Original Research Article

Comparison of Desensitizing Agents for Tooth Bleaching: Evaluating the Influence of Ozonated Sunflower Oil and Potassium Nitrate/Sodium Fluoride on Sensitivity and Shade Change

Abstract

Aims: Due to controversial results in relation to the influence of desensitizing agents on sensitivity during bleaching, the present study evaluated the degree of tooth bleaching sensitivity, color change, and patient acceptability of desensitizing agents containing potassium nitrate/sodium fluoride (PNF) or ozonized sunflower oil associated with in-office bleaching with 35% hydrogen peroxide (HP). **Place and Duration of Study:** The study described above was conducted at the Dentistry Clinics of a local university in Brazil. The duration of the study spanned from January to November 2021. **Sample:** The researchers recruited a total of 30 volunteer patients who met the eligibility criteria and agreed to participate in the study. **Methodology:** A clinical trial was conducted with a split-mouth design. The sample consisted of 30 volunteers randomized into 2 experimental groups according to the desensitization protocol. Potassium nitrate and sodium fluoride was applied to the hemiarch corresponding to the control group for 10 minutes, while in the hemiarch of the test group, ozonized sunflower oil was applied for 2 minutes with a rubber cup in low rotation, followed by a potassium nitrate and sodium fluoride application for 10 minutes. In sequence, both arcades were bleached with 35% hydrogen peroxide for 50 minutes. **Results:** Previous application of ozonated sunflower oil resulted in a significant reduction in sensitivity in the first 24 hours after removal of the bleaching gel, as well as the test group showed lower tooth sensitivity in times measured up to 1 hours after removal of the bleaching gel. Both experimental groups presented satisfactorily lighter shades, but no statistically significant differences were observed regardless of the desensitization protocol employed.

Keywords: Complementary therapies, Dentin sensitivity, Desensitizing agents, Ozonated sunflower oil, Ozone.

Introduction

Dentin hypersensitivity is a common condition of multifactorial cause and difficult treatment that, under favorable conditions, develops and progresses rapidly, being one of the

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most found clinical diseases, characterized by short-term, acute and sudden pain in response to thermal, tactile, osmotic or chemical stimuli that can not be attributed to another dental pathology[1]. Its treatment is based on the assumption that therapeutic agents can reduce or interrupt the transmission of stimuli by sealing tubular openings or penetrating dentinal tubules to modify the neural system of pulp responses. Despite the great variety of therapeutic agents available and desensitization procedures, dentin hypersensitivity remains a growing problem of difficult conclusion and uncertain prognosis[2].

Some patients that are asymptomatic in relation to dentin hypersensitivity present their signs and symptoms after dental bleaching, especially in the in-office technique. In this technique, the most used bleaching agent is 35% hydrogen peroxide. Hypersensitivity is a well-known side effect of dental bleaching[3]. Clinical studies show that more than 70% of the patients who undergo in-office bleaching report sensitivity, which can vary from mild to severe, with decreasing intensity over time[1,4].

The explanation to this is given to the fact that hydrogen peroxide results in an increased expression of inflammatory mediators such as Substance P, which interacts with a great variety of cells, inducing the release of inflammatory mediators such as prostaglandins and cyclooxygenases. Both have a recognized role in triggering nociceptive impulses for the perception of pain. Subsequently, both the concomitant increase in vascular permeability and the tissue pressure rise will result in pain, commonly known as post-bleaching hypersensitivity[3].

To minimize the side effects of the bleaching treatment, the application of desensitizing and remineralizing agents before, during or after the bleaching procedure has been enforced. These agents include fluoride, calcium, potassium nitrate and others[5].

Fluorides acts by obliterating the dentinal tubules exposed by the precipitation of calcium fluoride crystals, reducing fluid flow to the pulp and, consequently, pain[6]. Potassium nitrate causes the depolarization of nerve fibers by increasing the extracellular concentration of K^+ . This prevents the entry of sodium ions, delaying nerve repolarization and blocking the passage of painful stimulus to the central nervous system[7].

