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Ozonated Oil as a Desensitizing Protocol in External Dental Bleaching: A Clinical, Randomized and Double-Blind Study

Iara Fretta Wiggers [a*], Márcio José Mendonça [a], Rafael Vanolli [a], Francisco Campos [b], Julio Katuhide Ueda [a], Maria Ritha Veiga Colognese [a] and Veridiana Camilotti [a]

[a] Department of Restorative Dentistry, School of Dentistry, State University of West Parana – Unioeste, Cascavel, PR, Brazil.
[b] Sao Leopoldo Mandic College, Campinas, SP, Brazil.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research aimed to evaluate the action of ozonized oil associated with an obliterating desensitizing agent in reducing post-dental bleaching sensitivity.
Study Design: This was a randomized, prospective, double-blind, split-mouth study, in which the patient and evaluator were blinded to the distribution of the groups.
Place and Duration of Study: The study was conducted at the Dentistry Clinics of the Local University from June to December 2021.

**Corresponding author: E-mail: Iarafw_08@hotmail.com;*

Methodology: Forty patients were selected and divided into two groups according to the desensitizing agent used: control group (GC) - potassium nitrate and sodium fluoride and treatment group (GT) - sunflower oil ozonized and glutaraldehyde. In-office bleaching was performed with 35% hydrogen peroxide in a single clinical session. The following variables were evaluated: 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; IV - worse pain and V - color change, which was evaluated at the beginning of treatment and one week after the end of the treatment with the help of a VITA color scale. The risk analysis of tooth sensitivity of both groups was compared using McNemar's exact test. To analyze the intensity of tooth sensitivity, the Wilcoxon and Friedman tests were used, $P < 0,05$.

Results: The study revealed that there were significant differences in the risk and degree of tooth sensitivity between the test and control groups. Out of the total participants, 14 did not experience sensitivity, while 66 did. The test group exhibited lower pain scores at 35 and 40 minutes compared to the control group. In the intragroup analysis, significant differences were found within both the test and control groups across multiple time intervals. The analysis of global sensitivity showed significant differences between the treatment approaches, with the group receiving the specific treatment demonstrating lower sensitivity values compared to the control group. However, there were no significant differences in the worst pain between the treatment approaches. Regarding bleaching effectiveness, there were no statistically significant differences between the test and control groups. The comparison did not show a significant variation in bleaching effectiveness.

Conclusion: The protocol showed promising results as a less invasive and discomfort-free method. However, the study was limited to using only one bleaching agent, and further research with different bleaching agents could provide additional insights.

Keywords: Dentin sensitivity; hypersensitivity; ozone; tooth whitening.

1. INTRODUCTION

Dental whitening is one of the most frequently requested aesthetic procedures since it is a conservative and safe procedure and achieves good clinical results. It can be performed at home, with the use of trays, or by the in-office technique, with the dentist's supervision [1]. Regardless of the technique, the most common side effect is tooth sensitivity (TS), which developed during and/or after the procedure [2].

Studies show that the "TS induced by bleaching has been associated with the diffusion of hydrogen peroxide through enamel and dentin, reaching the pulp, where it produces an inflammatory reaction, activating the nociceptors responsible for pain" [3]. "The higher the peroxide concentration, the greater the oxidative stress generated in pulp tissue, which may be the factor responsible for tooth sensitivity. This oxidative stress generates an inflammatory process with the release of chemical mediators, such as adenosine triphosphate and prostaglandins, which excite nociceptors and trigger TS induced by bleaching. Even if the pain is transitory, it can often cause discomfort, irritation, and even the patient´s withdrawal from the treatment" [4,5].

The different strategies used to prevent or control TS are based on two paths: reducing the excitability of nerve fibers inside the pulp tissue (with neural agents) and/or obliterating dentinal tubules (with obliterating agents). Potassium nitrate alone or in combination with other agents increases the concentration of potassium ions inside the dentinal tubules in sufficient amounts to depolarize the nerve fibers of the pulp, thus preventing the passage of the painful stimulus to the central nervous system [6]. Obliterating agents, meanwhile, promote the sealing of dentinal tubules through protein precipitation, remineralizing the structure and hindering the movement of fluid flow inside the dentin. Examples of these agents are glutaraldehyde, oxalates, strontium, varnishes, and fluorides [7]. Currently, glutaraldehyde-based agents have been suggested for the treatment of tooth sensitivity. This component allows the coagulation of plasmatic proteins with the creation of cross-links with albumin and collagen, preventing the flow of tubular fluid, which differentiates it from other dentin adhesives [8]. However, there are still little evidence in the literature about its application in the control of sensitivity after dental bleaching.

