a greater effect (27). In addition, the biological response of each individual is different; this could explain the high deviation markers levels, but what is certain is that all individuals showed increased levels of both RANKL, such as IL-1β (12).

The effectiveness of the color change for both product equals the week and one month post-treatment, although the hydrogen peroxide has a faster effect. This may be due to the slower carbamide peroxide chemical decomposition and its lower penetration of dental tissues compared to hydrogen peroxide (6, 14, 22). This lower diffusion of hydrogen peroxide and carbamide peroxide could havedone would have less effect extraradicular level, but both products showed increased levels similar markers (Tables 3 and 4).

The “walking bleaching” technique is a procedure with a closed chamber (4, 28, 29), in which the chemical agent makes prolonged contact with the tooth period; this favors the spread of peroxide into the extra-radicular space, which

would cause an increase in the markers studied. Therefore, we suggest that an in-office technique with an open chamber may be less aggressive, especially in patients with a history of periodontal disease.

It is noteworthy that the two groups had significant differences in levels of IL-1β at baseline. This is why we decided to use the differences v/s baselines values (deltas) of IL-1β in comparisons (Table 4). Also, this difference can be seen by considering all the palatal sites or because the study with only vestibular sites presents no difference between groups. IL-1β is a potent osteoclast activity initiator (23) and generally stimulates macrophages, monocytes, or endothelial cells among others to produce metalloproteinases, prostaglandins, and other proinflammatory cytokines. (24, 25) Also, it stimulates osteoblasts' RANKL production, causing differentiation and maintenance of osteoclasts (26). Among the factors that trigger increased IL-1β are non-microbial factors such as tissue injury or the presence of inflammatory molecules among others (24), a situation generated by the peroxide released from the pulp chamber to the periodontium. The important factor is that there is an increase in markers responsible for the activation of osteoclasts, and this may be a predisposing factor for the marginal bone resorption and explanation for the phenomenon of external teeth cervical resorption mediated odontoclasts, which is still not explained in the literature.The bleaching intracoronal PH 35% and PC 37% results in increased levels of RANKL and IL1B in gingival crevicular fluid. The RANKL levels between the PH 35% and 37% PC were not different at different times. The IL1B levels between the PH 35% and 37% PC were different during treatment. Hydrogen peroxide is most effective in a walking bleach technique when measured subjectively.

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3.3 Publicação 3

Effectiveness and impact of non-vital bleaching on the quality of life, three months follow-up: Randomized clinical study*

Abstract

Objectives: The aim of this study was to evaluate the aesthetic perception and the psychosocial impact of patients at three months bleaching of non-vital teeth with hydrogen peroxide (35%) and carbamide peroxide (37%) using walking bleach technique. We also assessed the clinical effectiveness and the stability of the color change. Methods: Bleaching of the teeth was randomly divided into two groups according to the bleaching agent used: G1= hydrogen peroxide 35% (n=25) and G2= carbamide peroxide 37% (n=25). The non-vital bleaching was performed in four sessions using Walking Bleach technique. The color was objectively (ΔE) and subjectively (ΔSGU) evaluated. The aesthetic perception and psychosocial factors were evaluated before, one week and one month post- treatment using oral health impact profile (OHIP)-aesthetics and PIDAQ questionnaires. Results: The color change (ΔE) a month were G1= 16.80±6.07 and G2= 14.09±4.83, for both color groups remains stable until the third month post-treatment ($p>0.05$). There was a decrease in the values of OHIP-aesthetics and psychosocial impact of dental aesthetics questionnaire (PIDAQ) after treatment compared to the baseline ($p<0.05$), maintained at third month post-treatment. Conclusions: Both agents

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were highly effective and was a positive impact on the aesthetic perception and psychosocial impact of patients who also remained stable over time.

Clinical relevance: The non-vital bleaching produces positive and stable impact on the aesthetic perception and psychosocial factors.

Keywords: Non-vital bleaching, Carbamide peroxide, Hydrogen peroxide, Randomized clinical trial.

Introduction

One of the most important aspects of an esthetically pleasing smile is the color of the teeth. When the color change in a single tooth, the negative effect may be greater than a generalized (whole teeth) change color, because it is more evident that the color does not coincide with the rest of the teeth [1]. Therefore, bleaching should be part of any aesthetic smile planning required to improve tooth color, either as an individual treatment or prior to other procedures. It should beconsidered as a color rehabilitation treatment [2].

Intracoronary bleaching is widely used as a minimally invasive alternative treatment to solve esthetic non-vital teeth discoloration [2]. Also, It has a high rate of satisfaction among patients [3]; despite this fact, there are no studies on the assessment of the real impact of these procedures in the aesthetic perception of patients; recent studies have shown the positive effect on patients undergoing extra-coronary bleaching in vital teeth [4-6].

Today, the most common dental bleaching agents are hydrogen peroxide and carbamide peroxide in high concentration for non-vital teeth, but the mechanism of action is still the same in all cases, which is the oxidation of organic pigments

with the decomposition products of the chemical agent[2, 7] . Although non-vital whitening teeth has been documented since the mid of the 19th century, there is little clinical evidence with objective measurement and methodology showing the effectiveness of treatment and behavior over time.

The main objective of this study was to evaluate the psychosocial impact on and aesthetic self-perceptions of patients undergoing non-vital tooth bleaching and the stability of the color changewith hydrogen peroxide (35% conc.) and carbamide peroxide (37% conc.) gels using the walking bleach technique, at three months post-treatment.

The null hypothesis is: 1) There is no difference between the psychosocial impact on and aesthetic perception of patients undergoing non-vital bleaching using the technique walking bleach with 35% conc. hydrogen peroxide or 37% conc. carbamide peroxide at three months post-bleaching, and 2) there is no difference in the color change of non-vital bleached teeth with 35% conc. hydrogen peroxide or 37% conc. carbamide peroxide, at three months post-treatment.

Methods

This randomized clinical study was approved by the Ethics Committee of the Faculty of Dentistry, University of Chile (2016/04) and was conducted according to the Consolidated Standards of Reporting Trials Statement [8] and the Declaration of Helsinki [9] .

A randomized double-blind (patients and evaluator) study was designed; Randomization was performed using Excel 2013 (Microsoft, Washington, United State) software. Patients were recruited via flyers in dental school and through social media (such as Facebook and Twitter).

Prior to the beginning of the study, patients received prophylaxis with brush and prophylaxis paste; in addition, oral hygiene instructions were administered to standardize the conditions required for the removal of dental plaque.

Sample size

The sample size was determined using GPower 3.1 [10] software, with a 5% level of significance, 90% statistical power and a dropout of 25%, based on a previous study [5]. This study corresponds to atherapeutic equivalence type, where a color variation of ΔE tones in the range of 7-10 or more, based on the original color, was considered significant. We targeted a sample size of 20, but to compensate the drop-out rate reported in previous studies, we used a sample size of 25 per group.

A total of 74 volunteers were examined to assess whether they met the inclusion and exclusion criteria; 47 patients were selected with at least one non-vital tooth discolouration A2 or greater value, according to the Vita Classical (Vita Zahnfabrik, Bad Säckingen, Germany) scale, and they all accepted and signed an informed consent approved by the local ethics committee.

Selection criteria

Inclusion criteria: Patients over 18 years, with one or more non-vital teeth discoloration with tooth color A2 or greater value according to the scale vita classical, whose restoration does not include the vestibular surface of the tooth, endodontic treatment in good condition (adequate filled in amplitude and length, asymptomatic) and without previous bleaching experience.

Exclusion criteria: Patients who are pregnant or lactating; cancer patients, patients with enamel defects (enamel hypoplasia or fluorosis), dental staining by

tetracycline or metallic pigments derivatives amalgam, in orthodontic treatment with fixed appliances, patients with periodontal disease, teeth with caries lesions or periapical pathology, dental resorption (external or internal). Patients with pathology were referred to the appropriate clinical treatments in dental school.

Patients who met the study criteria and agreed to participate were randomly selected, and divided into two study groups according to the bleaching agent used:

G1 = Hydrogen peroxide 35% conc. (Opalescence Endo - Ultradent, South Jordan, Utah, United State).

G2 = Carbamide peroxide 37% conc. (Whiteness Superendo, FGM, Joinville, Santa Catarina, Brazil).

Bleaching protocol

An ambulatory technique (walking bleach) was used; the bleaching agent was applied in the pulp chamber followed by sealing the cavity; the agent was changed every week until after four weeks of treatment.

Preparation session: The root canal was prepared with absolute isolation (Rubber Dam Ash, Dentsply, Brazil, SP), endodontic filling was removed 3 mm below the cemento-enamel junction, and then sealed mechanically with resin-reinforced glass ionomer (Riva light cure, SDI, Bayswater, Victoria, Australia) with a thickness of 2 mm, light-cured for 60 seconds at a distance of 1 cm with the lamp Raddi Cal (SDI, Bayswater, Victoria, Australia). Radiographic control was taken to confirm proper sealing of the root canal.

Four bleaching sessions: The application of the bleaching agent was performed according to the manufacturer's instructions. The gel was left in the pulp chamber

with moisture (using the walking bleach technique); the cavity was sealed between sessions with temporary cement (Fermin, Detax, Baden-Württemberg, Germany). The replacement of the gel was made every seven days. At theend of the fourth week of bleaching, the access cavity was washed with water and temporarily sealed for seven days.

Final restoration: In control of one week post-bleaching, the final restoration was made with composite Brilliant NG (Coltène-Whaledent AG, Switzerland) using a multi-layered technique.

Color measurements (objective and subjective) were made prior to the commencement of treatment (baseline), immediately after each bleaching sessions, one week after treatment (pre-and post-restoration), one and three months post-bleaching, respectively. In addition, the color of the homologous tooth was used to record the general color of the teeth of the patients.