Recently, the use of ozone therapy has been suggested for the treatment of dental hypersensitivity, probably due to its effect on increasing tubular occlusion[2], and its positive effects reported in humans are based on three main functions: antimicrobial, antioxidant and oxidant balance and immunomodulatory effects[8].

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A controlled application of ozone increases the activity of antioxidant enzymes, including catalase, glutathione peroxidase and superoxide dismutase, thus preparing the host to face pathophysiological and injurious conditions mediated by hydrogen peroxide. Beneficial effects are commonly explained so that ozone has a strong oxidation potential on surfaces covered by calcium, allowing occlusion of dentinal tubules[9].

However, few controlled clinical studies have been conducted to evaluate the efficacy of ozone therapy in the treatment of dental hypersensibility[2,3,9]. Therefore, this study's hypothesis is that the combined use of ozone followed by a desensitizing agent based on potassium nitrate and sodium fluoride results in reduced sensitivity compared to the desensitizing agent alone.

Materials and Methods

Ethical aspects and protocol registration

The experimental design followed the statement CONSORT [11] and was registered in the Brazilian Registry of Clinical Trials (RBR-5sp6g). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Beings (4.251.188). All patients who met the selection criteria were informed about the objectives, procedures, risks, and benefits of the study and expressed their consent to participate by signing the Free and Informed Consent Form.

Clinical study design, randomization, allocation and recruitment

This was a randomized, prospective, double-blind, split-mouth study, in which the patient and evaluator were blinded to the distribution of the groups. This controlled clinical trial had an equal allocation rate to the groups. Simple randomization was performed using an open access online system (www.sealedenvelope.com) by a third person not involved in the implementation and evaluation steps.

The distribution of the group to be assigned for the first time was recorded sequentially on numbered cards and placed in sealed envelopes. The information contained in the envelope determined the treatment to be assigned to the upper right arch, while the other arch received the alternative treatment. Once the participant was fit for the procedure and all evaluations were completed, the allocation assignment was revealed when opening the envelope immediately after implementation.

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The recruitment of patients was carried out through disseminating research on the social network: Instagram. All participants were informed about the nature and objectives of the study. Before enrolling patients in the study, informed consent was obtained by asking the prospective patient to store and sign a form containing all information about the risks and benefits of treatment. The study was conducted from January to November 2021, at the Dentistry Clinics of the Local University.

This clinical trial evaluated the following variables: I- the intensity of sensitivity at different times in the same group; II- the intensity of sensitivity at different times in different groups; III- global sensitivity (GS) (sum of sensitivity throughout treatment, up to 48 hours); IV- worst pain (WP) and V- Bleaching effectiveness.

Eligibility criteria

Based on pre-established criteria, 30 volunteer patients were selected. general and oral health and aged between 18 and 35 years and had at least six caries-free upper anterior teeth, restorations, or endodontic treatment, with canine tone A2 or darker, according to the vita color scale (VITA ClassicalShade, VITA Zahnfabrik, BadSäckingen, Germany). Otherwise, participants with cognitive difficulties, that is, patients who did not understand the correct way of filling out forms for the registration of dental sensitivity were not included in the study, just as patients with orthodontic appliances, dental prostheses, and severe internal tooth discoloration, such as tetracycline, fluorosis or pulped teeth stains were not included in the study. Pregnant and lactating women, patients with bruxism or any pathology that could cause tenderness, such as recession, dentin exposure, visible clefts in the teeth, and patients who use anti-inflammatory drugs or analgesics.

Sample size calculation

The sample calculation was performed based on probability distributions of the t-test family (Wilcoxon and Mann-Whitney tests for comparison of two groups). The effect size used of 0.8, error type 1(α) of 0.05, power of analysis (β error) of 0.8 resulted in a total of 30 individuals per group. The sample calculation was performed in the GPower program, version 3.1.9.2 – University of Düsseldorf.

