©Operative Dentistry, 2019, **44-6,** 581-588

Clinical Research

Six-month Follow-up of the Effect of Nonvital Bleaching on IL-1β and RANK-L: A Randomized Clinical Trial

C Bersezio • J Estay • M Sáez • F Sánchez • R Vernal • E Fernández

Clinical Relevance

The "walking bleach" technique induces an increase in IL-1β and RANKL production in periodontal tissues, which persists for six months after treatment.

SUMMARY

Objectives: It has been reported that bleaching generates an increase in the activity of osteoclasts *in vitro*. We quantified the RANK-L and IL-1β biomarkers in a double-blind, randomized clinical trial evaluating the *in vivo* effect of hydrogen peroxide (35%) and peroxide carbamide (37%) six months after whitening.

Methods and Materials: Fifty volunteers participated, each with color change in a nonvital tooth. Fifty teeth were randomly divided into two groups (n=25), and the teeth were bleached using either 35% hydrogen peroxide (G1) or 37% carbamide peroxide (G2). Intracoronal bleaching was carried out by a technical "walking bleach" over four sessions. Gingival crevicular fluid samples were collected and used to quantify the IL-1β and RANK-L secreted levels. Samples of six periodontal sites (three vestibular and three palatal) were collected for up to six months (at the beginning of the study [baseline] and at one week, one month, and six months posttreatment). The color change was visually monitored using the Vita Bleached Guide (ΔSGU).

Results: Comparing each time to baseline assessment, a significant increase in the levels of IL-1β and RANK-L across time points was detected ($p<0.05$). The color change was 4 in G1 and G2, and a statistically significant difference ($p<0.05$) was found at the month time point between the groups. Using the Spearman test, a strong correlation (>0.8)

Cristian Bersezio, DDS, PhD, University of Chile, Restorative Dentistry, Santiago, Chile

Juan Estay, DDS, PhD, Universidad de Chile, Restorative Dentistry, Santiago, Chile

Mildri Sáez, DDS, University of Chile, Restorative Dentistry, Santiago, Chile and Universidad Politécnica y Artística del Paraguay, Paraguay

Francisca Sanchéz, DDS, University of Chile, Restorative Dentistry, Santiago, Chile

Rolando Vernal, DDS, MsC, PhD, Conservative Dentistry Departament, University of Chile, and Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Santiago, Chile

*Eduardo Fernandez, DDS, DS, PhD, Restorative Dentistry, Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Providencia, Chile

*Corresponding author: 11 de septiembre 1881 of 2108, Providencia, Chile 7500505; e-mail: edofdez@yahoo.com

DOI: http://doi.org/10.2341/18-023-C

between the IL-1β and RANK-L levels in both groups at all time points was detected.

Conclusions: Nonvital bleaching using a technical walking bleach induces an increase in the IL-1β and RANKL production in periodontal tissues, which persists for six months after treatment. Both biomarkers were highly correlated in both groups and at all time points.

INTRODUCTION

Whitening of nonvital teeth has proven to be an effective, minimally invasive treatment capable of restoring the esthetics of smile in cases that affect the self-esteem of the patient and his or her social interactions.[1,2]

High concentrations of hydrogen peroxide (H_2O_2; >30%), sodium perborate, and carbamide peroxide are commonly used for whitening. Each of these bleaching agents operates on the same oxidation mechanism: organic pigment by-products from the decomposition of H_2O_2.[3]

These whitening procedures have demonstrated significant success and patient satisfaction.[4,5] To the best of our knowledge, however, no follow-up of patients from a randomized clinical trial has been undertaken to analyze the possible adverse effects of whitening, such as external cervical resorption.[6] This effect is relatively unusual, but it typically leads to tooth loss.[7,8] The high oxidizing power of hydrogen peroxide can modify the histological and morphological properties of the tooth structure,[9] activate matrix metalloproteinases,[10] result in cytotoxicity, produce free radicals, and change the pH.[11] Similarly, a recent *in vitro* study demonstrated that bleaching results in an increase in the activity of matrix metalloproteinases and osteoclasts.[12,13]

Taken together, these data suggest that nonvital tooth bleaching could affect the levels of inflammatory markers, such as interleukin-1β (IL-1β) and the bone resorptive factor called receptor activator of nuclear factor kappa-B ligand (RANK-L), which have been associated with the regulation of resorption of the root.[14,15] A direct relationship between these two biomarkers during intracoronal bleaching has not been reported yet. Moreover, there have been no longitudinal follow-up studies about the effects of these biomarkers.

