Original Research Article

Effect of an experimental ozone-based desensitizing gel on tooth sensitivity and in-office dental bleaching

ABSTRACT

Aims: This study evaluated bleaching efficacy (BE), tooth sensitivity (TS), and enamel microstructure after in-office dental bleaching treatment with experimental desensitizing gel containing ozone (O_3).
Study design: For the TS and BE analysis, forty third molars were divided into two groups (n=20): OF-B, ozone-free desensitizer and bleaching agent; O-B, 16 ppm ozonized desensitizer and bleaching agent.
Place and Duration of Study: Dental Clinic of the State Western University of Parana, between June 2019 and April 2020.
Methodology: The calculation indicated that 20 teeth per group, totalizing 40 teeth (30 patients), would be sufficient to detect significant differences in TS and BE (power of 80%, significance level of 5%). Dental bleaching was performed with 35% hydrogen peroxide. TS was assessed using a visual analog scale, and BE was assessed using the Vitapan Classical color visual scale. After tooth extraction, the enamel microstructure was evaluated by micromorphological analysis using a scanning electron microscope (SEM). For this analysis, eighty third molars were used, divided into four groups (n=20): OF-B and O-B previously described; OF-WB, ozone-free desensitizer without bleaching agent; O-WB, ozonated desensitizer 16 ppm without bleaching agent. Statistical analyzes of all variables were performed with a significance level of $\alpha = 0.05$. The Mann-Whitney test was used for intergroup analysis for two outcomes, and the Friedman test was used for intragroup analysis. McNemar's exact test compared the risks of tooth sensitivity in the two groups ($\alpha = 0.05$).
Results: TS was higher in the OF-B group. No significant differences were observed in BE. No modification was demonstrated in the enamel microstructure by the action of O_3.
Conclusion: The experimental gel containing O_3 is a promising desensitizing agent for clinical use, reducing tooth sensitivity, without interfering with the color achieved by dental bleaching and in the microstructure of tooth enamel.

Keywords: ozone, tooth sensitivity, tooth whitening, dental bleaching, desensitizing gel.

1. INTRODUCTION

One of the most prevalent cosmetic dental procedures is external dental bleaching, which is performed in dental offices to enhance teeth esthetics [1]. The procedure typically involves the application of a bleaching gel, containing either hydrogen peroxide (H_2O_2) or carbamide peroxide ($CH_6N_2O_3$), onto the surface of the dental enamel [1,2]. In-office dental bleaching employs higher concentrations of hydrogen peroxide (ranging from 30% to 40%) than at-home dental

bleaching, usually performed with carbamide peroxide (ranging from 10% to 22%), resulting in a quicker bleaching effect [2,3].

Dental bleaching is an effective treatment; however, the most frequent side effect is tooth sensitivity, which affects an average of 70% of patients during and after the procedure [3-5]. Tooth sensitivity from bleaching is caused by the inflammatory response of the pulp tissue to the action of hydrogen peroxide during the bleaching process [4,5]. Studies indicate that using bleaching agents with high concentrations of hydrogen peroxide leads to increased production of inflammatory mediators that interact with various cells, generating nociceptive impulses that cause pain perception, which can persist for up to 48 hours [4-6].

Several techniques are suggested to decrease the tooth hypersensitivity induced by dental bleaching, including the use of topic desensitizing agents (i.e. potassium nitrate), and dentinal tubule-occluding agents (i.e. glutaraldehyde), combined with the administration of systemic anti-inflammatory medication [4,7]. Still, tooth hypersensitivity remains recurrent and there is an urgent need to develop new products to address the side-effect caused by bleaching agents [4,7,8].

Ozone gas (O_3) has been suggested as a new approach for treating tooth hypersensitivity induced by dental bleaching [8]. According to previous studies, ozone ions present potent anti-inflammatory and antioxidant effects, besides analgesic properties, due to a prolonged decrease in prostaglandin production and inactivation of cyclooxygenase, resulting in the suppression of inflammatory pathways [6,8]. With the controlled application, ozone increases the activity of antioxidant enzymes, and decreases the number and diameter of the open dentin tubules, by collagen degradation, reducing tooth sensitivity by mechanically blocking these tubules [6,8].