Study intervention

After the insertion of a lip reformer (Arcflex, FGM Dental Products, Joinville, Brazil), a light-curing gingival barrier (Top Dam, FGM Dental Products, Joinville, Brazil) was placed in the gingival tissue of the teeth to be bleached (from the second left premolar to the second right premolar of the upper arch). The gingival barrier was light curing by means of a light curing machine with a power of 1250 mW/cm² (Emitter NOW, Schuster Dental Equipment, Santa Maria, Brazil) according to the manufacturer's recommendations. After that, the right and left sides of the dental arch were separated with a Mylar matrix (Superdent, United States). In a hemiarched hemiarch was performed the process of the test group (GT), which consisted of the active application of ozonized sunflower oil Ozoncare Philozon was performed, with the peroxide index equal to 600 meq/kg (Philozon, BalnearioCambozo) with the aid of a disposable brush, rubber cup (American Burrs, Palhoça, Santa Catarina, Brazil) in low rotation (15000 rpm) for 2 minutes and removal of its excesses with saliva ejector. Follow by the application of a commercial desensitizing agent (Desensibilize KF, FGM, Joinville, Santa Catarina, Brazil) containing 5% potassium nitrate with 2% sodium fluoride for 10 minutes. In the other hemiarch, the control group (CG), a desensitizing gel based on potassium nitrate (Desensibilize KF, FGM, Joinville, Santa Catarina, Brazil) was applied for 10 minutes, and subsequent removal with water for 1 minute. Then, both arches were bleached with 35% hydrogen peroxide gel containing the commercial product Whiteness HP 35% Automixx (FGM, Joinville, Santa Catarina, Brazil). The bleaching gel was maintained for 50 minutes and removed with a saliva ejector, gauze, and rinse with water for 1 minute. After seven days, all participating patients were reassessed. The composition of the materials used in the study is described in Table 1.

Table 1 – Composition of the materials used in the study.

Material	**Manufacturer**	**Composition**
Bleaching Gel Whiteness HP 35%	FGM	35% Hydrogen Peroxide, thickener, red dye, glycol, and water.
Top Dam	FGM	HEMA, di-methacrylate urethane monomer, inert charge, pigments, and photo initiators.
Desensibilize KF	FGM	5% Potassium Nitrate with 2% Sodium Fluoride.
Ozonized sunflower oil	Philozon	Ozonized sunflower seed oil.

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Tooth sensitivity evaluation

Each patient received a form to evaluate the sensitivity experienced by them. This data collection instrument form for dental sensitivity registration every 5 minutes during the action of the bleaching gel, after 1 hour, 24 hours, and 48 hours after bleaching treatment. Patients were instructed in detail on how to record their most intense pain experience each day, in each hemiarch (right and left), based on the visual analogue scale (VAS) on Figure 1.

In addition, messages were sent daily to all research participants via WhatsApp Messenger, version 17.2.443 (WhatsApp Messenger, Social Networks. Facebook Inc., Menlo Park, CA, USA), informing them about completing the form, to ensure that the pain level was assessed correctly each day. All were instructed not to use any analgesic medication; if they did, they should notify the person responsible for the treatment. At the end of the treatment, the form was delivered by the patient to the researcher in charge.

If the participant scored 0 (without sensitivity) in all time evaluations of both bleaching sessions, this participant was considered insensitive to the whitening protocol (see Figure 1). In all other circumstances, participants were considered to have whitening-induced dental sensitivity.