Color evaluation

Objective assessment of color: Two calibrated evaluators (kappa = 0.85) measured tooth color in an area of 6 mm in diameter in the middle-third of the labial surface of the tooth, using the spectrophotometer Vita Easyshade Compact (VITA Zahnfabrik, Bad Säckingen, Germany). To standardize this evaluation, we made a silicone matrix (Zetaplus, Zhermack, Rovigo, Italy), with a window on the buccal surface of 6 mm in diameter, to the tip of the spectrophotometer. The color change was determined using the CIELab system with parameters L*, a* and b *. We calculated the color difference of each control with the baseline. The ΔE was calculated using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The evaluators considered the color of the tooth counterpart (treated tooth: upper

right central incisor; tooth counterpart: left central incisor), and the same area (middle) of each tooth was evaluated to compare color matching.

Subjective assessment of color: Two evaluators calibrated (Kappa = 0.85) was used to measure tooth color in the middle-third of the labial surface, according to the guidelines of the American Dental Association [11] , in which 16 color tablets were ordered by value, from the highest (B1) to the lowest (C4). Although the Vita Classic scale is not linear in its true sense, the investigators treated the changes as continuous using a linear ranking, as previously conducted in other dental bleaching clinical trials. [12-16] Color changes were recorded as the difference between the baseline and the different evaluation times, expressed in the number of color guide units (ΔSGU). If the results of the two evaluators did not match, the two evaluators will then discuss until a consensus is reached concerning the color. The color of the counterpart tooth was also recorded subjectively and compared to that of the treated tooth.

Oral health impact profile questionnaire (OHIP-aesthetics)

To evaluate the aesthetic perception, using the OHIP-aesthetics questionnaire was validated in Chilean Spanish [17]. The questionnaire was applied at the beginning (baseline), the control of the week, a month and three months post bleaching. Each statement was a response to a Likert scale, which generates a score of 4 to 0 (very often = 4, fairly often = 3, occasionally = 2, almost never = 1, never = 0). These individual scores were summed to obtain an overall score ranging from 0 (minimum) to 56 (maximum). The outcomes were considered to be the sum scores of the OHIP-aesthetics and dimension scores (functional limitation, physical pain,

psychological discomfort, physical disability, psychological disability, social disability and handicap).

The psychosocial impact of dental aesthetics questionnaire (PIDAQ)

PIDAQ questionnaire [18, 19] has 23 items, divided into four subscales (one positive and three negative), which corresponds to the dimensions evaluated: 1) self-confidence by dental appearance, 2) social impact, 3) psychosocial impact and 4) aesthetic concern. [14] Self-confidence by dental appearance consists of six items of the scale of self-confidence. The second dimension, social impact, contains eight items on the social aspects of the quality of life questionnaire. The third dimension, the psychosocial impact has six items primarily related to the psychosocial impact of dental aesthetics. The fourth dimension is on aesthetics with three items. The questionnaire is self-filling and is administered at the beginning of the session. It used a Likert scale of five points that range from 0 (no impact of dental aesthetics in quality of life) to 4 (maximum impact of dental aesthetics in quality of life) for each element. The responses option are: not at all= 0, a little= 1, somewhat= 2, strongly= 3 and very strongly= 4. The questionnaire was validated in Spanish; the confidence of 0.90 by Cronbach's alpha was reported [19] . The questionnaire was administered at the beginning (baseline), the control of the week, a month and three months post bleaching. The results were considered as the sum of PIDAQ and factor scores.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA) with $\alpha = 0.05$. For intra-group analysis, Wilcoxon test was used and for inter-group analysis test, we used Mann-Whitney.

Results

Forty-seven patients, with 50 non-vital teeth with discolorations, were recruited from the 74 patients evaluated. At three months, only 42 patients completed treatment and were compared with the control group; thus, a final sample of 44 non-vital teeth was obtained. The characteristics of the final sample are presented in Table 1.

Table 1. Participant baseline characteristics

Baseline features	Groups	
	Hydrogen Peroxide	Carbamide Peroxide
Age (years; mean ± SD)	30.24 ± 12.26	30.83 ± 11.25
Minimum age (years)	19	20
Maximum age (years)	65	65
Male (%)	42.9	39.1
Trauma (%)	52.38	39.13
SGU Baseline median (min; max) Vita Classical	15 (5; 16)	12 (7; 16)
L* (mean ± SD)	73.29 ± 8.65	75.91 ± 6.81
a* (mean ± SD)	4.84 ± 3.12	4.85 ± 3.39
b* (mean ± SD)	29.44 ± 3.61	31.79 ±6. 60

SD = standard deviation

Table 2. Color change by L, a and b (mean and standard deviation) at all time points.

Assessment times	Color change by ΔL			Color change by Δa			Color change by Δb		
	G1	G2	Mann-Whitney	G1	G2	Mann-Whitney	G1	G2	Mann-Whitney
Baseline vs. 1-week bleaching	8.58 ± 4.78	4.33 ± 4.31	**0.006**	-3.16 ± 1.97	-2.63 ± 2.32	0.359	-1.47 ± 3.63	-3.18 ± 4.18	0.269
Baseline vs. 2-week bleaching	10.46 ± 6.85	5.98 ± 4.70	**0.011**	-4.32 ± 2.89	-3.96 ± 2.97	0.715	-3.54 ± 4.67	-5.70 ± 5.97	0.200
Baseline vs. 3-week bleaching	12.00 ± 6.58	7.98 ± 4.26	**0.012**	-5.16 ± 2.56	-4.51 ± 2.69	0.488	-6.12 ± 6.10	-5.38 ± 5.96	0.698
Baseline vs. 4-week bleaching	13.66 ± 7.16	8.64 ± 3.53	**0.001**	-5.81 ± 2.94	-5.20 ± 2.85	0.716	-6.50 ± 6.11	-6.63 ± 5.85	1.000
Baseline vs. 1 week after bleaching (before restoration)	12.30 ± 7.50	7.64 ± 3.93	**0.015**	-5.74 ± 3.60	-5.45 ± 2.88	0.944	-7.01 ± 6.03	-8.00 ± 7.43	0.452
Baseline vs. 1 week after bleaching (after restoration)	11.17 ± 7.23	7.25 ± 4.53	**0.027**	-6.52 ± 2.80	-5.67 ± 2.75	0.424	-7.63 ± 6.02	-7.92 ± 7.75	0.581
Baseline vs. 1 month after bleaching	7.72 ± 6.20	4.24 ± 4.65	**0.028**	-6.72 ± 2.56	-6.07 ± 2.50	0.622	-9.98 ± 5.64	-10.12 ± 6.22	0.672
Baseline vs. 3 month after bleaching	7.76 ± 6.23	4.26 ± 4.44	0.072	-6.58 ± 2.63	-5.61 ± 3.70	0.724	-9.52 ± 5.50	-9.31 ± 6.45	0.860

Table 3. Color by L, a and b (mean and standard deviation) at all time points and the color of the Homologous Tooth.

Assessment times	Color change by L		Color change by a		Color change by b	
	G1	G2	G1	G2	G1	G2
Homologous Tooth	84.39 ± 4,76	84.77 ± 4.91	-0.91 ± 1.00	-1.16 ± 1.06	20.10 ± 3.47	19.43 ± 4.18
Baseline	73.29 ± 8.65 [a]	75.91 ± 6.81 [a]	4.84 ± 3.12 [a]	4.85 ± 3.39 [a]	29.44 ± 3.61 [a]	31.79 ± 6.60 [a]
1-wk bleaching	81.87 ± 7.47	80.24 ± 7.51 [a]	1.68 ± 3.23 [a]	2.22 ± 4.02 [a]	27.97 ± 4.44 [a]	28.61 ± 5.51 [a]
2-wk bleaching	83.75 ± 6.92	81.76 ± 7.26	0.52 ± 3.44	1.05 ± 4.48	25.90 ± 4.30 [a]	26.11 ± 5.53 [a]
3-wk bleaching	85.29 ± 6.00	83.87 ± 6.87	-0.31 ± 3.34	0.53 ± 4.09	23.32 ± 5.28 [a]	26.53 ± 5.96 [a]
4-wk bleaching	86.95 ± 6.14	84.34 ± 6.53	-0.97 ± 3.08	-0.16 ± 3.83	22.95 ± 5.34 [a]	25.26 ± 5.24 [a]
1 week after bleaching (before restoration)	85.60 ± 7.06	83.55 ± 6.23	-0.90 ± 3.61	-0.47 ± 4.08	22.43 ± 5.01 [a]	23.90 ± 6.12 [a]
1 week after bleaching (after restoration)	84.46 ± 6.03	83.08 ± 7.64	-1.68 ± 2.63	-0.72 ±3.91	21.81 ± 5.09 [a]	23.85 ± 6.07 [a]
1 month after bleaching	81.17 ± 6.01 [a]	80.15 ± 5.24 [a]	-1.88 ± 2.41	-1.23 ± 3.03	19.46 ± 5.10	21.67 ± 5.08
3 month after bleaching	81.05 ± 6.32 [a]	80.17 ± 5.22 [a]	-1.73 ± 2.41	-0.77 ± 3.30	20.29 ± 4.72	22.48 ± 4.54 [a]

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus Homologous Tooth.

Table 4. Color change expressed in units (E; mean and standard deviation) at all time points.

Assessment times	Color change by E		
	G1=Hydrogen Peroxide	G2= Carbamide Peroxide	Mann-Whitney
Baseline vs. 1-wk bleaching	10.08 ± 4.83	7.24 ± 4.79	0.065
Baseline vs. 2-wk bleaching	13.67 ± 5.34 [a]	10.84 ± 5.22 [a]	0.130
Baseline vs. 3-wk bleaching	16.19 ± 5.53 [a]	11.95 ± 5.04	**0.023**
Baseline vs. 4-wk bleaching	17.90 ± 6.03 [a]	13.01 ± 4.71 [a]	**0.011**
Baseline vs. 1 week after bleaching (before restoration)	17.04 ± 6.77	14.22 ± 4.92	0.235
Baseline vs. 1 week after bleaching (after restoration)	16.80 ± 6.07	14.45 ± 4.88	0.235
Baseline vs. 1 month after bleaching	16.04 ± 4.88	14.09 ± 4.83	0.307
Baseline vs. 3 month after bleaching	15.25 ± 5.28	13.71 ± 4.58	0.503

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus previous time point.