Dentin is formed of about 20% of the organic matrix, which is mainly composed of collagen, which in turn, is made for species containing multiple bonds [6,8]. Thus, previous studies showed that degradation can occur due to the oxidation of the multiple bonds present in the collagen by the action of O_3 [6,8]. Besides, recent evidence has suggested that using O3 can act as a whitening agent since it can go under oxygen dissociation and improve the H_2O_2 redox reaction [9,10]. Due to the additional generation of free radicals in the environment, when associated with H_2O_2, O_3 may increase the degree of tooth bleaching [11,12]. It is noteworthy that due to its gaseous state, O_3 is believed to diffuse more effectively through the tooth structure than other liquid/gel desensitizers. Despite the promising effects of O3, there is still little evidence on the effectiveness of O3-containing desensitizing agents, whether they can reduce tooth sensitivity, and whether they can affect bleaching efficacy and enamel microstructure.

Typically, when researching the impact of bleaching treatment on enamel surfaces, studies rely on *in-vitro* methods where extracted teeth are used and the treatment is conducted outside of the mouth. Nevertheless, there is a scarcity of research that mimics the dynamic conditions of the oral cavity, as well as the intricate interplay between teeth, tissues, and microbiota that takes place in vivo. Thus, the first aim of this study was to conduct a randomized-clinal trial to evaluate the effect of an experimental desensitizing gel containing O_3 on tooth sensitivity (TS) and bleaching effect (BE) during in-office dental bleaching. The second aim was to conduct an *ex-vivo* micromorphological enamel surface analysis posteriorly to the clinical trial intervention and teeth extract. The first research hypothesis was that the TS would be affected by the O_3 presents in the desensitizing gel; the second research hypothesis was that the O_3 would affect the BE; the third research hypothesis was the O_3 would generate micromorphological changes on the enamel surface.

2. MATERIAL AND METHODS

2.1. Ethics approval and protocol registration

The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement [13] and was registered in the Brazilian Clinical Trials Registry (RBR-6685wt). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Subjects (CAAE: 07934819.9.0000.0107). All patients who met the selection criteria were informed of the study's objectives, procedures, risks, and benefits and expressed consent to participate by signing the Terms of Free and Enlightened Consent.

2.2 Trial design, settings, and recruitment.

This randomized, prospective, double-blinded, and a parallel clinical trial was conducted between June 2019 and April 2020, at the Dental Clinic of the State Western University of Parana. This randomized clinical trial had dentin sensitivity as the principal outcome evaluated, and the variation factor was follow-up times and odds ratio. An additional *ex-vivo* micromorphological analysis was carried out using a scanning electron microscope (SEM). For this additional analysis, the third molars were submitted to the experimental desensitizer gel containing O_3 application and the bleaching agent. Then, were extracted 24 hours after this first step, and after, the enamel surface was prepared to be analyzed in SEM. The volunteers were recruited through advertisements published in the local community. All the patients were informed that the purpose of third molar extraction would be exclusively for scientific purposes. Only erupted third molars were selected for extraction.

2.3. Randomization, allocation concealment, and blinding.

This controlled clinical trial had an equal allocation rate to the groups. Based on the inclusion and exclusion criteria, the selected teeth were divided into two groups using the GraphPad.com program (https://www.graphpad.com/quickcalcs/randomize1.cfm). This was a double-blinded study. During the intervention's experiments, the patient did not know to which experimental group belonged, not allowing this to interfere with the patient's perception of sensitivity. To keep the operators blinded one of them performed the addition of O_3 to the desensitizing gel before the bleaching session. Both the syringe containing the ozonated desensitizing gel and containing the desensitizing gel in its conventional formulation had their packaging covered. Thus, the identification of the material was not carried out by the second operator who promoted the application of the product on the dental surface and made the color and TS and BE evaluation.