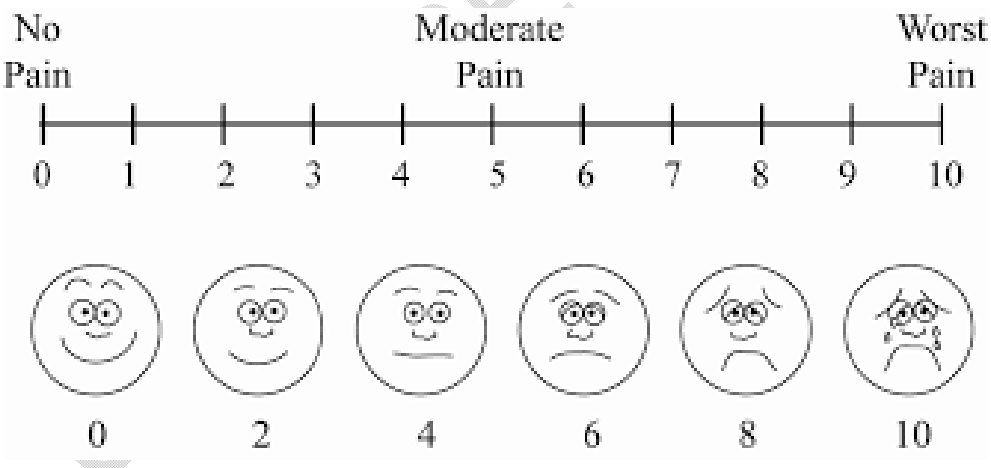

Figure 1 – Visual Analog Scale (VAS) used for evaluating dental sensitivity.

Bleaching effectiveness

Color taking was performed prior to bleaching treatment, using the upper central incisors as reference. The subjective evaluation was performed by comparison with the Vita Classical (Vita, Bad Säckingen, Germany). Seven days after the end of treatment, the color

recording procedure was repeated for final evaluation of tooth saturation. The color differences were calculated by the difference in the number of color guide units (SGU). The color scale has been assembled increasingly in relation to the luminosity, the brightest hue - B1 - at least luminous - C4. In this sequence, each hue will receive a score: B1 the score 1; A1 the score 2, and so on, which made the hue A3 the score 9. The scores are shown in Table 2.

Table 2 – Scores for color evaluation.

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 color change (ΔC) before (ΔI) and after (ΔF) bleaching in each experimental group was performed by calculating the difference between the two-color scores measured, using the following formula: $\Delta C = (\Delta I) - (\Delta F)$.

Statistical analysis

The statistical analysis was conducted by a blind researcher, who was not aware of which treatment protocol had been applied in each experimental group. The data collected in the study were tabulated in a digital spreadsheet (Microsoft1 Excel Windows 2010) and later analyzed using the BioEstat1 5.1 software (Sociedade Civil Mamirauá, Amazonas, Brazil). The risks of tooth sensitivity of both groups were compared using the exact McNemar test, used to compare the proportion of dependent data ($\alpha = 0.05$). The dental sensitivity reported by the patients was considered the primary outcome of this study, in which the scores recorded at different times using VAS were considered for statistical analyses. The analyses between the experimental groups (inter-groups) for the variables: global sensitivity, worse pain and dental sensitivity scores were evaluated using the Wilcoxon test, while the comparative analysis between the times evaluated in each experimental group (intra-group) was performed using the Friedman test. The evaluation of the degree of bleaching between the experimental groups was performed using the Mann-Whitney test. All variables were analyzed considering the significance level of $\alpha=0.05$. The demographic data collected were evaluated through descriptive statistical analysis with the aid of Bioestat® software, determining the frequencies related to gender, age, and color.

Results

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Characteristics of the study population

A total of 43 patients were analyzed for inclusion and exclusion criteria and after that 30 were included in the study. All participants attended the return consultations and none of them gave up on the research. Baseline color and gender distribution are described in Table 3.

Table 3 – Baseline characteristics of participants

Color (SGU; média ± DP*)		5.6 ± 1.9
Age (média ± DP)		23,3 ± 3.3
Gender (female; %)		60.0
Breed	White (%)	76,7
	brown(%)	23,3

*Abbreviations: SGU, color measurement unit by the Classical Vita Scale; DP, standard deviation.

Follow-up

All participants, except one, attended the return visit of the whitening protocol. Participants were followed and remembered via WhatsApp Messenger, version 17.2.443 (WhatsApp Messenger. Social Networks. Facebook Inc., Menlo Park, CA, USA) on the revaluation after 7 days. During this process, one patient did not attend the return. Figure 2 represents the participant's flow diagram in the different phases of the study design.