Studies on the use of ozone started after its discovery in 1840, considering that its use may be relevant in medicine, since its antimicrobial effect could be beneficial in various health specialties, such as dentistry [9].

Due to the ozone´s ability to control oxidative stress promoted by an inflammatory reaction, it has gained attention in recent decades for treating and controlling tooth sensitivity. Ozone therapy reduces the production of pro-inflammatory cytokines such as Interleukin-(IL-2), Interleukin-4(IL-4), Interferon-Gamma(IFN-Y), Tumor Necrosis Factor-Alpha (TNF-α); Interleukin 17 (IL-17), Transformative Growth Factor Beta (TGF-β), Interleukin 1 Beta (IL-1β) e Interleukin-6 (IL-6), that are increased in inflammatory processes (Bocci, 2004).

Ozone use in dentistry can be made possible via ozone gas, diluted in water or oil. For the control of tooth sensitivity, gas or oil are the most indicated. When applied to the dentin in addition to the production of cytokines, it is able to enlarge the diameter of the dentinal tubules, depolarize nerve fibers and stimulate the production of collagen fibers and reparative dentin. Inducing tubular obliteration, which leads to decreased dentin sensitivity by its neural and obliterating action. Ozone does not generate an effect on enamel and can be applied safely [10]. Venna et al. reported in an in vitro study that the use of ozonized oil with a desensitizing agent was able to promote tubular occlusion more effectively than the other methods evaluated in the study. There are still no clinical studies on the use of oil for the control of post-bleaching tooth sensitivity. Thus, the aim of this research is to evaluate the action of ozonized oil associated with glutaraldehyde in the reduction of TS after dental bleaching. The hypothesis is that the association of ozonized oil with glutaraldehyde is more effective than the use of conventional potassium nitrate-based technique, prior to office bleaching.

2. MATERIALS AND METHODS

2.1 Ethical Aspects and Protocol Registration

The experimental design followed the statement CONSORT [11] and was registered in the Brazilian Registry of Clinical Trials (RBR-3m4gbs). The study protocol was reviewed and accepted by the Local Ethics Committee on Investigations Involving Human Beings (4,544,943). 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.

2.2 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 in 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.

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 June to December 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.

2.3 Eligibility Criteria

Based on pre-established criteria, 40 volunteer patients were selected. General and oral health

and aged between 18 and 50 years and who 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 [12].

2.4 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 0.50, error type 1(α) of 0.05, and power of analysis (error β) of 0.90 resulted in a total of 36 volunteers per group. For convenience, due to the possible dropout of patients, 40 patients were selected per group. The sample calculation was performed in the GPower program, version 3.1.9.2 - University of Düsseldorf.

2.5 Study Intervention

After the insertion of a lip reformer (Arcflex, FGM Dental Products, Joinville, Brazil), a light-curing gingival barrier (Clàriant Angelus Dam, Angelus, Londrina, 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 application of glutaraldehyde Gluma Desensitizer® (Heraeus Kulzer, Hanau, Germany) for 60 seconds, application of the dry air jet until the brightness of the product disappeared completely, and finally, its removal was performed with plenty of water. In the same hemiarch, following in the footsteps of the GT, the active application of ozonized sunflower oil OzoncarePhilozon 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. In the other hemiarch, the control group (CG), a desensitizing gel based on potassium nitrate (Clàriant Angelus D-Sense, Angelus, 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 Clàriant Angelus Office 35% (Angelus, Londrina, Brazil). The bleaching gel was maintained for 45 minutes and removed with a saliva ejector, gauze, and rinse with water for 1 minute. After seven days, all participating patients were reassessed.

2.6 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) [13].

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. In all other circumstances, participants were considered to have whitening-induced dental sensitivity.

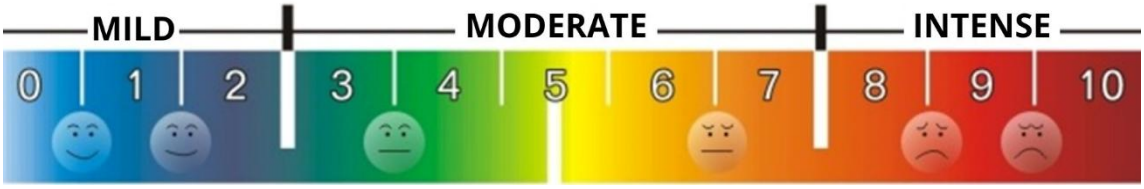

Fig. 1. Visual analog scale (VAS)

Table 1. Scores for color assessment

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

2.7 Bleaching Effectiveness

The color assessments were performed during the bleaching treatment, using the upper central incisors as a reference. An operator, previously calibrated, conducted these using the visual comparison method with the aid of the Vitapan Classical color scale (Vita, Bad Säckingen, Germany). Before the initial application of the bleaching gel and seven days after treatment completion, color assessments were performed. The 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 Table 1.