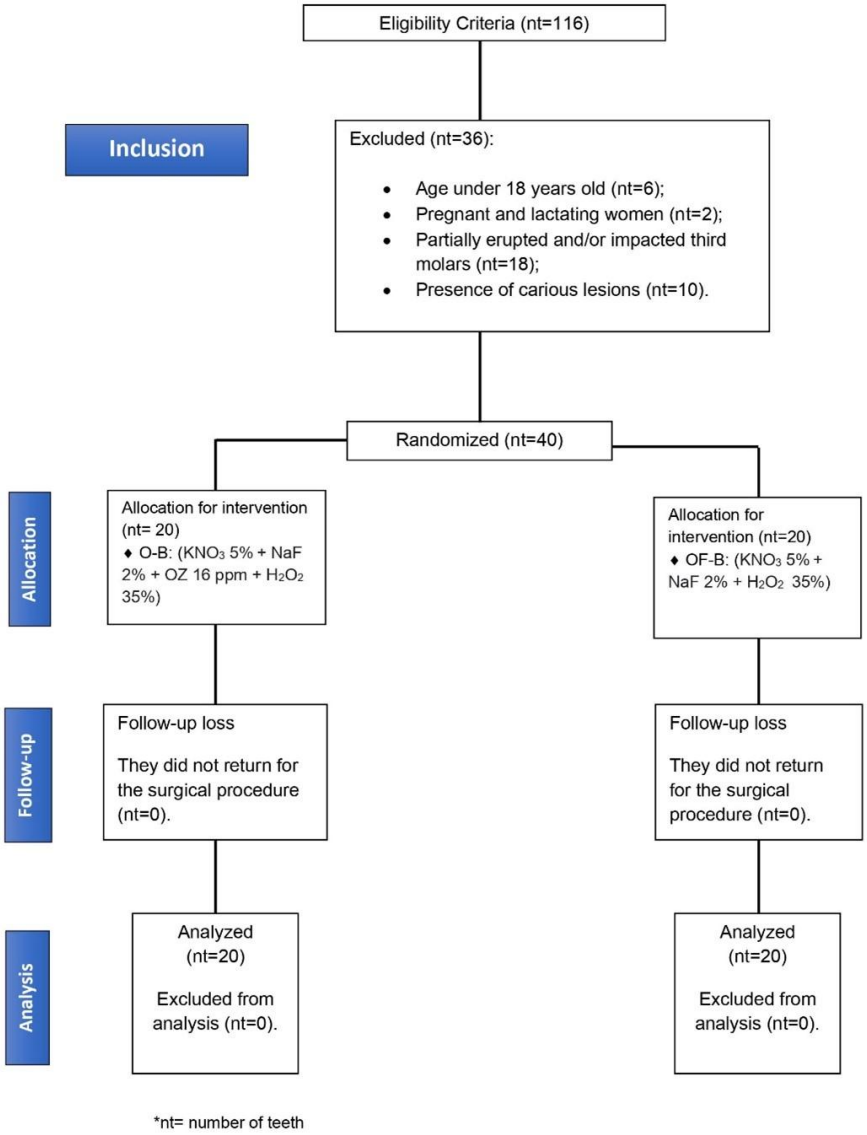
The analyzes of the influence of O_3 on TS and BE were divided according to the desensitizer agent used and bleaching performance: OF-B, use of desensitizer ozone-free following bleaching agent application (as an experimental control); and O-B, 16 ppm ozonated desensitizer following bleaching agent application. The micromorphological analysis of the enamel microstructure was analyzed through four groups (n=20): OF-WB, use of ozone-free desensitizing agent without application of bleaching agent (desensitizer control); O-WB, ozonated desensitizer 16 ppm without application of bleaching agent, and the same two groups were used to carry out the clinical trial, in which the TS and BE outcomes were analyzed (OF-B and O-B). Table 1 demonstrates the composition of the materials used and the application protocol. According to the CONSORT flow diagram, the distribution and dynamics of the groups are shown in (Fig. 1).

Table 1: Composition of the materials used.

Group	Product	Trade Mark	Composition
_____	Bleaching agent.	Whiteness HP MAX (FGM, Joinville, Santa Catarina, Brazil).	35% hydrogen peroxide.
OF-WB	Desensitizer agent ozone-free (without bleaching performance).	Manipulated.	5% potassium nitrate + 2% sodium fluoride.
OW-B	Desensitizer ozone-free	Manipulated.	5% potassium nitrate +

	agent (and bleaching performance).		2% sodium fluoride.
OF-B	Ozonated desensitizer agent (without bleaching performance).	Manipulated.	5% Potassium nitrate + 2% Sodium fluoride + Ozone 16 ppm.
O-B	Ozonated desensitizer agent (and bleaching performance).	Manipulated.	5% Potassium nitrate + 2% Sodium fluoride + Ozone 16 ppm.

Fig. 1: Flowchart of the CONSORT clinical trial.



2.4. Eligibility criteria

The inclusion criteria of the volunteers were: (1) age equal to or over 18 years old; (2) the presence of erupted and healthy third molars; and (3) no history of tooth hypersensitivity. Volunteers with severe internal discoloration (spots of stains by tetracycline, fluorosis, or endodontic treatment), with teeth in potential conditions for sensitivity, such as gingival recession, dentinal exposure, and enamel malformation; those who reported sensitivity that already existed at the time of

the anamnesis; use of orthodontic appliances; recent use of medications that could interfere with pain perception (e.g., analgesics, anti-inflammatories, and muscle relaxants), pregnant, and lactating subjects were excluded [14].

2.5. Pilot study and sample size calculation

A pilot study was carried out to calibrate the operator for the application of each material tested and the clinical evaluation of the patients. In addition, the results served as a basis for carrying out the sample size calculation. The calculation indicated that 20 teeth per group, totalizing 40 teeth (30 patients), would be sufficient to detect significant differences in TS and BE (power of 80%, significance level of 5%). The sample size was calculated using the software BioEstat 5.1 (Sociedade Civil Mamirauá, Amazonas, Brasil).