The purpose of this investigation was to assess the *in vivo* effect of hydrogen peroxide (35%) and carbamide peroxide (37%) on the levels of periodontal markers (IL-1β and RANK-L) up to six months postwhitening and to determine whether there is any correlation between their levels.

We hypothesized that the "walking bleach" technique increases the levels of IL-1β and RANK-L, which remains stable for up to six months after treatment. Also, we hypothesized that there is no correlation between these biomarkers.

METHODS AND MATERIALS

This clinical trial was randomized, double-blinded (patient and evaluation), and approved by the Ethics Committee of the Faculty of Dentistry of the Local University. The participants were informed of the goals and risks of the treatment as well as their freedom to leave the study at any time. Clinical procedures were explained in detail, and the subjects of the study were required to provide informed consent according to the regulations of the institutional Ethics Committee of the Faculty of Dentistry of the Local University. This research was conducted in full accordance with the World Medical Association Declaration of Helsinki of 1975 (revised 2000) and independently reviewed and approved by a local ethics committee/institutional review board.

Sample Size

We used the program 3.2 G-Power to determine the sample size (power statistic of 80%, 5% significance level, 15% abandonment). Participants were invited via social networks (Facebook and Twitter) to visit the dental school. Seventy-four volunteers were examined to assess whether they satisfied the study criteria. Fifty patients were selected for the study, and a total of 50 (1 per patient) discolored nonvital teeth satisfied the study criteria.

The inclusion criteria were patients aged at least 18 years with one or more nonvital teeth with color change of A2 or higher, without vestibular restoration covering the middle third of the tooth, no apical lesions, and no prior experience with whitening.

Exclusion criteria included pregnant or breastfeeding patients, the presence of enamel hypoplasia, spots of tetracycline or fluorosis, orthodontic braces, periodontal disease, or active caries lesions.

The volunteers were examined clinically and radiographically to determine the presence of periapical lesions, internal or external root resorption, dental caries, or periodontal disease. If any of these conditions was detected, the participants were briefed and referred to a specialist for treatment.

We divided the patients into two groups according to the bleaching agent used (n=25); the patients were divided randomly using Microsoft Excel (Microsoft, Seattle, WA, USA). Patients in group 1 (G1) were treated with 35% hydrogen peroxide (Opalescence Endo, Ultradent, South Jordan, UT, USA), and patients in group 2 (G2) were treated with 37% carbamide peroxide (Superendo Whiteness, FGM, Brazil).

Participants received a pumice and water prophylaxis. They also received oral hygiene instructions to maintain and enhance periodontal health status.

Bleaching Protocol

The bleaching agent was applied according to the manufacturer's instructions over four sessions via an outpatient technique; each session was separated by one week.

Preparation Session—The root canal was prepared under rubber dam isolation. 3-mm of endodontic filling was removed apical to the cementoenamel junction (CEJ), and a sealing of 2 mm was applied using a resin-reinforced glass ionomer cement (Riva Light Cure, SDI, Bayswater, VIC, Australia).

The cement was cured for 60 seconds at a distance of approximately 1 cm, with a 1200-mW lamp intensity (Cal Raddi, SDI). Due to the italic s-shape of dentin tubules in the cervical region, and to guarantee the whitening of this area of the tooth, the coronal limit of the seal was located 1 mm below the CEJ. The proper sealing of the endodontic treatment was verified radiographically.

A small amount of whitening gel remained in the pulp chamber in the presence of moisture (walking bleach technique). The cavity was sealed with temporary cement (Fermín, Detax, Germany) until the next session.