Objective assessment of color

The result of the parameters ΔL, Δa and Δb parameters CIELab, obtained using the spectrophotometer are presented in Table 2. Statistically significant difference was obtained with the Mann Whitney test between the two groups at all times in the ΔL parameter ($p<0.05$), except for the control in the third month of post-bleaching ($p=0.072$). Table 3 shows the evolution of bleached tooth color compared to the homologous tooth. Table 4 shows the values obtained for ΔE; the effectiveness was similar between groups ($p>0.05$) in all evaluation times, except for 3ª and 4ª bleach sessions ($p<0.05$). Both groups showed high effectiveness, with at least an average color change of 14 units ΔE, at the first month after bleaching; the color remains stable for the third month (Wilcoxon test $p>0.05$ between the first month and third month after bleaching).

Subjective assessment of color

Table 5 shows the scale ΔSGU vita classic, which showcase the greater effectiveness of hydrogen peroxide group ($p<0.05$) in the second bleaching session. However, based on the Mann Whitney test, there is no significant difference ($p=0.56$) in the final measurement at third month post-bleaching. Table 6 shows the evolution of bleached tooth color compared to the homologous tooth.

Table 5.- Comparison of ΔSGU values at different times using the Vita Classic scale (Median (min:max))

Assessment points	Color change by ΔSGU		
	G1=Hydrogen Peroxide	G2=Carbamide Peroxide	Mann-Whitney test
Baseline vs. 1-wk bleaching	4 (0:10)	3 (0:9)	0.347
Baseline vs. 2-wk bleaching	8 (0:14) [a]	4 (0:11) [a]	**0.017**
Baseline vs. 3-wk bleaching	10(0:14) [a]	7 (0:13) [a]	**0.005**
Baseline vs. 4-wk bleaching	11(2:14) [a]	7 (1:14) [a]	**0.004**
Baseline vs. 1-wk after bleaching (before restoration)	10(2:13) [a]	7 (1:13)	**0.013**
Baseline vs. 1-wk after bleaching (after restoration)	10 (2:13)	7 (2:13)	**0.011**
Baseline vs. 1-mth after bleaching	9(2:13)	7 (1:13)	**0.021**
Baseline vs. 3-mth after bleaching	9 (2:13)	7 (1:12)	0.056

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus previous time point.

Table 6. Comparison of SGU values at different times using the Vita Classic scale (Median (min:max))

Assessment times	Color change by SGU	
	G1	G2
Homologous Tooth	5 (1:9)	3 (2:10)
Baseline	15 (5:16) [a]	12 (7:16) [a]
1-wk bleaching	11 (2:16) [a]	9 (5:16) [a]
2-wk bleaching	5 (1:15)	7 (1:15) [a]
3-wk bleaching	4 (1:15)	5 (2:15) [a]
4-wk bleaching	2 (1:12) [a]	5 (1:15)
1 week after bleaching (before restoration)	4 (1:12)	5 (1:15)
1 week after bleaching (after restoration)	3 (1:12)	4 (1:15)
1 month after bleaching	4 (1:12)	6 (1:14) [a]
3 month after bleaching	4 (1:12)	6 (1:14) [a]

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus Homologous tooth.

OHIP-aesthetics

OHIP-aesthetics Questionnaire values are presented in Table 7. There is no statistical difference between the two groups ($p>0.05$); all dimensions had significant difference ($p<0.05$) in time, except for physical limitation.

Table 7.- Effect of intracoronary bleaching in the esthetic self-perception evaluated with the OHIP questionnaire

	Baseline			1 week after bleaching			1 month after bleaching			3 month after bleaching		
Dimension	G1	G2	Mann Whitney	G1	G2	Mann Whitney	G1	G2	Mann Whitney	G1	G2	Mann Whitney
Functional limitation	5(2:8)	5(2:8)	0.659	3(0:6) [a]	4 (1:6) [a]	0.357	2(0:7) [a]	4 (0:8) [a]	0.435	4 (0:6) [a]	3.5 (1:7) [a]	0.291
Physical pain	4(1:6)	3(0:5)	0.439	3 (1:6)	3 (1:6)	0.575	3(0:6)	2(0:8)	0.829	2 (0:5) [ab]	2.5 (0:6) [ab]	0.630
Psychological discomfort	5(2:6)	5(0:7)	0.870	4(0:6) [a]	3.5 (2:7) [a]	0.974	4(0:6) [a]	4(0:8)	0.754	4 (0:6) [abc]	3 (1:7) [a]	0.931
Physical disability	2(0:6)	2(0:5)	0.585	2 (0:4)	1 (0:6)	0.893	1(0:5)	0(0:6)	0.672	1(0:3)	1 (0:6)	0.982
Psychological disability	3(0:6)	3(0:8)	0.203	2 (0:6)	1.5 (0:7) [a]	0.905	2(0:5) [a]	2 (0:6) [a]	0.533	2 (0:4) [a]	1 (0:6)	0.683
Social disability	0(0:5)	0.5 (0:6)	0.900	0(0:4) [a]	0 (0:6)	0.921	0(0:6)	0(0:4)	0.950	0(0:4)	0 (0:4) [b]	0.598
Handicap	1(0:6)	0.5 (0:6)	0.718	0(0:5) [a]	0 (0:6)	0.690	0(0:6) [a]	0 (0:3) [a]	0.933	0(0:5)	0 (0:4) [a]	0.692
Sum	(5:38 19)	(5:42 19)	0.915	(3:33) 13[a]	14 (4:41) [a]	0.564	(2:31) 14[a]	12.5 (5:41) [a]	0.983	13 (1:27) [a]	11 (4:35) [ab]	0.601

[a] Statistically significant difference (Wilcoxon test, $p<0.05$) versus baseline
[b] Statistically significant difference (Wilcoxon test, $p<0.05$) versus 1 week after bleaching
[c] Statistically significant difference (Wilcoxon test, $p<0.05$) versus 1 month after bleaching

PIDAQ

PIDAQ Questionnaire values are presented in Table 8.

Table 8. PIDAQ results at different time point

	Baseline			1 week after bleaching			1 month after bleaching			3 month after bleaching		
Dimension	G1	G2	Mann Whitney	G1	G2	Mann Whitney	G1	G2	Mann Whitney	G1	G2	Mann Whitney
Dental Self-Confidence	16 (6:26)	14 (10:26)	0.386	21 (11:28 [a])	23 (12:30) [a]	0.653	19 (10:30) [a]	22.5 (6:29) [a]	0.708	21.5 (12:27) [b]	22 (12:29) [a]	0.815
Social Impact	24 (8:40)	22.5 (8:34)	0.693	18 (8:31) [a]	16 (8:26) [a]	0.315	17 (8:31) [a]	17 (8:33) [a]	0.764	17 (8:31) [a]	15 (8:27) [a c]	0.260
Psychological Impact	20 (8 26)	17 (6:24)	0.347	14 (6:24) [a]	14.5 (6:21) [a]	0.949	14 (8:24) [a]	13.5 (6:25)	0.991	15 (6:23) [a c]	13.5 (6:24) [a b]	0.856
Esthetic Concern	10 (3:14)	9.5 (3:14)	0.604	9 (3:12) [a]	4.5 (3:12) [a]	0.094	6 (3:12) [a b]	6 (3:12) [a b]	0.320	9 (3:12) [a]	6 (3:14) [a]	0.741
Sum	72 (38:98)	65.5 (39:81)	0.365	65 (40:79) [a]	56.5 (39:74) [a]	0.287	60 (39:79) [a]	57 (40:94) [a]	0.733	60.5 (35:78) [a]	52 (36:78) [a]	0.888

[a]Statistically significant difference (Wilcoxon test, p<0.05) versus baseline

[b]Statistically significant difference (Wilcoxon test, p<0.05) versus 1 week after bleaching [c] Statistically significant difference (Wilcoxon test, p<0.05) versus 1 month after bleaching

PIDAQ values were significantly different when comparing the baseline to week, month and three months post-treatment ($p<0.05$; Wilcoxon test), except the dimension of confidence in the group of hydrogen peroxide in the third month ($p=0.34$). When the week and the month after treatment were compared, the only statistically significant difference found was in the aesthetic concern ($p<0.05$) for both groups. When the week with the control of the third month hydrogen peroxide group was compared, there was no difference in self-confidence, and the psychosocial impact for peroxide carbamide group ($p<0.05$). Within the first month and the third month post-treatment, there was significant difference in the dimension of psychosocial for peroxide hydrogen groups, and in social impact for peroxide carbamide ($p<0.05$) with Mann Whitney test.

Discussion

This randomized clinical study shows the aesthetic perception and psychosocial impact of internal bleaching, and the effectiveness of intracoronary bleaching with two bleaching agents (hydrogen peroxide, 35% conc. and carbamide peroxide, 37% conc.) and their behavior up to three months. Both gels were highly effective with technical walking bleach in non-vital teeth; the color achieved was stable at three months post-treatment; till date, positive effect was maintained in the aesthetic perception and psychosocial impact of the patients. Therefore, both null hypothesis are accepted, as the two gels were widely effective according to objective and subjective measurements at three months follow-up, and they had similar positive effects on the esthetic perception and psychosocial impact of patients in this clinical trial.

Presently, there are few randomized clinical trials on the effectiveness of non-vital teeth whitening, and in general, it has been studied that sodium perborate can be used as a bleaching agent [2] . In a study by Amato et al. [20] , who evaluated a series of 50 cases of patients who had undergone intracoronary bleaching, and determined that after 16 years, 62.9% of the cases had satisfactory results. In monitored cases at 5 years, about 79% of cases of internal bleaching with different techniques were considered satisfactory by the evaluators when comparing the color with the adjacent tooth [21] .

Bleaching non-vital teeth is a minimally invasive treatment; it has a good effectiveness and stability over time [20]. Our results demonstrate the stability of results of treatment with two most common gels in three months with an objective methodology. It reported that a change of 5 ΔE with bleaching can be considered an effective procedure [22], a change of about 14 ΔE post-treatment was obtained and remained stable with the control in the third month; this is also because non-vital teeth may have extreme discoloration.