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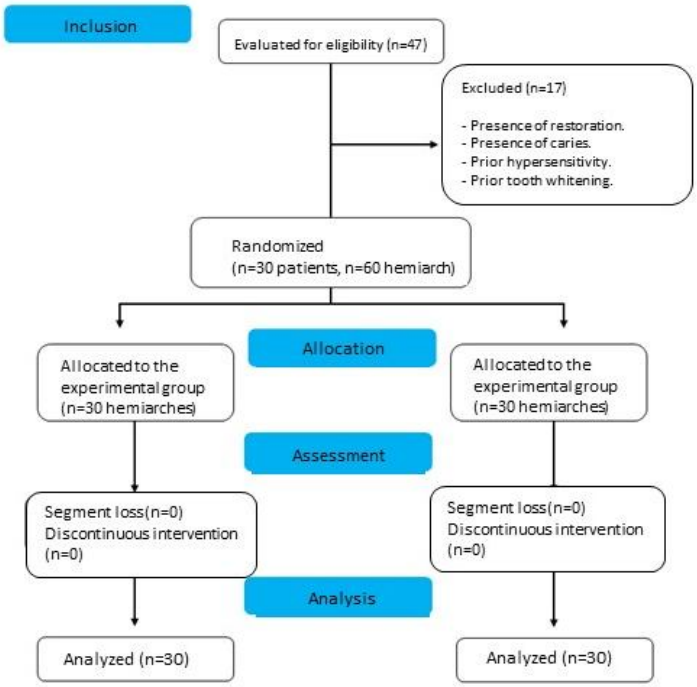

Figure 2 – Flowchart of distribution and dynamics of experimental groups.

Risk of tooth sensitivity

The analysis of the risk of dental sensitivity is described in Table 4, in which there is a higher risk of tooth sensitivity in the control group compared to the test group, according to the McNemar's test ($p < 0.001$).

Table 4 – Combined tabulation of the results obtained in the treatments of the two experimental groups.

	Absence of pain	**Presence of pain**	**Total**
Control group	3	27	30
Test group	5	25	30
Total	8	52	60

Degree of tooth sensitivity

The statistical analysis of the differences between the scores of the intensity of dental sensitivity intergroups (different groups) and intragroups (different times) is described in

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Table 5, in which it is observed in the times 20, 25, 30, 35, 50 minutes and 1h statistically significant differences in the comparison between the scores for the treatment and control groups, at the same time, with median values higher than pain scores for the control group.

The intragroup analysis revealed that in the test group statistically significant differences were found with higher pain score for the time of 1-hour, intermediate pain values for the times 45 and 50 minutes and 24 hours, and the lowest pain scores for the times 5 to 40 minutes and from 48 hours to 7 days. The comparative analysis between the times for the control group showed statistically significant differences between several sets of times, and a progressive increase in pain was observed after 10 minutes, peaking at 1 hour, and from 24 hours there was a progressive decrease in pain scores.

Table 5 - Medians and interquartile intervals of NRS (Numerical Rating Scale), according to the experimental group and evaluation time.

Time	**GT**	**GC**	p value[§]
5 min	0 (0 - 1) A	0 (0 - 1) A	1,000
10 min	0 (0 - 0) A	0 (0 - 2) AC	0,431
15 min	0 (0 - 0) A	0 (0 - 2) AC	0,277
20 min	0 (0 - 0) A	0 (0 - 1) AC	0.043*
25 min	0 (0 - 0) A	0 (0 - 2) AC	0,008*
30 min	0 (0 - 0) A	0 (0 - 2) AC	0,005*
35 min	0 (0 – 2) A	0 (0 - 3) AC	0,018*
40 min	0 (0 – 2) A	0 (0 – 3) AC	0,2012
45 min	0 (0 – 3) AB	0 (0 – 3) AC	0,006
50 min	0 (0 – 4) AB	0.5 (0 – 4) BC	0,028*
1 hour	1 (0 - 4) B	2 (0 - 4) B	0,001*
24 hours	0 (0 - 4) AB	1 (0 - 4) BC	0,093
48 hours	0 (0 - 1) A	0 (0 - 2) C	0,109
72 hours	0 (0 - 1) A	0 (0 - 1) A	0.317
7 days	0 (0 - 0) A	0 (0 - 1) A	0,317
p value[€]	< 0,0001	< 0,0001	

§: Wilcoxon Test for comparison between groups within each evaluation time: *Statistically different ($p < 0.05$).