2.6. Study intervention

2.6.1 Ozonated desensitizing gel

Sixteen ppm of ozone gas was added in 20 g of the experimental desensitizing gel 5 minutes before the use of it, at room temperature (25 ± 1 ℃), with an ozone generator (O&L3.0 RM; Ozone & Life, São José dos Campos, Brazil). The ad of the ozone was made by using pure oxygen, which was dispensed from a cylinder coupled to the generator and regulated at a flow rate of 1 L/min for 6 min as recommended by the International Ozone Association (*https://www.ioa-pag.org/*). The desensitizing gel was manipulated and contained 5% potassium nitrate and 2% sodium fluoride. For the control groups, O_3 was not added.

2.6.2 Dental bleaching

After positioning the labial retractor (Arcflex, FGM, Joinville, Santa Catarina, Brazil), the cured gingival barrier (Top Dam, FGM, Joinville, Santa Catarina, Brazil) was used for the protection of the gingival tissues. After that, the barrier was light-cured using an LED curing unit (Bluephase N, Ivoclar Vivadent, Schaan, Liechtenstein) with 1200 mW/cm2, for 40 seconds. The desensitizing gels were evenly used on the vestibular enamel surface of the upper and lower third molars according to the description of each group, with the aid of a disposable micro-applicator (Cavibrush, FGM, Joinville, Santa Catarina, Brazil) for 10 minutes. After removing the desensitizing gels, the bleaching agent was based on 35% hydrogen peroxide (Whiteness HP MAXX, FGM, Joinville, Santa Catarina, Brazil). Two applications were made in a row, each lasting 15 minutes, with an average interval of 3 minutes between each one, totalizing 30 minutes. This period (30 minutes) was used because of the similar BE time used in three 15-minute applications (totalizing 45 minutes) [15] and variables such as patient discomfort due to the location of the teeth, as well as limitation of mouth opening and

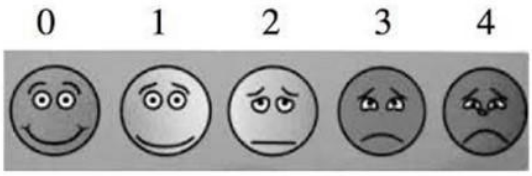
difficulty in applying and controlling the bleaching gel acts in the region of the third molars [15]. The bleaching gel was removed with cotton and gauze during the interval. Abundant water was carried out only after the end of the second application.

2.7 Outcomes

2.7.1 Tooth sensitivity evaluation

During the bleaching procedure, each volunteer received a scale form to assess the possible tooth sensitivity experienced. This data collection instrument (Analogic Visual Scale [VAS]) englobes five scores: 0 – the absence of sensitivity; 1 - smooth sensitivity; 2 – moderate sensitivity; 3 – considerable sensitivity; and 4 – severe sensitivity [16]. To facilitate understanding of the test by the patients, illustrative figures were inserted for each degree of sensitivity (Fig. 2). Data collection was performed every 5 minutes during the entire time the bleaching gel was on the teeth' surface [17].

Fig. 2. Tooth Sensitivity data collection instrument.



0 - Absence of sensitivity; 1 – Soft sensitivity; 2 - Moderate sensitivity; 3 - Considerable sensitivity; 4 - Severe sensitivity.

Evaluation periods: 5 min; 10 min; 15 min; 20 min; 25 min; 30 min.

2.7.2 Bleaching effectiveness

The color assessments were performed during the bleaching treatment, using the upper central incisors as a reference. A previously calibrated operator conducted the assessments using the visual comparison method with the aid of the Vitapan Classical color scale (Vita, Bad Säckingen, Germany) [3,17]. Before the initial application of the bleaching gel and seven days after treatment completion, color assessments were performed. A shade guide was mounted in a sequence of increasing luminosity, from the most luminous shade (B1) to the least luminous (C4). Each shade received a score in this sequence: B1, score 1; A1, score 2; and so on, with A3 being a score of 9. The scores are shown in Chart 1.

Chart 1: Scores assigned to the color scale.

B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3,5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

The bleaching effectiveness (∆B) was calculated by the difference between the color assessment initial (∆I) and the color assessment final (∆F), in each patient, according to the following formula:

$$\Delta B = (\Delta I) - (\Delta F)$$

2.8. Surgical procedure

Twenty-four hours after the bleaching procedure, the patients were submitted to a surgical procedure to extract the third molars. Immediately after the extraction, the teeth were washed with saline (0.9%), cleaned with pumice powder and a rubber cup, stored in chloramine solution (0.5%), and kept at a temperature of 4ºC until the beginning of the laboratory procedures for the micromorphological analysis of the enamel microstructure.