During the whitening sessions, the access cavity was washed with water and temporarily sealed for seven days before the final restoration with composite Brilliant (Coltene Whaledent AG, Altstätten, Switzerland).

Patients were instructed to avoid foods that can stain teeth, such as coffee, tea, or wine.

Evaluation of Color

Tooth color was visually recorded by two independent evaluators (with a Kappa test agreement of 80%). This procedure was done before (baseline) treatment and at one week, one month, and six months after the treatment had concluded. The evaluation of color was done in the middle third of the tooth according to the recommendations of the American Dental Association.[16] The patients were examined under the same conditions independently by each reviewer, using the color scale Vita Bleachedguide (Vita Zahnfabrik, Bad Säckingen, Germany). In case of disagreement, the two referees reached a consensus in the presence of the patient. Each color on the scale was assigned a numeric value from which we could compute scalar color changes in units (ΔSGU).

Quantification of IL-1β and RANK-L Levels in Gingival Crevicular Fluid

After the tooth was isolated with a cotton roll, the supragingival plaque was removed using a Gracey 3/4 curette without touching the marginal gingiva. The gingiva was dried gently using air from a syringe, and the gingival crevicular fluid (GCF) was collected using paper strips (Periopaper, Ora-Flow Inc, New York, NY, USA) placed in the periodontal sulcus, and left in position for 30 seconds. Paper strips contaminated with saliva or blood were discarded. The GCF samples of six periodontal sites were obtained: three vestibular and three palatal (mesial, middle and distal), before the whitening treatment (baseline) and at one week, one month, and six months after the whitening treatment. The GCF samples were then eluted twice in 120 μL of buffer saline with 0.05% Tween-20 (Fluka, Sigma Aldrich Chemie GmbH, Buchs, Switzerland), centrifuged at 10,000 g for five minutes at 4°C, and stored at −80°C until analysis.

Total protein levels were quantified using the Bradford method (R&D Systems Inc, Minneapolis, MN, USA), and 100 μL elution was used for RANK-L and IL-1β quantification using an enzyme-linked immunosorbent assay–based assay (Quantikine test, R&D Systems). Absorbance was measured at 450 nm with a correction at 540 nm for RANKL and 620 nm for IL-1β, using an automated plate reader (ELx800, Bio-Tek Instruments Inc, Winooski, VT, USA). The concentration of each marker in each sample was calculated using a four-parameter logistic equation.

Statistical Analysis

Statistical analyses were performed using SPSS version 25.0 (SPSS Inc, Chicago, IL, USA) with an α-value of 0.05 indicating statistical significance. For intragroup analysis, we used the Wilcoxon test; the Mann-Whitney test was used for analyzing the variables between the groups. Correlations were calculated using Spearman R in Graph Pad Prism