When measuring objectively the color change, there is significant difference between groups only at weeks 3ª and 4ª bleaching ($p<0.05$), unlike the subjective methodology in which a significant difference is seen in all sessions up to three months post-bleaching. The VITA Classical scale has a order according to the suggested luminosity but not equidistant from one another, which makes the changes from one unit to another not proportional or linear. Furthermore, color discrimination by the human eye is highly influenced by the brightness of the tooth [22] ; this is consistent with the measurement of the spectrophotometer, since evaluating color parameters separately with the CIELab system parameter ΔL,

which corresponds to the change in brightness and the significant difference ($p<0.05$), is observed up to the post-bleaching month.

One of the disadvantages of the subjective measurement is the inter-observer difference greatly influenced by the environment[5] solved withthe objective measurement of the spectrophotometer [23] , which can also detect minor changes. Thus, it is recommended to use objective methodologies for scientific studies, though subjective methodology has a greater clinical application since it graphically present the results to the patients. In both methods, hydrogen peroxide shows greater effective evaluated times, but also a regression of slightly more color, which is not significant ($p>0.05$); this is an evidence that both gels are stable up to three months (Tables 2 and 4).

The aim of non-vital teeth bleaching is to achieve a tooth color similar to the adjacent teeth color, within of normal procedure; a huge color change is achieved though discoloration is quite intense, this will also depend on the origin, since stains of organic origin have a better prognosis than inorganic origin [2] . Tables 3 shows the evolution of color with CIELAB parameters, during and after the bleaching, and we compared the color of the tooth counterpart in the L* parameter group of hydrogen peroxide from the first week of bleaching; there is no difference in the parameter with the homologous, but post-treatment presents a regression ($p<0.05$). Despite this, all parameters color (ΔE) remains stable after the treatment, but the luminosity (ΔL) presents a regression of =5.78 units hydrogen peroxide group and =4.19 in the carbamide peroxide group. When comparing the color of teeth homologous with the evolution of the color of teeth treated subjectively (Table 6), hydrogen peroxide can match the color of the tooth in the second week of treatment ($p>0.05$) compared with the carbamide peroxide that

achieved the matching in four-bleaching sessions, but the color regression for the first month and the third month showed homologous tooth difference ($p<0.05$). The color in hydrogen peroxide group remained stable with the tooth homologous until the third month ($p>0.05$). These results show that hydrogen peroxide (35% conc.) should be the recommended agent for this type of procedure in order to get faster results and greater stability over time.

Being a randomized clinical study, the protocol was standardized in four bleaching sessions with a walking bleach technique. The color of the adjacent tooth should be considered in order to customize treatments that meet this objective. For this reason, the color difference obtained for the teeth studied compared with the homologous teeth of even the hydrogen peroxide group in the fourth week of bleaching is lighter than the homologous. The color correlates with the color regression at week. Therefore, depending on factors specific to the teeth with discoloration, the preferences of the patient should be considered during the whitening technique and the number of sessions or repetitions. For the clinician, it is important to evaluate the success of the treatment based on the opinion of the patients, but does not necessarily by comparing the neighboring tooth. Alghazali et al. reported that the human eye has a threshold of perception of color ΔE, under controlled conditions [24] .

It is clear that the objective of non-vital teeth bleaching is to match the tooth color with the color of the other teeth, thus achieving a harmonious color; in spite of not achieving the exact or same color, patients are usually satisfied with the results. In a follow up study conducted on a number of cases of non-vital teeth whitening with different techniques, Glockner et al. considered 79% of the cases at five years successful; but when considering the opinion of the patients, the success rate

increased to 98% [21] . The extra-coronary bleaching vital tooth has a positive effect on the aesthetic perception of the patients and the psychosocial impact of patients [5, 6] ; In our study, similar results were obtained in the intracoronary bleaching, in which the positive effect lasts up to three months post-treatment, this reaffirms the importance of this "rehabilitative color" treatment of teeth color for people.

The aesthetic perception assessed with the questionnaire OHIP- aesthetics showed a positive effect that remained stable even when compared to the baseline up to three months in the sum of the score and the dimension of functional limitation and psychological discomfort, which accompanied longevity color. The other dimensions showed variations in the times thus proving that quality of life is complex and there are multiple factors influencing it; this correlate with those reported by Meireles et al. [4] .

The psychosocial impact questionnaire shows that the positive effect is maintained until the third month in all dimensions ($p<0.05$). Although not designed to evaluate the effect of bleaching, it shows a positive effect especially in the dimension of self-confidence.

This is reflected in the high rate of satisfaction among patients undergoing this type of bleaching, seeing how a minimally invasive procedure manage to solve an aesthetic problem affecting the quality of life with a stable result is impressive.

Conclusions

Both agents, hydrogen peroxide (35% conc.) and carbamide peroxide (37% conc.), are highly effective with walking bleach technique in non-vital teeth; the color remains stable up to three months. There is a positive impact on the aesthetic

perception and the psychosocial impact of the patients, as it is consistent over time.

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3.4 Publicação 4

3-month follow-up of the effect of non-vital bleaching on IL-1β and RANK-L*

Abstract

Objetive: It has been reported that bleaching generated an increase in osteoclast activity, “in vitro”. This randomized double-blind clinical trial aims to assess "in vivo" the effect of hydrogen peroxide 35% and carbamide peroxide 37% with the following of biomarkers: RANK-L and IL-1β, three months after bleaching with the technical Walking Bleach. Methods: A total of 47 volunteers with color change in a non-vital tooth with root canal treatment in good condition. 50 teeth were randomly divided into two groups (n=25) Study according to the whitening agent: G1= 35% hydrogen peroxide and carbamide peroxide G2= 37%. The intracameral bleaching was performed with the walking bleach technique for 4 sessions. Gingival fluid samples were taken to measure the IL-1β levels and RANK-L by ELISA test, samples were collected six periodontal sites (3 vestibular and 3 palatine) on 8 opportunities: at the beginning of the study (baseline), after 4 sessions of intracameral bleaching, a week, a month and three months post-treatment. The color change was evaluated at the same time visually with Vita bleach guide (ΔSGU). Results: Significant increases were found in IL-1β and RANK-L, comparing each evaluation time to baseline ($P<0.05$); there was no difference in IL-1β levels between month with the third month post-treatment ($p>0.05$). The

* Bersezio C, Vildósola P, Sánchez F, Sáez M, Vernal R, de Oliveira OB Jr, Fernández E. 3-month follow-up of the effect of non-vital bleaching on IL-1β and RANK-L. J Dent 2017. Under Revision.

color change was from 5 to G1 and 4 to G2, statistically significant difference ($p<0.05$) was found at all time points. Conclusion: The non-vital bleaching with Walking Bleach technique induces an increase in IL-1β and RANKL levels in periodontal tissues, which maintained until the third month post-treatment.

Introduction

The color change from a anterior tooth produces greater dissatisfaction widespread change coloration of the teeth whitening to this non-vital teeth has proven to be a very good option for minimally invasive treatment to rehabilitate the aesthetics of the smile (1,2).

Today the most common agents for tooth whitening are hydrogen peroxide (H_2O_2), sodium perborate and carbamide peroxide in high concentration, but the mechanism of action remains the same in all cases, that is oxidation of organic pigments by subproducts of H_2O_2 decomposition (1,2).

It has been reported a high success rate and patient satisfaction with this type of procedure (3-5), but no randomized clinical trials with patient follow-up on possible adverse effects the literature has reported that a possible adverse effect of intracoronary bleaching is the external cervical resorption (2,6), which is presented with a low incidence (7,8). The literature has extensively reported that the high oxidizing power of hydrogen peroxide can alter the histological and morphological properties of the tooth structure (9-11), has been activation of metalloproteins in the tooth structure (12), the cytotoxicity generate free radicals in the periodontal cells and increased pH extra-root (13). In addition, a recent in vitro study showed that bleaching generated an increase in osteoclast activity (14).

This suggests that bleaching of non-vital teeth also have an effect on levels of inflammatory markers such as interleukin-1β (IL-1β) and bone destruction as the Receptor Activator for Nuclear Factor Kappa β Ligand (RANK-L), which it has been associated with the regulation of the process of root resorption (15,16).

The aim of the study was to evaluate "in vivo" the effect of hydrogen peroxide 35% and carbamide peroxide 37% in periodontal markers (IL-1β and RANK-L) levels at 3 months post-bleaching with Walking Bleach technique.

Our working hypothesis is that the "Walking Bleach" technique generates an increase in baseline levels of IL-1β biomarkers and RANK-L, which would remain stable at 3 months post-treatment.

Material and method

A randomized double-blind trial (patient and evaluator) approved by the Ethics Committee of the Faculty of Dentistry at the University of Chile was conducted (2016/04).

Sample size: To determine the sample size the G-Power 3.2 program was used, considering a significance level of 5%, a statistical power of 80% and 15% of abandonment.

It has made an invitation to attend dental school and through social networks like Facebook and Twitter. A total of 74 volunteers were examined to assess if they met the criteria for inclusion and exclusion. 47 volunteers were selected for the study with a total of 50 non-vital teeth discoloration that met criteria.

Inclusion criteria: patients older than 18 with one or more non-vital teeth color change of A2 or higher, without vestibular restoration covering the middle third of the tooth, endodontic treatment should be in good condition, should not present apical lesion or peri-radicular pathology, and no prior bleaching experience.

Exclusion criteria: patients who are pregnant or lactating, with hypoplasia of enamel, with tetracycline staining or fluorosis, in orthodontic treatment with fixed appliances, patients with cancer or periodontal disease or in the presence of caries lesions.

The volunteers were examined clinically and radiographically to see the presence caries, periapical lesions, external or internal root resorption, or periodontal disease, should find something patients were informed and referred to a specialist for treatment.

Two study groups were formed according to the bleaching agent used, were randomized with Excel 2010 (Microsoft, Seattle, USA) software, each group with a n=25 teeth:

G1= Teeth bleaching with Hydrogen Peroxide 35% (Opalescence Endo, Ultradent, USA).

G2= Teeth bleaching with Carbamide Peroxide 37% (Whiteness Superendo, FGM, Brazil)

Previous to the beginning of the study patients signed an informed consent approved by the ethics committee of the faculty of dentistry at the University of Chile. They received prophylaxis with a slurry of pumice and water. In addition, oral hygiene instruction was made to standardize oral conditions of each volunteer and maintain periodontal health status baseline.