2.9. Micromorphological analysis of the enamel microstructure

An *ex-vivo* micromorphological analysis on the SEM was done for the enamel microstructure evaluation. Eighty third molars (CAAE: 07934819.9.0000.0107) were divided into four groups (n=20): OF-WB, use of desensitizer ozone-free without bleaching agent application; O-WB, 16 ppm ozonated desensitizer without bleaching agent application; OF-B, use of desensitizer ozone-free following bleaching agent application; O-B, 16 ppm ozonated desensitizer following bleaching agent application. The same teeth that were used for TS and BE (OF-B and O-B groups) were used for this analysis. The teeth used for the OF-WB and O-WB were obtained as previously described for OF-B and O-B. However, TS and BE were not evaluated in these two groups because the bleaching agent was not applied. After the extraction, the teeth were cut in half along the longitudinal axis in a buccolingual direction to obtain the vestibular surface where the desensitizer and bleaching agent application was performed. The samples were placed on metallic stubs, gold-coated (MED 010, Balzers, Balzer, Liechtenstein), and examined using SEM (JSM IT 300; Jeol, Tokyo, Japan). Qualitative changes in the enamel surface were analyzed and compared with the healthy structure of the dental enamel.

2.10. Statistical analysis

The statistical analysis followed the intent-to-treat protocol and involved all participants, who were randomly divided (Fig. 1). The statistician was also blinded to the groups. The sensitivity tooth data reported by the patient and bleaching effectiveness collected in this study were tabulated in a digital spreadsheet (Microsoft Excel Windows 2010) and subsequently analyzed using the software BioEstat 5.1 (Sociedade Civil Mamirauá, Amazonas, Brazil). Considering this study's qualitative and independent variables, the Mann–Whitney test was used for intergroup for two outcomes, and the Friedman test was used for intragroup analysis. The McNemar exact test compared both groups' tooth sensitivity risks, used to compare the proportion of dependent data ($\alpha = 0.05$). The odds ratio and the confidence interval (CI) for the effect size were also calculated. The phi correlation coefficient was calculated for pairs of binary data of the risk of pain between the two groups. Descriptive statistics and comparative analysis between the groups were used to analyze demographic data. An alpha level of 5% was considered for all analyses.

3. RESULTS

3.1 Tooth sensitivity and bleaching efficacy

Eighty teeth were obtained from 30 volunteers for this study. Forty teeth were selected for the TS and the BE analysis (OF-B and O-B groups), and the other forty were selected for micromorphological analysis *in vitro*. The demographic data, the mean, and the standard deviation of the initial color are shown in Table 2.

Table 2: Baseline characteristics of participants.

Initial color (scale: mean and standard deviation)	6.30 (2.67)
Age (years: mean and standard deviation)	24.23 (3.53)
Gender (female: %)	60

Table 3 presents the analysis of risk for TS. The phi correlation coefficient for the binary data pairs was moderate and significant ($r = 0.46$; $p = 0.01$). In total, 28 third molars analyzed did not show TS during the experiment, while 12 showed it. This evaluation was made individually at each third molar. There were statistically significant differences between the groups evaluated (Mcnemar's test $p = 0.001$; OR = 15.54; IC 95%: 1.73 a 139.66), in which the group containing O3 (O-B) had significantly less pain than the group O3 free (OF-B) ($p = 0.01$).

Table 3: Combined tabulation of results with two treatments, jointly with the odds ratio.

	Absence of pain	Presence of pain	Total	Odds ratio (95% CI interval*)
O-B	19	1	20	
OF-B	11	9	20	15.54 (1.73 a 139.66)
Total	30	10	40	

* McNemar's test (p = 0.006). The correlation coefficient between paired data = 0.46. p = 0.01

The statistical analysis of the difference of scores intra and inter-group is described in Table 4. In this, there is an absence of statistically significant differences in all the comparisons, except for the time of 15 minutes, which showed statistically significant differences in the comparisons between the scores (p = 0.02) for the OF-B and O-B groups.

Table 4: Medians and interquartile ranges of NRS scores (Numerical Rating Scale), according to the experimental group and time of evaluation.