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€: Friedman Test for comparison within column (intragroup), significant differences ($p < 0.05$) are represented by distinct uppercase letters within the same column.

Dental sensitivity in the first hour

The analysis of Figure 3 reveals that statistically significant differences were found between the treatment approaches ($p<0.001$), and the test group presented lower dental sensitivity in the times measured up to 1 hour after bleaching gel removal.

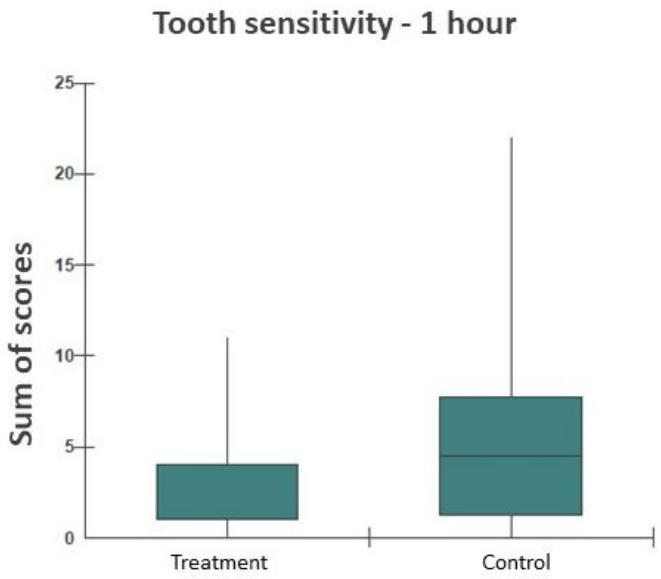

Figure 3 - Dental sensitivity in the first hour after the desensitizing agent according to each treatment approach evaluated ($p<0.001$).

Dental sensitivity after 24 hours

The analysis of Figure 4 reveals that no statistically significant differences were found between treatment approaches ($p = 0.093$) considering the comparative analysis of tooth sensitivity after 24 hours.

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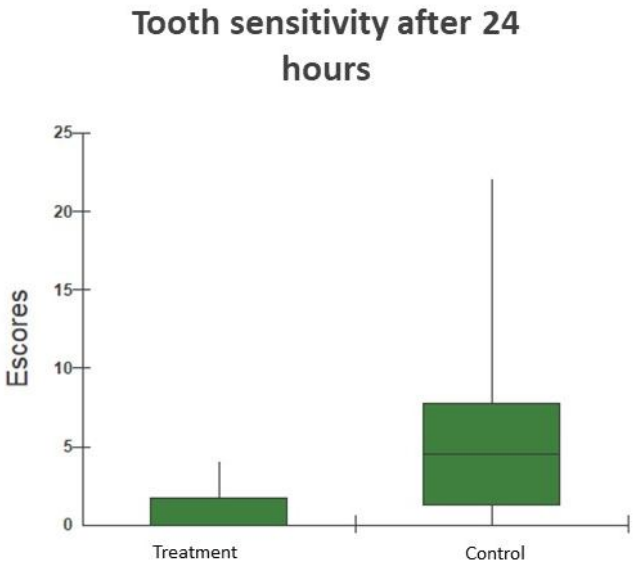

Figure 4 – Dental sensitivity after 24 hours of desensitizing agent according to each treatment approach tested. (p = 0.093).

Overall dental sensitivity

The analysis of Figure 5 reveals that statistically significant differences were found between the treatment approaches ($p < 0.001$), considering the overall dental sensitivity, and the control group presented lower overall dental sensitivity after bleaching gel removal.

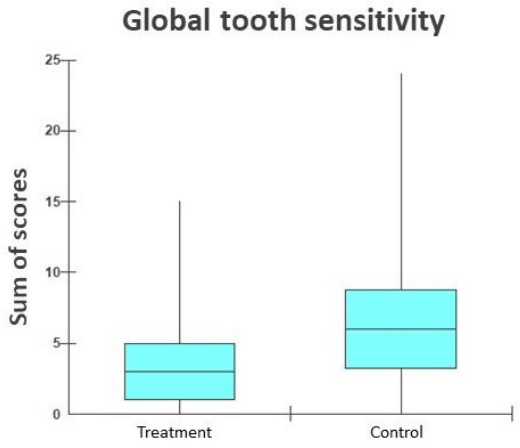

Figure 5 – Overall dental sensitivity ($p < 0,001$).