Bleaching Procedure

The application of the bleaching agent was made according to the manufacturer's instructions in 4 sessions with an ambulatory technique (Walking Bleach); each session was one week between each.

Preparation session: The root canal was prepared with absolute isolation with rubber dam, endodontic sealing was removed 3 mm from the limit amelo-cemetary. The final seal was improved with resin-reinforced glass ionomer (Riva Light Cure, SDI, Australia) of 2 mm thickness and light cured for 60 seconds (Cal Radii, SDI, Australia) glass ionomer. Radiographic Control was taken to corroborate the seal.

Four bleaching sessions: The application of the bleaching agent was performed according to manufacturer's instructions. An amount of bleaching gel was left in the pulp chamber in the presence of moisture (Walking Bleach Technique). The cavity was sealed with a temporary cement (Fermin, Detax, Germany) until the next session.

Restoration sessions: The access cavity was washed with water, and temporarily sealed for 7 days before the final restoration with composite Brilliant NG (Coltène-Whaledent AG, Switzerland).

Patients were instructed to avoid foods that have elements that could stain your teeth like coffee, tea, wine, etc.. during the study. Additionally hygiene Instruction was conducted to maintain oral health. They were given the instructions in writing and contact information for any questions or problems.

Color Evaluation

Two evaluators calibrated with an agreement of 80% (Kappa test) tooth color they recorded visually, prior to treatment (baseline), immediately after each whitening session, one week, one month and 3 months after treatment ends. Color evaluation was in the middle third of the tooth in the vestibular surface of the tooth bleaching according to the recommendations of the ADA (17). Patients were examined under the same conditions independently by each evaluator, using the color scale Vita Bleach Guide (Vita Zahnfabrik, Bad Säckingen, Germany). In case of non present a correspondence in the evaluation, the two evaluators should reach a consensus in the presence of the patient. For each color on the scale I was assigned a numerical value in order to calculate changes color in scalar units (ΔSGU).

Quantification of IL-1β and RANK-L levels in gingival crevicular fluid (GCF)

Recollection of GCF sample

After isolating the tooth with a cotton roll, supragingival plaque was removed with a curette Gracey 3/4 without touching the marginal gingival. The crevicular site was dried gently with air from the triple syringe without pointing directly to the groove, the FCG was collected using paper strips (Periopaper, OraFlow Inc., New York, USA), this was introduced in the periodontal sulcus until mild tissue resistance and remained in position for 30 seconds. The paper strips contaminated with saliva or blood were discarded. GCF samples were obtained in 6 periodontal sites: 3 vestibular and 3 palatine (mesial, middle and distal). The samples were taken before bleaching (baseline), after each of the four bleaching session, and week, one and three months after treatment. Then the sampling GCF, the Periopaper was stored in eppendorf tubes. It has been made a centrifugal

elution in 100 µl buffer saline with 0.05% Tween-20 (Fluka, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland), and centrifuged at 10,000 xg for 5 minutes at 4 ° C. The elution centrifuge process was repeated 2 times elution, samples obtained were stored at -80 °C until analysis.

Quantification of RANKL and IL-1β

The total protein levels were quantified using the Bradford system (R & D Systems Inc., Minneapolis, USA) and from 100 ul of eluted shows the levels of RANK-L and IL-1β were measured by ELISA test (Quantikine®; R&D Systems Inc., Minneapolis, USA) following the manufacturer's protocol. Absorbance was measured at 450 nm with correction at 540 nm for RANKL and 620 nm for IL-1β, using an automated plate reader (ELx800 Microplate Reader Universal, Bio-Tek Instruments Inc., Winooski, VT, USA). The concentration of each marker in each sample was calculated by a four-parameter logistic equation.

Results

Of the 74 patients evaluated, of which 47 were recruited with a total of 50 teeth discolouration. At 3 months, 42 patients completed treatment and met the controls, making a final sample of 44 teeth (Fig. 1) was obtained. Table 1 shows the characteristic of the final sample.

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

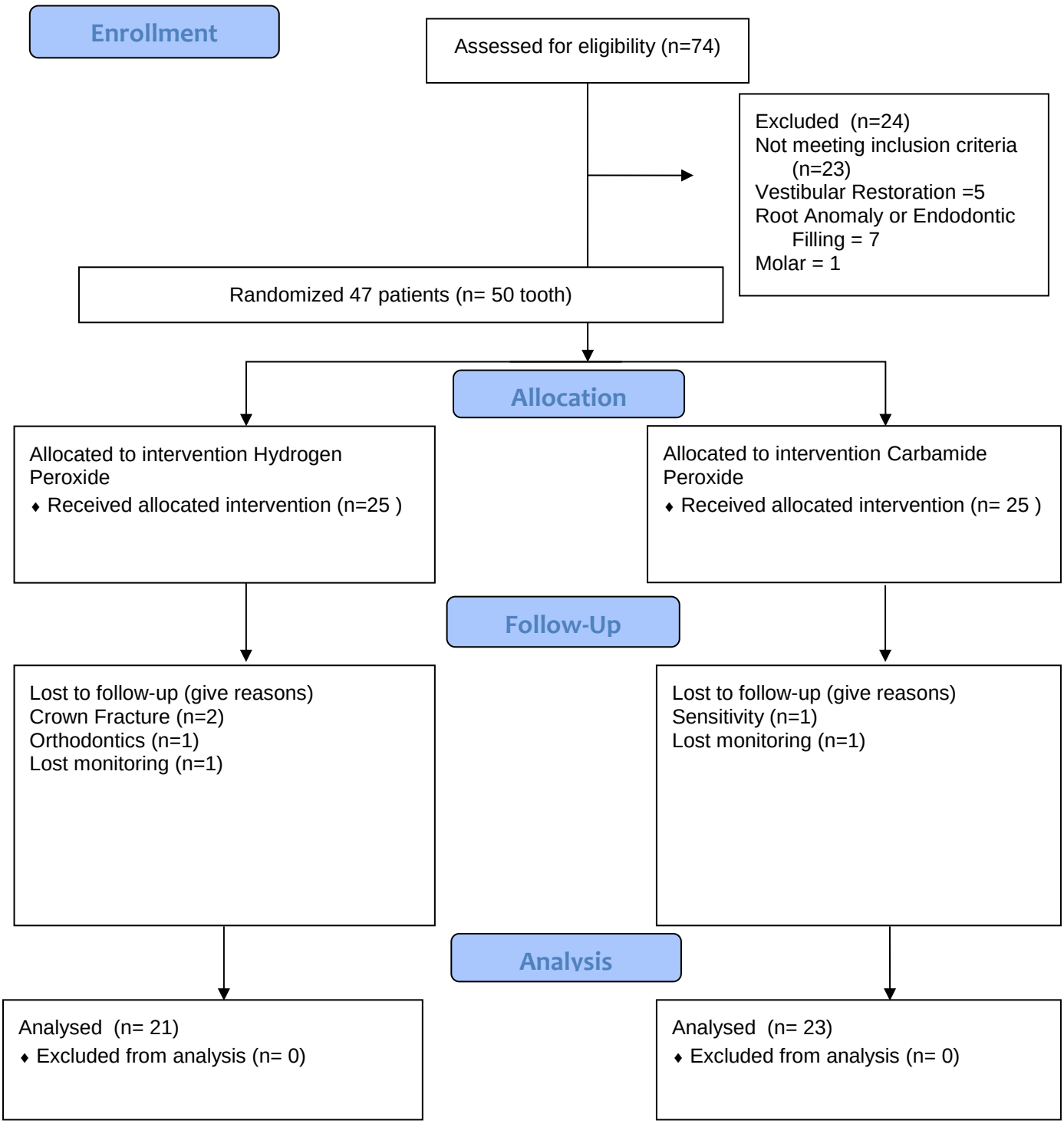

Table 1. Participant baseline characteristics

Baseline features	Groups	
	Hydrogen Peroxide	Carbamide Peroxide
Age (years; mean ± SD)	30.24 ± 12.26	30.83 ± 11.25
Minimum age (years)	19	20
Maximum age (years)	65	65
Male (%)	42.9	39.1
Trauma (%)	52.38	39.13
Caries (%)	47.62	60.87
Baseline Vita Bleach SGU median (min ; max)	15 (5 ; 16)	12 (7 ; 16)

SD = standard deviation

Subjective measurement of color

The color change was measured subjectively and expressed in units ΔSGU with bleach scale guide. Statistically significant difference ($p<0.05$) was found with the Mann Whitney test at all times evaluated, showing a greater color change in the group Hydrogen Peroxide. (Table 2)

Table 2. Comparison of ΔSGU values at different times using the Vita Bleach scale

Assessment points	Color change by ΔSGU		
	G1=Hydrogen Peroxide	G2=Carbamide Peroxide	Mann-Whitney test
Baseline vs. 1-wk bleaching	3 (0:5)	1 (0:4)	**0.032**
Baseline vs. 2-wk bleaching	4 (0:8) [a]	3 (0:5) [a]	**0.001**
Baseline vs. 3-wk bleaching	5 (0:9) [a]	4 (1:5) [a]	**0.004**
Baseline vs. 4-wk bleaching	6 (1:9) [a]	4 (2:8) [a]	**0.011**
Baseline vs. 1-wk after bleaching (before restoration)	5 (1:9)	4 (2:7)	**0.025**
Baseline vs. 1-wk after bleaching (after restoration)	5 (1:9)	4 (2:7)	**0.027**
Baseline vs. 1-mth after bleaching	5 (1:9)	4 (2:7)	**0.023**
Baseline vs. 3-mth after bleaching	5 (0:7)	4 (2:7)	**0.024**

[a] Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus previous time point.

IL-1β levels and RANKL

Six sites per tooth with a total of 1008 samples for the group of hydrogen peroxide and 1104 for carbamide peroxide were measured. Table 3 shows the levels of IL-1β expressed in pg/ul. All evaluation times were statistically significant difference between them until the month post-bleaching ($p<0.05$) with the Wilcoxon test. All evaluation moments had a statistically significant difference ($p<0.05$), except for the month to the third month after treatment ($p>0.05$). After the month are stabilized values until the third month post-treatment. There was no difference between the two groups ($p>0.05$) with Mann Whitney test.