Times	OF-B	O-B	p value [§]
5 min	0 (0 - 0)	0 (0 - 0)	0.14
10 min	0 (0 - 0)	0 (0 - 0)	0.21
15 min	0 (0 - 1)	0 (0 - 0)	0.02*
20 min	0 (0 - 1)	0 (0 - 0)	0.06
25 min	0 (0 – 0.75)	0 (0 - 0)	0.14
30 min	0 (0 – 0.87)	0 (0 - 0)	0.09

p value €	0.25	0.99

* : Statistically different (p < 0.05)

§ : Mann Whitney test for comparison if groups within each assessment time

€: Friedman test for comparison within the column (intragroup)

There were no statically significant differences between the two groups for BE evaluated before and after the dental bleaching (p = 0.30). The baseline assessment of the patients also did not reveal any statistically significant differences (p = 0.45) (Table 5).

Table 5: Mean and standard deviation (±) of the color change (ΔE) for the two experimental groups and the baseline.

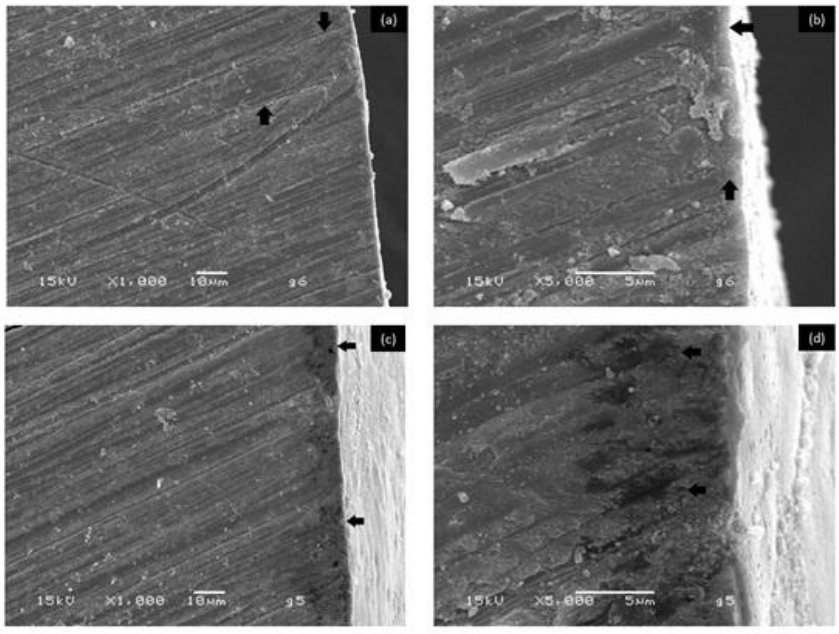
	OF-B	O-B	p value *
Baseline	6.30 (± 2.08)	6.30 (± 3.21)	0.45
Color change	4.75 (± 2.10)	5.05 (± 3.05)	0.30

* : Statistically different = p < 0.05.

3.2. Micromorphological analysis of the enamel microstructure

For the enamel microstructure evaluation, eighty third molars were selected, the same forty teeth used for TS and BE followed by extraction and divided into four groups OF-WB, O-WB, OF-B, and O-B. Fig. 3 shows the changes in the enamel microstructure of the OF-B and O-B groups. In these images, it is possible to observe different standards of mineral loss on the microstructure, which is attributed to changes in the organic and inorganic composition of the dental enamel after the treatment using agents based on hydrogen peroxide and ozone.

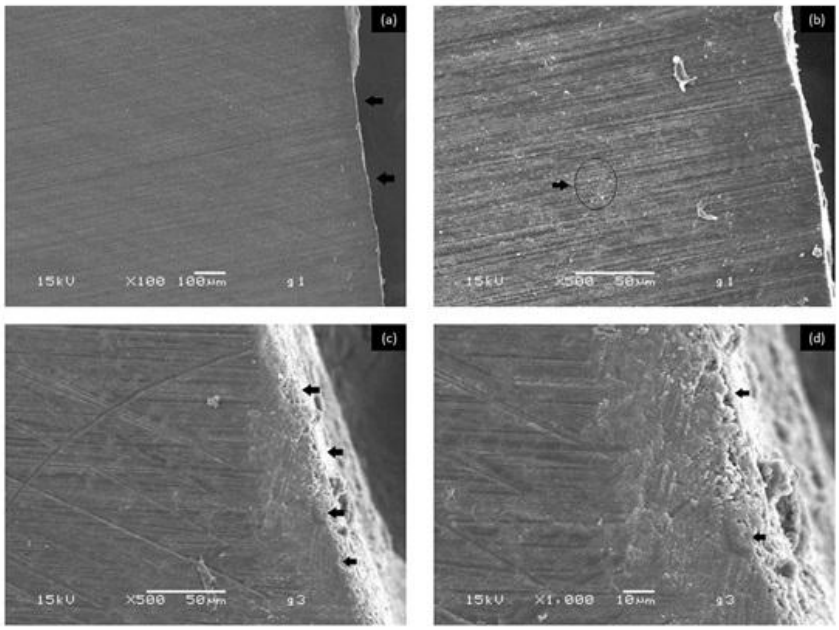
Fig. 3. Changes in the enamel microstructure of the different groups (OF-B and O-B).