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Worst pain

The analysis of Figure 6 reveals that statistically significant differences were found between the treatment approaches ($p < 0.001$) for the comparative analysis of the worst experimental pain in the experimental groups, and that in the teeth of the test group the pain manifestation was significantly lower than that reported in the teeth of the control group.

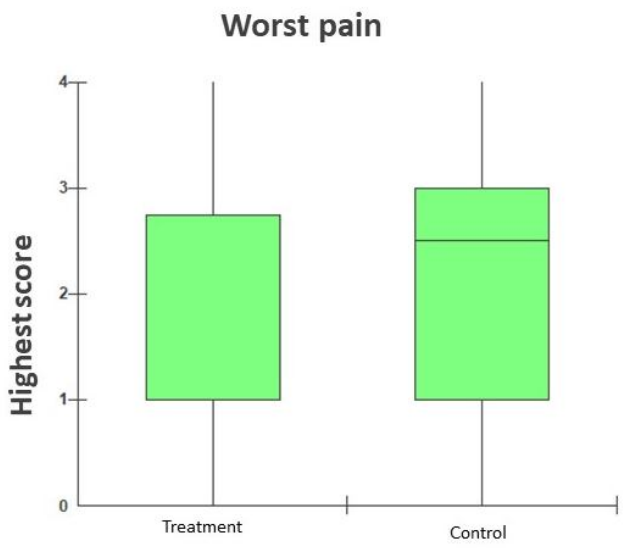

Figure 6 – Worst experimental pain ($p<0.001$).

Color evaluation

The color taking performed previously was compared to that performed 7 days after bleaching treatment for final evaluation of tooth saturation. The color differences were calculated by the difference in the number of color guide units.

Both experimental groups presented satisfactorily lighter tones. Among the experimental groups, no statistically significant differences were observed, regardless of the desensitization protocol employed ($p < 0.05$), as shown in Table 6.

Table 6 - Means and standard deviation of SGU obtained by the Vita Classical scale, comparing initial color and after 7 days.

Color evaluation	**Groups**	**Value p**

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	Teste	Controle	
Initial	5.6 ± 1.9	5.6 ± 1.9	0,559
Final	2.3 ± 0.0294	2.43 ± 0.069	0,559

Discussion

Dental whitening is the most prescribed procedure in cosmetic dentistry because it is an effective and minimally invasive technique[11]. Its mechanism of action is based on the production capacity of free radicals by hydrogen peroxide (H2O2) that oxidizes the pigmented organic molecules of the tooth structure. Thus, its low molecular weight causes its diffusion in the interprismatic spaces of enamel to occur rapidly, reaching the pulp chamber and producing oxidative stress in the cells present there that release inflammatory mediators with consequent sensitization of noceptors, causing the most common adverse effect in bleaching treatments, dentin hypersensitivity[12].

There is still no "gold standard" protocol established in the literature for the control of sensitivity after dental bleaching. Therefore, the present clinical trial evaluated whether the combination of ozonized oil followed by a desensitizing agent based on potassium nitrate and sodium fluoride would provide greater efficiency in the control of sensitivity after dental bleaching. For this, we opted for a split mouth design so that the interindividual variability of each patient can be controlled, because the two interventions were applied in the same patient[13].

According to the results found, the hypothesis that the combined use of ozonized oil followed by a desensitizing agent based on potassium nitrate and sodium fluoride results in reduced sensitivity compared to the desensitizing agent alone was accepted. Since statistically significant differences were observed when comparing the test group in relation to the control group at times 20, 25, 30, 35 and 50 minutes ($p<0.05$) during the application of the bleaching gel, as well as 1 hour after the end of the session ($p<0.001$).