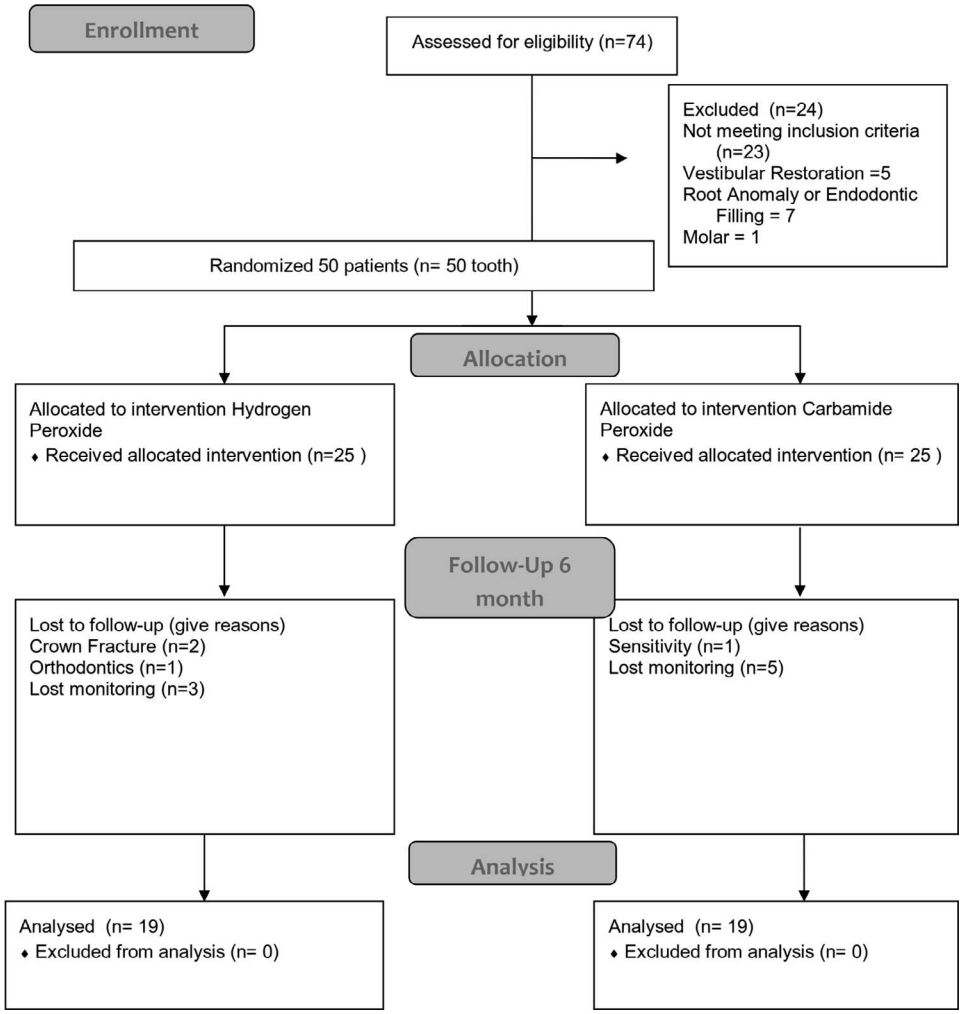

Figure 1. *Flow diagram of the clinical trial, including detailed information on the excluded participants.*

version 7.00 (Graphpad , La Jolla, California, United States).

RESULTS

Six months after treatment, 38 of the 50 recruited patients completed treatment and satisfied the study criteria, resulting in a final sample of 38 teeth (19 teeth per group; Figure 1). The characteristics of the final sample are reported in Table 1.

Subjective Color Measurement

A statistically significant difference in color ($p<0.05$) was detected at the one-month time point. The teeth in the G1 group were bleached a bit more than those in the G2 group (Table 2).

Levels of IL-1β and RANKL

The biomarker data were analyzed using a media by tooth collection or pooled complete data; the statistical results were similar, so we decided to present the data as a pool. Six sites per tooth were measured (114 sites for the hydrogen peroxide group), and 113 sites were measured in the carbamide peroxide group. Table 3 lists the levels of IL-1β (pg/μL). All of the time points exhibited a significant difference ($p<0.05$). Table 4 lists the levels of RANK-L

Table 1: *Baseline Features of the Participants*

Baseline Feature	Group	
	Hydrogen Peroxide	Carbamide Peroxide
Age, y (mean ± SD)	30.6 ± 11.7	30.8 ± 11.3
Minimum age, y	19	20
Maximum age, y	65	65
Male, %	47.83	39.13
Trauma, %	56.52	39.13
Caries, %	43.48	60.87
VITA Bleachedguide 3D-MASTER Median (minimum;maximum)	12 (7;15)	11 (9;15)

Table 2: *Comparison of ΔSGU Values at Different Time Points Using the Vita Bleach Guide (Mean ± SD)*

Assessment Point	Color Change by ΔSGU		
	Hydrogen Peroxide	Carbamide Peroxide	Mann-Whitney Test
Baseline vs one week after bleaching (before restoration)	5.00 ± 2.26[a]	4.16 ± 1.26	0.093
Baseline vs one week after bleaching (after restoration)	5.00 ± 2.24[a]	4.16 ± 1.26	0.080
Baseline vs one month after bleaching	4.84 ± 2.14[a]	3.89 ± 1.20	**0.037**
Baseline vs six months after bleaching	4.37 ± 2.29	3.89 ± 1.15	0.180