(a) and (b) microstructure of the dental enamel of the OF-B group in a 1.000× and 5.000× magnification, respectively - the arrows indicate regions of alteration on the enamel microstructure; (c) and (d) microstructure of the dental enamel of the O-B group in a 1.000× and 5.000× magnification, respectively - the asterisks and arrows represent the regions of change on the enamel microstructure.

Changes in the dental enamel microstructure of the OF-WB and O-WB groups were also observed between the two (Fig. 4). The OF-WB group was normal. In contrast, the standard of the dental enamel microstructure was observed, containing only areas of crystal deposits that correspond to potassium nitrate and sodium fluoride content on the desensitizing gel. At the same time, in the O-WB group, it is possible to observe a slight change in the microstructure, with an incidence of surface porosity and a standard of mineral loss, which is, however, less than that observed in Fig. 3.

Fig. 4. Changes in the enamel microstructure of the different groups (OF-WB and O-WB).



(a) microstructure of the dental enamel of the OF-WB group in a 100× magnification - the arrows indicate the pattern of normal mineral composition of the dental enamel and absence of changes; (b) microstructure of the dental enamel of the OF-WB group in a 500× magnification - the circled area indicated by the arrow shows a region of deposition of crystals (sodium fluoride and potassium nitrate) components of the desensitizing gel; (c) and (d) microstructure of the dental enamel of the O-WB group in a 500× and 1.000× magnification, respectively - the arrows indicate smaller alterations of the enamel microstructure in its most superficial regions.

4. DISCUSSION

In this study, it was observed that the use of the ozonated desensitizing gel promoted, in general, fewer TS when compared to the ozone-free desensitizing gel commonly used in in-office dental bleaching with 35% hydrogen peroxide, then the first hypothesis that the TS would be affected by the O_3, was accepted. This result corroborates the founds of Al-Omiri et al. [18] that evaluated bleaching outcomes with O_3 or hydrogen peroxide. A possible explanation is that the ozone added to the desensitizing gel presents synergism with their components, promotes collagen degradation, and reduces the diameter of the dentinal tubules opened. In addition, we believe that it compromises the capacity of nerve endings without transmitting the stimulus pain, similar to the sodium fluoride and potassium nitrate contained in the desensitizing gel. Those alterations could decrease TS by the mechanical obstruction of dentinal tubules, besides promoting suppression of inflammatory pathways through its analgesic action [4,8,19-21]. Besides, the higher TS observed in the OF-B group might occur due to the induction of inflammatory mediators by the hydrogen peroxide, such as Substance P,

responsible for the activation cells that produce cyclooxygenases and prostaglandins generate nociceptive impulses for pain [18].

Besides the evaluation performed during all the dental bleaching procedures, the TS was also evaluated during each time of the procedure (30 min in total). The results obtained on this evaluation did not show differences between the two experimental groups, except for the time of 15 minutes, which showed higher TS values. More intense sensitivity at this time could result from the whitening treatment's increased dentinal and enamel permeability. The rise of permeability favors the passage of peroxide through these tissues to the pulp, promoting a painful stimulus, which takes about 15 minutes to occur [22-24]. This difference between the groups may be due to the summative desensitizing action promoted by O3 on the O-B group, by generating the obliteration of the dentinal tubules and analgesic action [4,18].

An absence of significant changes in BE between OF-B and O-B groups was observed; then the second hypothesis was rejected. This outcome corroborates with the founds of Dietrich et al. [25], which may be due to the oxidative potential of hydrogen peroxide [25]. Hydrogen peroxide produces hydroxyl radicals (OH-), per hydroxyl radicals, superoxide anions, and hydrogen peroxide anions that increase the environment's pH [25]. This condition is responsible for the bleaching ability or BE due to the action of these free radicals converting chromophores — pigments of complex molecular structure present in the dentin — within hard dental tissues into more superficial structures or changing their optical properties [25]. Ozone acts by forming oxygen molecules that oxidize the components responsible for tooth discoloration, as chromophore groups may be broken by O_3, forming smaller molecules, and resulting in tooth bleaching, similar to that occurs by hydrogen peroxide action. However, the BE by O_3 action depends on the concentration and time of application [25]. Thus, it is speculated that both variables were insufficient to generate a significant BE in the O-B group by O_3 action jointly with hydrogen peroxide in this study. Our findings go against Al-Omiri et al. [6] and Zanjani et al. [26] who found significant differences between groups when O_3 was used jointly with hydrogen peroxide [6,26]. The findings of the in vitro study carried out by Mantom et al. [27] showed that the O_3 applied for 40 s every 4 h in a layer of 16% carbamide peroxide did not significantly increase the effectiveness of the 52-h whitening [27]. The absence of significant differences in color results observed in this study may be associated with an insufficient concentration of O_3 (16 ppm) and/or application time since O_3 was added to the desensitizing gel applied for a time of only 10 m, affecting its penetration and efficiency [26].