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: ∆B= (∆I) - (∆F).

2.8 Statistical Analysis

The analysis followed the protocol of intent to treat and involved all participants who were randomly divided. 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 BioEstat 5.1 software (Civil Society Mamirauá, Amazonas, Brazil). The risks of TS 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 variable's 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 Wilcoxon test. All variables were analyzed considering the significance level of $\alpha=0.05$. The collected demographic data were evaluated through descriptive statistical analysis with the aid of the Bioestat® 5.3 software, determining the frequencies related to gender, age and color.

3. RESULTS AND DISCUSSION

TS is considered a common side effect related to office bleaching [14]. "During the process of dental bleaching, hydrogen peroxide, which has a low molecular weight, can permeate dental enamel and break down pigment macromolecules. At this time it can also come into contact with the nerve endings of the dentin and pulp, and trigger an inflammatory reaction, causing TS during and/or after bleaching" [15, 16, 17].

According to the results found in the present study, the hypothesis that the association of ozonized oil with glutaraldehyde is more effective than the use of conventional potassium nitrate-based technique, prior to office bleaching was accepted. It was observed that at times 35 and 40 minutes there were statistically significant differences in the comparison between groups ($P < 0.05$), finding that the test group presented

lower sensitivity when compared to the control group.Similar results regarding the use of the glutaraldehyde-based obliterating agent to control TS were found by Mehta.4

As in the present study, Al-Omiri [18] revealed that sensitivity to bleaching was not observed in the participants of the group who used ozone. This observation can be attributed to the documented analgesic properties of ozone. When in contact with the dentinal tubules stimulates the production of collagen and restorative dentin that can potentially block the passage of fluids. In this context, in 2013, Ozgul [19] observed a significant reduction in hypersensitivity of teeth affected by hypomineralization when ozonized oil was used as a desensitizing agent after 3 months of clinical follow-up.

Tubular obliteration was explained in the in vitro study conducted by Veena [20]. In this work, they found that the adjuvant application of ozonized oil with an obliterating desensitizing agent (Colgate Sensitive Pro-Relief containing 8% arginine, calcium carbonate, and 1450 ppm of fluoride as sodium monofluorophosphate) caused more compact deposition of particles in the mouth of the dentinal tubules compared to the application of the desensitizing agent isolated.

This may justify the associative use of desensitizing agents to control TS after dental bleaching in the office. In the present study, the global sensitivity (sum of sensitivity throughout the treatment) showed statistically significant differences between treatment approaches (P = 0.0294), where the test group presented a lower degree of sensitivity compared to the control.

In this study, the use of ozonized sunflower oil associated with Gluma showed positive results in relation to tooth sensitivity. This finding can be explained by the glutaraldehyde action as an obliterating agent, blocking the micromovement of the dentinal tubules, thus preventing the painful stimulus [21].

The ozonized extra virgin oil, because it has in its composition, a predominance of fatty acids with double unsaturation is more reactive to ozone. Thus, the peroxide index (which is directly related to the antimicrobial potential of the product) in ozonized sunflower oil is a higher one. The application of ozone in dentistry has been reported in the literature due to its desensitizing properties. Its inflammatory response in the pulp, in addition to blocking painful nerve stimuli, leads to a change in local pH and also allows the remineralization process in an accelerated manner in order to obliterate the tubular structure [22,23,24].

The greatest difference that occurred in the present study between the test group and the control group was in the times 35 and 40 minutes. This finding can be explained by the presence of a desensitizing agent in the composition of the bleaching agent. This can help control TS for any agent used prior to external dental bleaching. Tam [25] concluded after a randomized clinical trial that the addition of the desensitizing agent to the agent significantly decreased the reported sensitivity. Obtaining the same results, Jorgensen et al. [26] reported that, in a study conducted with 50 adults, sensitivity decreased over time. Regarding potassium nitrate, a study by Browning et al. [27] compared the efficacy of potassium nitrate, added to the dental bleaching gel, at concentrations of 3% and 0.5%. Although desensitizing agents have been in the dental market for some time, there are still few clinical studies on this subject.