Table 3. IL 1 β expressed in pg/ul, Median (min ; max)

Assessment points	Hydrogen Peroxide	Carbamide Peroxide	ΔPH	ΔPC	Mann Whitney
Baseline	92.90 (20.67 ; 206.01)	98.47 (30.65 ; 279.54)			
1-wk bleaching	95.86 (16.34 ; 217.53) [ab]	103.53 (33.41 ; 298.32) [ab]	2.83 (-1.69 ; 17.32)	2.75 (-1.91 ; 30.03)	0.330
2-wk bleaching	100.00 (17.54 ; 231.27)[ab]	111.75 (37.54 ; 298.23) [ab]	9.97 (0.77 ; 35.36) [a]	9.29 (0.30 ; 57.92) [a]	0.333
3-wk bleaching	107.53 (20.14 ; 254.36) [ab]	127.51 (42.36 ; 303.64) [ab]	20.69 (2.75 ; 73.01) [a]	18.87 (0.49 ; 107.03) [a]	0.269
4-wk bleaching	122.28 (25.21 ; 301.16) [ab]	141.35 (48.81 ; 325.11) [ab]	36.30 (7.86 ; 147.22) [a]	35.09 (3.12 ; 177.58) [a]	0.419
1-wk after bleaching	142.25 (35.52 ; 371.71) [ab]	160.82 (55.24 ; 352.13) [ab]	60.00 (13.10 ; 201.01) [a]	65.49 (9.91 ; 222.92) [a]	0.623
1-mth after bleaching	159.04 (51.51 ; 436.76) [ab]	182.12 (61.12 ; 391.12) [ab]	78.59 (21.79 ; 237.21) [a]	82.22 (16.47 ; 254.03) [a]	0.524
3-mth after bleaching	133.06 (18.75 ; 405.82) [b]	145.49 (16.13 ; 435.21) [b]	68.95 (-143.46 ; 212.50)	59.99 (-123.63 ; 276.25) [a]	0.910

Δ (Assessment points vs baseline)
[a] Statistically significant difference intragroup
(Wilcoxon test, $p<0.05$) versus previous time point.

Table 4 shows the levels of RANK-L expressed in pg/uL, considering all sites. All evaluation moments had a statistically significant difference with baseline ($p<0.05$) with the Wilcoxon test. All evaluation moments had a statistically significant difference ($p<0.05$). There was no difference between the two groups ($p>0.05$) with Mann Whitney test.

Table 4. RANK-L expressed in pg/ul, Median (min ; max)

Assessment points	Hydrogen Peroxide	Carbamide Peroxide	ΔPH	ΔPC	p
Baseline	12.31 (3.39 ; 30.35)	13.74 (4.42 ; 27.84)			
1-wk bleaching	12.46 (3.25 ; 31.39)	13.70 (4.36 ; 27.76)	0.51 (-2.61 ; 2.80)	0.52 (-0.88 ; 4.77)	
2-wk bleaching	13.74 (3.48 ; 32.38)	15.48 (4.47 ; 28.64)	1.82 (-0.88 ; 6.57)	1.69 (-0.70 ; 9.24)	
3-wk bleaching	16.05 (4.83 ; 37.29)	17.37 (6.13 ; 31.27)	4.09 (-0.47 ; 13.81)	3.29 (0.18 ; 13.19)	
4-wk bleaching	18.87 (5.53 ; 44.23)	19.69 (8.31 ; 37.42)	7.88 (0.30 ; 22.76)	6.37 (1.72 ; 18.19)	
1-wk after bleaching	22.48 (6.18 ; 53.42)	24.59 (9.67 ; 47.26)	11.89 (1.91 ; 37.67)	11.09 (2.61 ; 29.83)	
1-mth after bleaching	25.42 (8.47 ; 60.31)	27.75 (10.34 ; 53.84)	13.89 (0.80 ; 46.45)	14.09 (4.18 ; 36.41)	
3-mth after bleaching	27.56 (10.18 ; 68.05)	29.53 (11.27 ; 63.61)	17.08 (0.17 ; 43.48)	16.73 (4.83 ; 41.10)	

Δ (Assessment points vs baseline) * Statistically significant difference intragroup (Wilcoxon test, $p<0.05$) versus previous time point.

Discussion

Intracoronary bleaching is a highly effective minimally invasive procedure, because it allows solving aesthetic problems keeping the tooth remaining teeth and achieving high satisfaction among patients (5). Our results also show high effectiveness in color change average four to five units ΔSGU. Despite this, it is not a completely safe process has been widely reported cytotoxicity of Hydrogen Peroxide (18-21). Already it reported an increased proliferation and resorptive activity of osteoclasts after bleaching (14).

Our study is the first randomized clinical study to evaluate the biological effect of intracoronary bleaching, according the results obtained this causes an increase until the third month post-bleaching levels of RANKL, of the RANK-RANKL-OPG system, regulates bone metabolism (22). Furthermore, we found a significant increase in pro-inflammatory cytokine (IL-1β), to a post-bleaching month, values which have a tendency to decrease for the third month post-treatment, but have no significant difference with the post month -treatment. The very active inflammatory process itself RANK-L expression, the IL-1β markers are more sensitive than RANK-L, so it is logical to reduce first. It is possible that there should be a decrease in the level of RANKL if IL-1β levels begin to descend.

When comparing both gels (Table 2), hydrogen peroxide has a greater effectiveness than carbamide peroxide in all times evaluated until the third month post-bleaching ($p<0.05$ with Mann Whitney test). When comparing the different times, in both groups there is no difference from the post-bleaching week with the previous time ($p>0.05$ with Wilcoxon test), suggesting that the color change remains stable until the third month, this is consistent with previous studies that

showed high stability in color non-vital teeth bleaching (3,4). The greater effectiveness of hydrogen peroxide may be due to the greater availability the same peroxide, as carbamide peroxide is to be decomposed, reaching a lower concentration of hydrogen peroxide is the bleaching active agent.

There is no relationship between the effectiveness of bleaching with increased levels of markers (IL-1β and RANK-L), and that when comparing both groups no significant difference between them ($p>0.05$ with the Mann Whitney).

The levels found did not reach the levels of active periodontal disease (23,24), but levels may be sufficient to generate some alteration in individuals most susceptible, because there is great variability in the inflammatory response. Patients with a history of periodontal disease may be more susceptible to the imbalance of these markers, which would generate a contraindication for this type of treatment was not described in the literature.

IL-1β is a potent osteoclast activity initiator (25) and generally stimulates macrophages, endothelial monocyte among others to produce metalloproteinases, prostaglandins and other pro-inflammatory cytokines (26,27). Also it stimulates the osteoblasts to produce RANKL, causing differentiation and maintenance of osteoclasts (28). Another important aspect in relation to non-vital teeth are the external cervical resorption (2), which despite having a low incidence is described as a possible adverse effect of intracoronary bleaching.

The cause is not completely described, and these results may be a clue to how the resorption process is triggered, since the RANK-RANKL-OPG axis also regulates the process of root resorption (15,16,29,30). We must consider that there is a high anatomic variability between teeth endodontically treated, anatomical defects that could lead to greater diffusion of peroxide into the root space, generating an

inflammatory process that could become chronic and culminate in a resorptive process. The Walking Bleach technique, is a procedure with closed chamber (2), wherein the chemical agent is in contact for prolonged tooth time and being closed chamber pressure favors the diffusion of peroxide to the extra-root space, which causes more of the markers studied. It is recommended to study techniques to assess whether open chamber would have less biological effect.

The non-vital bleaching with hydrogen peroxide 35% or carbamide peroxide 37% generates an imbalance in the levels of RANK-L and IL-1β in gingival fluid cervicular to 3 months after treatment. Both groups showed no significant difference. The effectiveness of bleaching is higher for the group with hydrogen peroxide, both groups maintained the stability of the color to 3 months.

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4 CONSIDERAÇÕES FINAIS

Nessas quatro pesquisas clínicas autorizado pela Comissão de Ética Científico da Faculdade de Odontologia da Universidade do Chile (Anexo 2), foram realizadas para estudar o efeito da técnica WB com concentração reduzida de peroxido (PH13) foi encontrado que PH13 tem eficácia estética final tão boa quanto o tratamento com peroxido de alta concentração (PH35) e impacta positivamente a auto-percepção estética e a qualidade de vida dos participantes. No entanto, tanto PH35, quanto PH13, provocam aumento nos níveis de IL-1β e RANK-L do fluido crevicular, demontrando efeito sub-clinico com potencial de reabsorção óssea e/ou radicular, que se mantem alterado após 3 meses de acompanhamento.

Muito embora o produto mais utilizado na técnica tradicional de clareamento WB seja o perborato de sódio, [27] nos estudos realizados neste projeto, decidimos adotar como grupo controle o gel de maior concentração de peróxido (PH35 em função do estudo in vitro conduzido por Lim, que concluiu que o peróxido de carbamida de 37%, que origina o PH13 e o PH35 são mais eficazes para clarear os dentes escurecidos por tratamento endodontico do que o perborato de sódio. [27]

Já foi demonstrado que o clareamento dental pode ser considerado eficaz, a partir de uma diferença minima de de 5 unidades de ΔE a partir da cor inicial. [28] Após 4 sessões da tecnica WB, nossos resultados mostraram uma redução do escurecimento dental da ordem de 15,48 ± 5,17 unidades de ΔE para PH35 e 14,02 ± 4,85 para PH13, o que é altamente significativo e se mantem nos 3 meses após o tratamento.

Devido as difereças de concentração e ao uso do peróxido de carbamida como precursor do PH13 na formulação de concentração reduzida, a cinética de clareamento durante o tratamento foi diferente. PH35 induziu efeito clareador mais rapido e evidente logo na primeira sessão de clareamento, que evolui pouco mas progressivamente nas 3 sessões subsequentes de tratamento, enquanto que no grupo PH13 a cinetica foi mais gradual Nas avaliações realizadas após o final do tratamento (1 mês e 3 meses após), a eficácia estética de PH35 e PH13 foi estatísticamente semelhante.