Results similar to the present study, in relation to the reduction of sensitivity associating the use of ozone with dental bleaching, were found by Al-Omiri, et al.[3] (2018). These findings are justified by the action of ozone in increasing the activity of antioxidant enzymes, preparing the host for pathophysiological conditions mediated by hydrogen peroxide.

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It is emphasized that in our study, after the removal of the bleaching gel, a statistically significant difference was found between the two protocols on the first day of treatment, when patients in the control group reported a perceptibly higher mean of pain. This data follows according to the literature, in which dental sensitivity due to bleaching usually occurs in the first 24 hours[14]. Sensitivity was reduced over time and in the evaluation of return in 7 days no participant of the two experimental groups reported pain.

The primary factor of dentin hypersensitivity is the exposure of the dentin by tooth surface wear, but not all exposed dentin is sensitive, this occurs due to the smear layer that obliterates the mouth of some dentinal tubules[15]. Due to its oxidizing property, ozone acts by removing this layer, opening, and extending the mouth of the tubules. Thus, by applying a desensitizing agent, it will be able to enter the tubules more quickly and deeply, obstructing them and preventing fluid exchange that could lead to hypersensitivity[16].

Moreover, it is known that sodium fluoride, present in the composition of the desensitizer used in the present study, acts so that the acidic property of fluoride gel is able to condition peritubular dentin. Therefore, ionized calcium in the tubular fluid reacts with the active ingredient of sodium fluoride gel leading to the formation of calcium fluoride crystals, which are deposited in the dentinal tubules[17].

Thus, the combined use of ozone and fluoride-based desensitizing agent was probably responsible for increasing tubular occlusion, assuming that the pretreatment with ozone provides a better pathway for fluoride precipitation within the dentinal tubules. In addition, oxidation of the organic part of the dentin provided by ozone leads to subsequent exposure of hydroxyapatite and with the application of a fluoride desensitizer applied after ozone there is an increase in the precipitation of fluorapatite crystals in addition to calcium fluoride crystals, thus doubling its precipitation effect[15].

With the results found, lower overall sensitivity was observed in the test group in which ozonized sunflower oil was initially applied and in sequence a desensitizing agent based on potassium nitrate with sodium fluoride. Thus, it is suggested that the synergistic effect of ozone opening the mouth of the tubules followed by the denser deposition of the desensitizing agent inside these tubules provide a more efficient tubular occlusion and consequent decrease in trans sensitivity and after dental bleaching[19].

The color evaluation was performed by a subjective method, and this has great relevance for clinical studies with evaluation of dental color. However, this technique can be influenced by patient characteristics, environment, eye fatigue and evaluator experience[20]. To

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eliminate this bias, the two initial and final color shots were standardized, being performed by the same operator, in the same environment and conditions.

Both experimental groups were associated with changes in color values observed through the Vita Scale acquiring lighter shades, and no statistically significant differences were found between them, concluding the previous application of the desensitizing agent did not compromise the efficacy of the bleaching gel, both in the control group and in the test, in which the ozonized oil was also applied[21,22]. This data has also been observed in similar studies. The non-interference in the final color saturation between the experimental groups can be explained by the ability of both materials, ozone, and hydrogen peroxide, to produce free radicals that have strong whitening effects allowing changes in tooth color[23,24], causing its previous application not to decrease the bleaching effect of peroxide.

Some limitations should be emphasized. Regarding dental sensitivity, the present study did not evaluate the direct influence of the desensitizing agent on dental sensitivity, as it did not have a group without desensitizing application before bleaching treatment. Finally, despite the promising results presented in this study, more controlled clinical trials are needed using different protocols in order to evaluate with greater external validity the effectiveness of ozone in reducing pulp hypersensitivity due to dental bleaching.

Conclusions

In view of the above, the results found in this study show that the combined use of ozone to a desensitizing agent based on potassium nitrate and calcium fluoride proved beneficial in reducing sensitivity after office dental bleaching when compared to the use of desensitizing alone.

Declarations Section

Ethics Approval and Consent

Informed consent was obtained from all study participants in written form. Written consent was deemed necessary to ensure participant understanding and compliance. The study was conducted following the ethical guidelines, and the study protocol and consent procedures were approved by the National Commission for Research Ethics.

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