[a] *Statistically significant intragroup difference (Wilcoxon test, p<0.05), baseline vs six months after bleaching.*

expressed in pg/μL for all of the sites. According to the Wilcoxon test, the measurements of all time points were significantly different from those obtained at baseline ($p<0.05$). Using the Mann Whitney test, we did not detect any significant difference between the two groups ($p>0.05$). Figure 2 shows the linear correlation between the two biomarker tests (Spearman test); all correlations were greater than 0.8 with a p-value less than 0.0001 (all time points) and for both treatment groups (Figure 2).

DISCUSSION

Intracoronal bleaching is a very efficient and minimally invasive protocol that is currently widely used by endodontists to solve esthetic problems and achieve high levels of patient satisfaction.[17] Our results also demonstrate high effectiveness in the average of the color change units (ΔSGU; Vita Bleached Guide). However, there are safety concerns associated with this process (eg, hydrogen peroxide has been reported to be cytotoxic).[18,19] Another study reported a resorptive increase in the proliferation and activity of osteoclasts after bleaching.[13] This study is the first randomized clinical study to assess the biological effects of intracoronal whitening. Our data show that intracoronal bleaching results in an increase in the levels of RANKL and IL-1β that persists for six months after whitening.

RANKL is part of the RANK/RANKL/OPG axis that regulates bone metabolism.[20] We also detected an increase in the levels of IL-1β for six months after whitening. The levels of IL-1β and RANK-L were highly correlated among them and during all the periods assessed. We detected a positive and proportional correlation of both markers that increased during the sessions and persisted for up to six months of follow-up. Both biomarkers are recognized to be very sensitive to inflammation and the resorption process. Our results are conclusive, and the first hypothesis is accepted whereas the second was rejected, because the levels of markers increased and persisted up to six months after treatment. In addition, there was a positive correlation between them.

Even though this study cannot answer whether root resorption is produced by intracoronal bleaching, it provides some clues that could help clarify the question.

Comparing treatments, hydrogen peroxide was more effective (color change) than carbamide peroxide at all time points up to six months after whitening (Mann-Whitney test, $p<0.05$; Table 2). There was a little rebound at the sixth month, consistent with the results of previous studies of bleaching in nonvital teeth.[21,22] The greater efficacy of hydrogen peroxide may be due to its greater availability; carbamide peroxide should first be

Table 3: *IL-1β Levels Expressed (pg/μL), Mean ± SD*[a]

Assessment Point	Hydrogen Peroxide	Carbamide Peroxide	Δ Hydrogen Peroxide	Δ Carbamide Peroxide	Mann-Whitney Test
Baseline	102.20 ± 48.61	114.81 ± 52.50			
One week after bleaching	160.02 ± 70.74[b,c]	174.91 ± 76.71[b,c]	60.06 ± 31.09	60.11 ± 42.02	0.323
One month after bleaching	177.35 ± 76.00[b,c]	193.74 ± 83.26[b,c]	77.10 ± 36.27[c]	78.93 ± 47.79[c]	0.709
Six months after bleaching	192.19 ± 79.17[b,c]	213.20 ± 88.79[b,c]	91.97 ± 39.19[c]	98.39 ± 52.42[c]	0.581

[a] *Δ indicates difference between time point and baseline value.*
[b] *Statistically significant difference from baseline using the Wilcoxon test (p<0.05).*
[c] *Statistically significant difference from the previous time using the Wilcoxon test (p<0.05).*

Table 4: *RANKL Levels Expressed (pg/μL), Mean ± SD*[a]

Assessment Point	Hydrogen Peroxide	Carbamide Peroxide	Δ Hydrogen Peroxide	Δ Carbamide Peroxide	Mann-Whitney Test
Baseline	13.03 ± 5.88	14.04 ± 5.77			
One week after bleaching	25.70 ± 11.46[b,c]	25.89 ± 10.42[b,c]	13.68 ± 7.28	11.84 ± 5.88	0.078
One month after bleaching	28.67 ± 12.40[b,c]	29.32 ± 11.36[b,c]	16.70 ± 8.25[c]	15.28 ± 6.87[c]	0.201
Six months after bleaching	31.77 ± 12.85[b,c]	33.44 ± 12.55[b,c]	19.75 ± 9.02[c]	19.40 ± 8.28[c]	0.755