As diferenças na cinética inicial de clareamento devem-se, provavelmente, as diferenças quimicas entre os dois produtos clareadores testados. O gel de alta concentração (PH35), é composto somente por peroxido de hidrogênio e espessante. A molécula de peroxido de hidrogênio tem baixo peso molecular e, devido a isso, permeia facilmente atraves dos canaliculos dentinários e pelo espaço interprismático, atuando rápida e diretamente sobre os cromógenos que estão comprometendo a estética dental.

Por sua vez, o gel de concentração reduzida (PH13), é composto por peroxido de carbamida a 37% e espessante. Para ter ação clareadora, este produto primeiro precisa ser catalizado por ação térmica e/ou enzimática, dessociando-se em ureia, agua e peroxido de hidrogênio [29, 33] para depois ter ação clareadora, que é determinada pela quantidade de peróxido dissociado. Devido a isso, o clareamento por este processo é mais lento.

A eficácia estética também foi avaliada pelo indice visual (ΔSGU). Cabe ressaltar que nos nosso estudos a avaliação visual foi realizada por 2 examinadores previamente treinados e calibrados (k=0.85), o que possibilitou que os resultados observados neste estudo fossem, no geral, semelhantes aos

registrados na analise instrumental objetiva realizada com espectrofotometro de reflectancia Vita Easyshade, apesar das limitações de acurárica e reprodutibilidade inter e intra-avaliadores deste tipo de analise. [14, 23, 28, 36]

Cabe ressaltar que estes resultados não validam a substituição da avaliação objetiva instrumental pela avaliação subjetiva visual em estudos de clareamento dental. Nós acreditamos que a avaliação subjetiva visual possa ser utilizada como analise complementar a avaliação instrumental e sirva para verificar se os achados da avaliação instrumental podem também ser clinicamente percebidas por profissionais e pacientes. [5, 12, 26, 31, 46]

Também foi observado que o parametro L* (luminosidade) do sistema de cor CIELab, apresenta maior relevancia para a discriminação de cor em estudos de clareamento dental. Isto é explicado porque o olho humano tem maior ocurácia para detectar variações de luminominosidade (tons de cinza) do que para discriminar variações de matiz e croma, chegando a apresentar desempenho semelhante ao obtido pela avaliação instrumental realizada com espectrofotometros. [17]

A manutenção dos resultados estéticos após 3 meses do tratamento indica que o clareamento realizado pela tecnicas WB com PH13 apresenta potencial de longevidade. Estudos publicados têm demonstrado um sucesso do clareamento WB de 79% dos casos, após 5 anos de acompanhamento [16] e 62,9% após 16 anos do tratamento[1].

Devido a alta eficácia estética demostrada tanto pelo PH13 como pelo PH35, não foi surpresa o o alto grau de satisfação com a estética final evidenciado pelos resultados dos questionários PIDAQ e OHIP Estetico, [3] mesmo levando em consideração que a alta eficácia estética observada não representou, em sua

maioria, a exata correspondência de cor entre entre o dente clareado e a cor natural dos dentes adjacente.

Mesmo com esta limitação da tecnica WB, notou-se efeito psicosocial positivo na percepção estética dental e na qualidade de vida dos participantes. Este benefício foi mantido até 3 meses do final do tratamento clareador.

A manutenção deste efeito psicosocial positivo já havia sido observado anteriormente em pacientes submetidos ao clareamento de dentes vitais, [12] mas foi pela primeira vez comprovado para a tecnica WB no presente estudo.

Embora o questionário PIDAQ não tenha sido desenhado para pacientes submetidos a clareamento dental, sua aplicação neste estudo pode evidenciar melhoria na auto percepção da estética dental ao longo das consultas de avaliação para ambos os agentes clareadores. Todas as dimensões psicosociais analisadas pelos dois questionários, foram impactadas positivamente pelas técnicas WB utilizadas.

O PIDAQ também indicou um aumento da auto-confiança do paciente, estatisticamente significativo, o que reflete os benefícios psico-emocionais promovidos pela técnica minimamente invasiva de clareamento WB. [12]

O questionário OHIP-Estético também mostrou um efeito positivo sobre a qualidade de vida e auto-percepção estética dos pacientes. Estes resultados são apoiados por outros estudos,[23,12] que avaliaram a influência da técnica de clareamento de dentes vitais na auto-percepção estética dos pacientes. Os resultados mostraram um efeito positivo, inclusive após 9 meses de tratamento.

Não houve diferenças nas pontuações de OHIP-Estética entre os grupos, mas houve diferenças progressivam em função do tempo, resultando em um efeito

mais pronunciado e positivo para o PH35. o que pode ser explicado pelo efeito mais rápido na modificação da luminosidade e da variação total de cor (ΔE).

O peróxido de hidrogênio utilizado nas técnicas de clareamento dental não é um agente inocuo, já que tem um alto potencial citotóxico. [6,7,10] Estudos recentes também demonstram que as técnicas de clareamento provocam um aumento na atividade de citoquinas inflamatórias e metaloproteinases.[40,43] Na tecnica WB, devido ao seu baixo peso molecular, o peroxido de hidrogênio tem alta capacidade de permear os tecidos dentais e se exteriorizar a partir da camara pulpar em direção a superfície da estrutura dental. [29, 30, 34, 37, 38] Nossos resultados mostram que o clareamento WB possui um efeito biológico que provoca aumento nos níveis de IL-1β e RANK-L, comparado com o observado antes da realização do clareamento dental. Este aumento já é observado a partir da primeira sessão de clareamento e se mantem persistente após o final do tratamento. (1 e 3 meses após). Estes achados são suportados pelo estudo de Torres-Rodriguez et al.,[44] mostrou aumento da atividade osteclástica provocado por clareamento dental em estudo in vitro.

Os níveis encontrados não aumentam ao nível de doença periodontal ativa, [4, 15] mas podem indicar a ativação do processo de reabsorção óssea em pacientes mais suscetíveis ou em risco periodontal, além de ser um possível gatilho de RCE ou então de predisposição da reabsorção óssea marginal (ROM). Todas estas hipoteses precisam ser testadas em estudos futuros e pelo segmento deste grupo de voluntários por mais tempo.

A IL-1β é uma importante citocina pro-inflamatória, além de ser um potente iniciador da atividade odontoclástica.[45] A expressão desta proteina tem sido associada com a reabsorção radicular devido ao tratamento ortodôntico.[21] Neste

estudo, foi encontrado um aumento significativo e persistente nos níveis desta citocina demonstrando que a tecnica WB desencadeia um processo inflamatório,[11] que pode ser sub-clínico. Por sua vez, este processo inflamatório pode ser a causa do aumento nos níveis de RANK-L observados.[42]

Ao isolar as causas que determinaram a necessidade do tratamento endodontico (traumatismo ou cáries) não se encontrou diferença entre os níveis de IL-1β e RANK-L para os dentes considerados nestes estudos, confirmando que o aumento na expressão dos marcadores de inflamação e reabsorção foram determinados pelo clareamento WB.

Os niveis de IL-1β e a RANK-L expressados devido a ação de PH35 e PH13 foram semelhantes e igualmente persistentes, demonstrando que a redução de peroxido utilizada não foi suficiente para evitar os possiveis efeitos deletérios do uso do peroxido de hidrogênio no interior da camara pulpar. Além disso, o mecanismo que desencadeia a expressão destes marcadores ainda não é conhecido e pode não ser dependente da concentração do peroxido utilizado, e sim de outro fator não considerado neste estudo. A manutenção da expressão de IL-1β e RANK-L em niveis aumentados após 3 meses de acompanhamento foi um resultado inesperado a ainda não compreendido. Pode ser que indique a instalação de um processo crônico ou efeito deletério permanente na estrutura dental. Estas hipoteses poderão ser confirmadas com o seguimento dos nossos estudos. É esperado que estes marcadores retornem aos niveis observados antes do tratamento WB no controle de 6 meses.

Dentro das limitações desses estudos, as diferentes causas da desvitalização dos dentes não poderam ser padronizadas; alguns dentes precisaram receber tratamento endodontico devido a trauma dental e outros foram comprometidos

por cárie dentária. Outra limitação é que não foi constituido nenhum grupo controle negativo, no qual os pacientes receberiam um tratamento clareador placebo. Assim, os efeitos da simples realização de um tratamento, independente de sua eficácia não puderam ser mensurados pelos questionários PIDAQ e OHIP-Estética.

Os nossos dados confirmam que a qualidade de vida é complexa, multifatorial e relevante para a saúde, uma vez que esta condição é definica pela OMS como "um estado de completo bem-estar físico, mental e social e não somente ausência de afeções e enfermidades". Assim, os estudos de eficácia e desempenho deveriam sempre também avaliar o impacto de produtos e técnicas sobre as diferentes dimensões de qualidade de vida dos pacientes uma vez que este é um componente é fundamental para a promoção da saúde.

Por outro lado, o aumento e persistência da expressão dos marcadores de inflamação e reabsorção são uma preocupação adicional que deve ser considerada quando se indica a realização do clareamento dental pela técnica WB.

5 CONCLUSÃO

O clareamento WB com peróxido de hidrogênio de 35% (PH35) ou com o peróxido de carbamida de 37% (PH13) causa um desequilíbrio nos níveis de RANK-L e IL-1β do fluido crevicular que persiste até 3 meses pós-tratamento, independente da concentração do peróxido utilizado. Ambos agentes clareadores utilizados na tecnica WB (PH35 e PH13), são altamente eficazes para reduzir a discrepancia de cor de dentes escurecidos devido ao tratamento endodontico. Em função disso, as duas concentrações testadas impactam positivamente nos fatores psicosociais de qualidade de vida avaliados por PIDAQ e OHIP Estético.