Consecutive bleaching sessions with high concentrations of hydrogen peroxide may cause micromorphological and chemical changes in the enamel structure, which depending on the concentration and duration of the procedure, make the enamel more porous, with a significant loss of phosphate ions on its surface [16,28-30]. A pattern of changes in

the enamel microstructure in the groups submitted to bleaching was observed in the SEM images (Fig. 3). This loss pattern may be attributed to the oxidative capacity of hydrogen peroxide (O-B group), to the pH of the bleaching gel used, and due to the acid characteristics of its components [28,30,31]. According to Abouassi et al. [31] and Loguercio et al. [16], among the observed alterations, stand out: increased enamel porosity, decreased enamel microhardness values, and changes in mineral content [16,31]. Besides, a similar mineral content of the enamel in the O-B group was observed. The study carried out by Santana et al. [28] showed a decrease in the enamel microhardness of teeth that were subjected to O_3 bleaching, similar to what occurs when hydrogen peroxide is applied, which may have resulted from morphological changes resulting from the O_3 molecule, due its oxidizing act, able to readily react with organic and inorganic structures [28]. Then, it is speculated that the changes observed in the O-B group can result from both the action of hydrogen peroxide and ozone but not showing significant differences when compared to the use of hydrogen peroxide alone (conventional in-office bleaching protocols).

In addition, the changes observed in the experimental groups caused by the hydrogen peroxide, it was observed in this study that the O_3 also made minor changes in the enamel microstructure when used in isolation (Fig. 4). However, more superficial, and minor morphological changes were observed than those in the OF-B group, where the desensitizer without O_3 was applied in association with hydrogen peroxide (conventional protocol). As previously described, O_3 has an oxidative ability, which allows it to participate in chemical reactions with organic and inorganic substances [26,28]. Thus, similarly to hydrogen peroxide, the O_3 can interact with the dental enamel microstructure with less incident, promoting only more minor alterations than those generated by hydrogen peroxide (Fig. 4) [26,28,32]. This find is in accordance with the study carried out by Celiberti et al. [33], where they assessed the effects of the highly reactive molecule of O_3 on sound enamel physical properties and found no significant differences regarding microhardness, contact angle, and acid resistance properties [33]. Thus, the third hypothesis of this study that the O_3 would generate changes on the enamel surface was accepted.

Finally, within the limitations of this study, it can be affirmed that the experimental desensitizer gel containing O_3 promoted a significant reduction in the TS induced by dental bleaching, especially during the treatment. Concerning the BE, our results showed no differences between hydrogen peroxide and the experimental desensitizer gel containing O_3 use. This can be due to an insufficient O_3 concentration on the desensitizing gel and/or insufficient application time. Regarding enamel microstructure alterations, our findings demonstrated that O_3 acted similarly to hydrogen peroxide conventionally used in in-office bleaching protocols, promoting no changes when used alone and when associated with hydrogen peroxide. Therefore, future studies using different concentrations and application times should be carried out *in*

vitro to evaluate possible changes in the results regarding BE and changes in the enamel microstructure in these situations. This study was conducted using only third molars instead of anterior teeth. The bleaching was performed only for 30 minutes in total, and the TS was evaluated only during the procedure, instead of 48 hours. All these situations consist of the limitations of this study. Other studies should be made to improve these variables.

5. CONCLUSION

It was concluded that the intensity of TS during dental bleaching using the desensitizer modified with O_3 was reduced. Besides, O_3 did not influence the BE and the enamel microstructure.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The experimental design followed the Consolidated Standards of Reporting Trials (CONSORT) statement and was registered in the Brazilian Clinical Trials Registry (RBR-6685wt). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Subjects (CAAE: 07934819.9.0000.0107). All patients who met the selection criteria were informed of the study's objectives, procedures, risks, and benefits and expressed consent to participate by signing the Terms of Free and Enlightened Consent.

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