[a] *Δ indicates difference between time point and baseline value. Δ (assessment points vs baseline).*
[b] *Statistically significant difference from baseline using the Wilcoxon test ($p<0.05$).*
[c] *Statistically significant difference from the previous time using the Wilcoxon test ($p<0.05$).*

decomposed (10% carbamide peroxide yields approximately 3.5% of hydrogen peroxide), resulting in a lower concentration of hydrogen peroxide, the active ingredient of whitening.

Biomarkers did not attain the levels of active periodontal disease.[23] However, the detected levels may be sufficient to generate some alteration in the most susceptible individuals. It is very striking that the markers were present six months after treatment. This finding implies that the stimulus generates a mediating effect, which has not been reported in the literature.

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. To the best of our knowledge, this has not been described in the literature.

IL-1β is an initiator of potent activity of the osteoclasts[24] and stimulates macrophages and endothelial monocytes (among others) to produce matrix metalloproteinases, prostaglandins, and other proinflammatory cytokines.[24,25] IL-1β also stimulates osteoblasts to produce RANKL, causing differentiation and maintenance of osteoclasts.[23]

External cervical resorption is another important relevant aspect in nonvital teeth,[1] which, despite its low incidence, has been described as a possible adverse effect of intracoronal whitening, and almost always leads to tooth loss. The cause is not clear, and the results of this work can offer a clue as to how the process of resorption is activated from the RANK/RANKL/OPG axis, which also regulates the process of resorption of the root.[20,23] It should be noted that there is significant anatomic variability between endodontically treated teeth; anatomical defects can lead to wider dissemination of the peroxide in the space of the root, creating an inflammatory process that could become chronic and culminate in a resorptive process. Whitening treatments have been lessening in their concentrations and are free of