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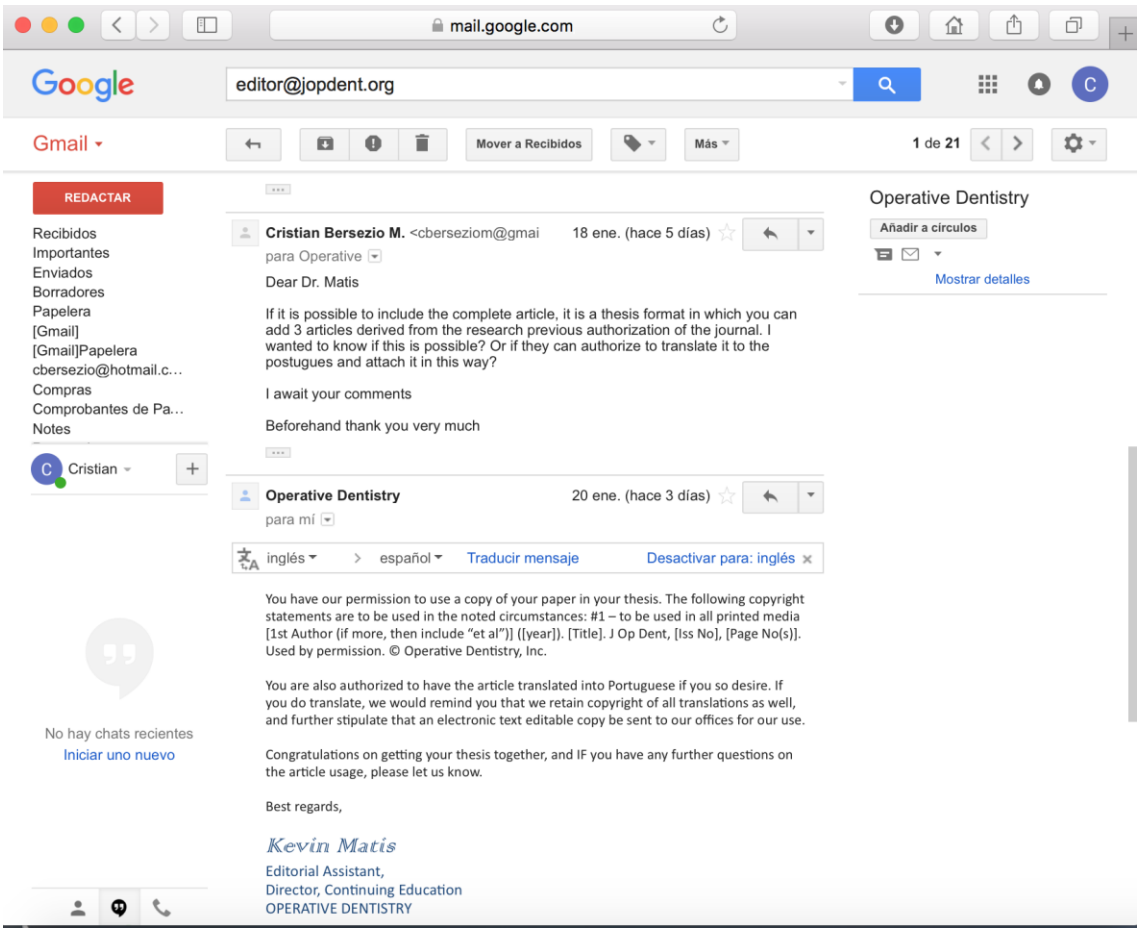
ANEXOS

Anexo 1: Autorização de Operative Dentistry para adicionar artigo para a tese



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Anexo 2: Certificado de aprovação do Comitê de Ética

FACULTAD DE ODONTOLOGÍA UNIVERSIDAD DE CHILE | COMITÉ ÉTICO CIENTIFICO

Ed 29/01/2016

ACTA DE APROBACION DE PROTOCOLO DE INVESTIGACIÓN

Dr.Eduardo Fernández Pdte./ Dr.Marco Cornejo / Dr. Rodrigo Cabello/ Dr.Mauricio Baeza/ Sra. Paulina Navarrete/ Sr. Roberto La Rosa

ACTA N°: 2

1. **Acta De Aprobación De Protocolo De Estudio N° 2016/04**

2. **Miembros del Comité Ético-Científico de la Facultad de Odontología de la Universidad de Chile participantes en la aprobación del Proyecto:**

Dr. Eduardo Fernández Godoy Presidente CEC	**Sra. Paulina Navarrete** Secretaria Ejecutiva CEC	**Sr. Roberto La Rosa** Miembro permanente CEC
Dr. Rodrigo Cabello Ibacache Miembro permanente CEC	**Dr. Marco Cornejo Ovalle** Miembro permanente CEC	**Dr. Mauricio Baeza Paredes** Miembro permanente CEC
Dr. Alfredo Molina Miembro Alterno CEC	**Dra. Patricia Hernández** Miembro Alterno CEC	**Dra. Paola Llanos** Miembro Alterno CEC

3. **Fecha d Aprobación: 29 de Enero de 2016**
4. **Titulo completo del proyecto:** "Niveles de RANKL-OPG extraradicular y Efectividad del Blanqueamiento Intracoronario en Dientes No Vitales"
5. **Investigador responsable:** Cristian Bersezio Miranda
6. **Institución Patrocinante:** Universidad de Chile
7. **Documentación Revisada:**
   - Proyecto
   - Consentimiento Informado (CI)
   - Currículo del investigador responsable y Coinvestigadores
   - Nómina de los coinvestigadores y colaboradores directos de la investigación.
   - Carta de aceptación de la autoridad administrativa de la Clínica Odontológica donde se realizará el estudio.

8.- Carácter de la población: Los sujetos que serán invitados a participar de este estudio pertenecen a la población consultante de la Clínica Odontológica de la FOUCH.

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9.- Fundamentación de la aprobación

En los últimos años la demanda por odontología estética ha aumentado enormemente y dentro de los aspectos más considerados por la población se encuentra el color de los dientes. La decoloración o el oscurecimiento de una pieza unitaria en el sector anterior, genera una insatisfacción mayor que el oscurecimiento generalizado de los dientes, ya que atrae más la atención del observador generando una mayor inconformidad a la persona. El color del diente se ve determinado por las propiedades de la dentina y del esmalte dentario y se ve modificado por el efecto combinado de coloraciones extrínsecas e intrínsecas.

El blanqueamiento de dientes tratados con endodoncia o no-vitales, que presentan alguna alteración de color, es una alternativa conservadora para mejorar la estética, en comparación con tratamientos más invasivos, tales como la colocación de coronas o carillas Los agentes blanqueadores comúnmente usados para el blanqueamiento de dientes no-vitales son peróxido de hidrógeno, peróxido de carbamida y perborato sódico.

El blanqueamiento es un procedimiento seguro y conservador, pero no deja de tener posibles efectos adversos, tanto localizados como sistémicos. Dentro de los efectos localizados están los que afectan a los tejidos blandos y duros, entre los más comunes se encuentra la sensibilidad dentaria además se han reportado efecto en las propiedades mecánicas y en la resistencia de unión de los materiales restauradores. Otro efecto adverso reportado en los tratamientos de blanqueamiento de dientes no-vitales es la reabsorción radicular externa, que es una respuesta inflamatoria que puede ocurrir en la región cervical externa de las raíces, generalmente asociado al blanqueamiento con altas concentraciones de Peróxido de Hidrógeno en combinación con calor en una técnica termocatalítica. El proceso de la reabsorción envuelve una compleja interacción entre células inflamatorias, células reabsortivas y las estructuras de los tejidos duros. Las células responsables de la reabsorción del tejido duro dental son los odontoclastos, estas son células multinucleares cuya morfología y mecanismo de acción son similares a los osteoclastos. Actúan como macrófagos específicos, como células inflamatorias especializadas en todo tipo de reabsorción dentaria. Aparecen sobre las estructuras mineralizadas de los dientes definitivos solamente en condiciones patológicas.

Se ha reportado que la regulación del proceso de reabsorción de los tejidos dentarios esta mediado por el sistema RANK-RANKL-OPG similares al de la fisiopatología ósea. Este sistema de señales se basa en que el RANKL induce la activación del receptor RANK, constituyendo un sistema de segundo mensajero, el RANK provoca la activación de la diferenciación osteoclástica, la OPG funciona como un factor inhibidor de la osteoclastogénesis, actúa como un receptor señuelo neutralizando el RANKL. Por lo que se propone evaluar el efecto del blanqueamiento intracoronario en los niveles de marcadores de destrucción ósea como es el sistema RANK-RANKL-OPG, en diferentes tiempos (previamente al blanqueamiento, luego de las sesiones y

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controles), además de evaluar su eficacia como agentes blanqueadores en un contexto de un trabajo clínico randomizado.

Los miembros del Comité declararon que uno de sus miembros tiene conflicto de interés, por lo que no ha participado de la evaluación.

Los antecedentes curriculares del Investigador Principal garantizan la ejecución del Ensayo Clínico dentro de los marcos éticamente aceptables.

En consecuencia, el Comité Ético Científico de la Facultad de Odontología de la Universidad de Chile, Aprueba por unanimidad de sus miembros el estudio: "Niveles de RANKL-OPG extraradicular y Efectividad del Blanqueamiento Intracoronario en Dientes No Vitales"; bajo la conducción de Cristian Bersezio Miranda del Depto. Odontología Restauradora. Facultad de Odontología, Universidad de Chile.

El Dr. Bersezio asume el compromiso de enviar a este Comité cualquier enmienda realizada durante la ejecución del protocolo y una copia del Informe final de resultados. Este Comité se reserva el derecho de monitorear este proyecto si lo considera necesario y el investigador deberá, bajo mutuo acuerdo, presentar los antecedentes solicitados.

Dicho estudio se llevará a cabo en la clínica odontológica, dependiente de la Facultad de Odontología de la Universidad de Chile, bajo la supervisión de Cristian Bersezio como Investigador Principal.

Dr. Eduardo Fernández Godoy
Presidente Comité Ético Científico

UNIVERSIDAD DE CHILE
COMITÉ DE ETICA
FACULTAD DE ODONTOLOGIA

C/C.
Investigador Principal.
Secretaría C.E.C.

Autorizo a reprodução deste trabalho

Araraquara, 16 de novembro de 2016

Cristian Alejandro Bersezio Miranda