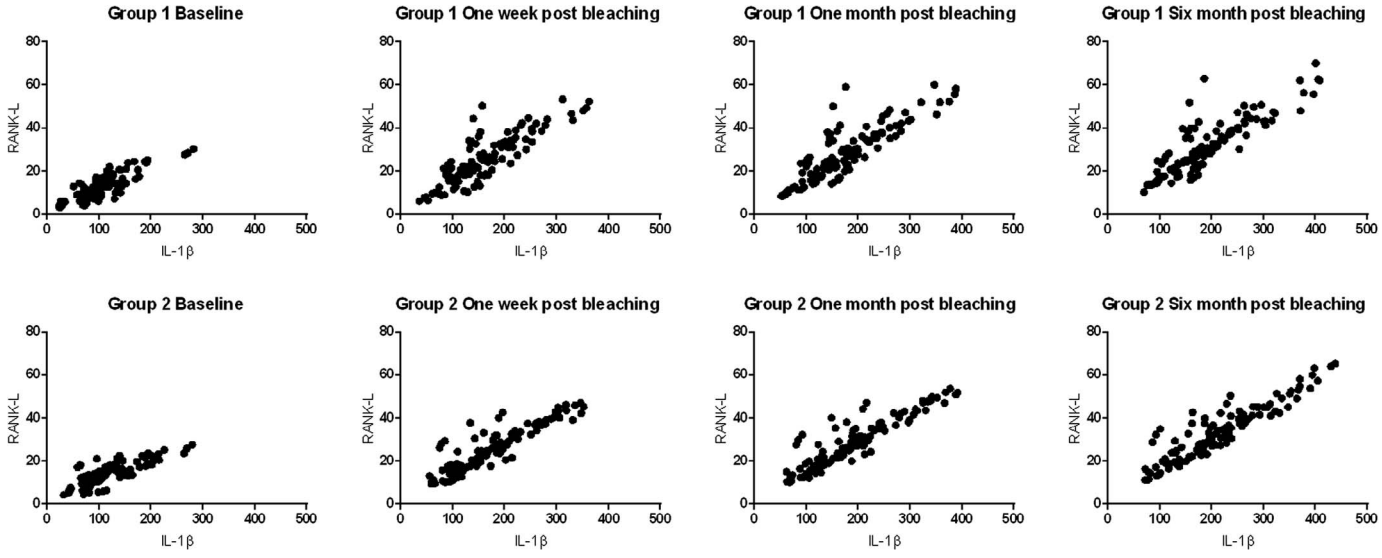

Figure 2. *Linear correlation between two biomarkers (Spearman test).*

activators such as heat, which is used to accelerate chemical reactions and obtain faster results.

The technique of walking bleach uses a closed chamber,[26] wherein the chemical agent is in contact with the tooth for a prolonged length of time. A closed-chamber pressure favors the dissemination of peroxide into the extraradicular space.

A limitation of this study was its inability to have real negative controls. Unfortunately, that protocol was not approved by the local ethics committee in any form (instead of a placebo within the tooth, serum, or something similar). However, comparisons with the reference data were significant for both biomarkers at all time points. On the other hand, the strong correlation between biomarkers suggests a negative effect of the whitening gels that persisted for weeks after the treatment. Despite not being the objective of this work, the question remains whether the cement used to seal the canal could be a factor in the persistence of inflammation.

CONCLUSIONS

Nonvital bleaching with hydrogen peroxide (35%) or carbamide peroxide (37%) generates an imbalance in the levels of fluid gingival RANK-L and IL-1β persisting up to six months after treatment with a strong and positive correlation between the biomarkers. Hydrogen peroxide is more effective than carbamide peroxide bleaching, but the color change was stable in both groups for up to six months.

Acknowledgements

The work was supported by Fondecyt 1140904 and Fondecyt 1170575. The corresponding author dedicates this article to his two kids, Elisa and Eduardo, for their enormous inspiration.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Comité de Etica FOUCH. The approval code for this study is 2016/04.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 3 July 2018)

REFERENCES

1. Plotino G, Buono L, Grande NM, Pameijer CH, & Somma F (2008) Nonvital tooth bleaching: a review of the literature and clinical procedures *Journal of Endodontics* **34(4)** 394-407.
2. Joiner A (2006) The bleaching of teeth: a review of the literature *Journal of Dentistry* **34(7)** 412-419.
3. Joiner A (2007) Review of the effects of peroxide on enamel and dentine properties *Journal of Dentistry* **35(12)** 889-896.
4. Fernandez E, Bersezio C, Bottner J, Avalos F, Godoy I, Inda D, Vildosola P, Saad J, Oliveira OB Jr, & Martin J (2017) Longevity, esthetic perception, and psychosocial impact of teeth bleaching by low (6%) hydrogen peroxide concentration for in-office treatment: a randomized clinical trial *Operative Dentistry* **42(1)** 41-52.
5. Martin J, Vildosola P, Bersezio C, Herrera A, Bortolatto J, Saad JR, Oliveira OB Jr, & Fernandez E (2015) Effectiveness of 6% hydrogen peroxide concentration for tooth bleaching: a double-blind, randomized clinical trial *Journal of Dentistry* **43(8)** 965-972.
6. Lado EA, Stanley HR, & Weisman MI (1983) Cervical resorption in bleached teeth *Oral Surgery, Oral Medicine, and Oral Pathology* **55(1)** 78-80.
7. Cvek M & Lindvall AM (1985) External root resorption following bleaching of pulpless teeth with oxygen peroxide *Endodontics & Dental Traumatology* **1(2)** 56-60.
8. Latcham NL (1986) Postbleaching cervical resorption *Journal of Endodontics* **12(6)** 262-264.
9. Jiang T, Ma X, Wang Y, Zhu Z, Tong H, & Hu J (2007) Effects of hydrogen peroxide on human dentin structure *Journal of Dental Research* **86(11)** 1040-1045.
10. Sato C, Rodrigues F, Garcia D, Vidal C, Pashley D, Tjäderhane L, Carrilho M, Nascimento F, & Tersariol I (2013) Tooth bleaching increases dentinal protease activity *Journal of Dental Research* **92(2)** 187-192.
11. Lee GP, Lee MY, Lum SO, Poh RS, & Lim KC (2004) Extraradicular diffusion of hydrogen peroxide and pH changes associated with intracoronal bleaching of discoloured teeth using different bleaching agents *International Endodontic Journal* **37(7)** 500-506.
12. Toledano M, Yamauti M, Osorio E, & Osorio R (2011) Bleaching agents increase metalloproteinases-mediated collagen degradation in dentin *Journal of Endodontics* **37(12)** 1668-1672.
13. Torres-Rodriguez C, Portoles MT, Matesanz MC, Linares J, Feito MJ, Izquierdo-Barba I, Esbrit P, & Vallet-Regi M (2016) Effects of bleaching on osteoclast activity and their modulation by osteostatin and fibroblast growth factor 2 *Journal of Colloid and Interface Science* **461** 285-291.
14. Kikuta J, Yamaguchi M, Shimizu M, Yoshino T, & Kasai K (2015) Notch signaling induces root resorption via RANKL and IL-6 from hPDL cells *Journal of Dental Research* **94(1)** 140-147.
15. Nakano Y, Yamaguchi M, Fujita S, Asano M, Saito K, & Kasai K (2011) Expressions of RANKL/RANK and M-CSF/c-fms in root resorption lacunae in rat molar by heavy orthodontic force *European Journal of Orthodontics* **33(4)** 335-343.
16. American Dental Association (2012) Tooth whitening products: ADA statement on safety and effectiveness;

Retrieved online from: http://www.ada.org/en/about-the-ada/ada-positions-policies-and-statements/tooth-whitening-safety-and-effectiveness

17. Martin J, Rivas V, Vildosola P, Moncada L, Oliveira Junior OB, Saad JR, Fernandez E, & Moncada G (2016) Personality style in patients looking for tooth bleaching and its correlation with treatment satisfaction *Brazilian Dental Journal* **27(1)** 60-65.
18. Soares DG, Basso FG, Hebling J, & de Souza Costa CA (2014) Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy *Journal of Dentistry* **42(2)** 185-198.
19. Goldberg M, Grootveld M, & Lynch E (2010) Undesirable and adverse effects of tooth-whitening products: a review *Clinical Oral Investigations* **14(1)** 1-10.
20. Tunyogi-Csapo M, Kis-Toth K, Radacs M, Farkas B, Jacobs JJ, Finnegan A, Mikecz K, & Glant TT (2008) Cytokine-controlled RANKL and osteoprotegerin expression by human and mouse synovial fibroblasts: fibroblast-mediated pathologic bone resorption *Arthritis and Rheumatism* **58(8)** 2397-2408.
21. Bersezio C, Martin J, Pena F, Rubio M, Estay J, Vernal R, Junior OO, & Fernandez E (2017) Effectiveness and impact of the walking bleach technique on esthetic self-perception and psychosocial factors: a randomized double-blind clinical trial *Operative Dentistry* **42(6)** 596-605.
22. Patil AG, Hiremath V, Kumar RS, Sheetal A, & Nagaral S (2014) Bleaching of a non-vital anterior tooth to remove the intrinsic discoloration *Journal of Natural Science, Biology, and Medicine* **5(2)** 476-479.
23. Belibasakis GN & Bostanci N (2012) The RANKL-OPG system in clinical periodontology *Journal of Clinical Periodontology* **39(3)** 239-248.
24. Dinarello CA (2011) Interleukin-1 in the pathogenesis and treatment of inflammatory diseases *Blood* **117(14)** 3720-3732.
25. Dinarello CA (1996) Biologic basis for interleukin-1 in disease *Blood* **87(6)** 2095-2147.
26. Bizhang M, Heiden A, Blunck U, Zimmer S, Seemann R, & Roulet JF (2003) Intracoronal bleaching of discolored non-vital teeth *Operative Dentistry* **28(4)** 334-340.