3.1 Characteristics of the Study Population

A total of 122 participants were examined, but only 40 participants met the inclusion and exclusion criteria for the clinical trial, of which 15 were male and 25 females. Final analyses were performed on 39 patients. No volunteers reported discomfort due to medication.

3.2 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. Fig. 2 represents the participant's flow diagram in the different phases of the study design.

3.3 Risk of Tooth Sensitivity

The analysis of the risk of tooth sensitivity is described in Table 3. In total, 14 hemiarches did not present painful sensitivity during the

experiment, while 66 presented painful sensitivity. Statistically significant differences were observed between the groups evaluated (McNemar test P <0,001) (Table 3).

Table 2. Characteristics of the baseline of the participants

Initial color	9 (±2,68)
Age (years: mean and standard deviation)	22 (±2,68)
Gender (female:%)	70

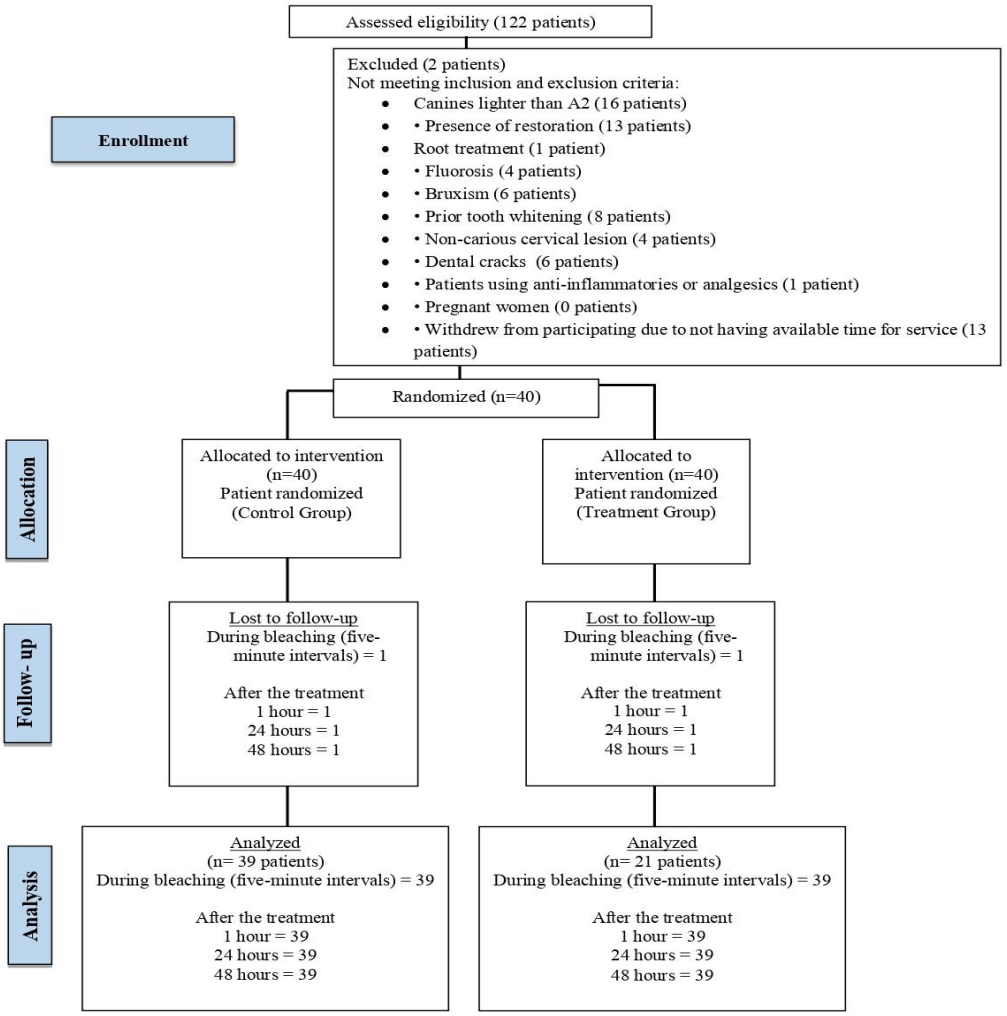

Fig. 2. Flowchart of the clinical trial. Subtitles: Control group – desensitizing agent based on potassium nitrate (Clàriant Angelus D-Sense); Treatment group - ozoned oil associated with the desensitizing agent based on glutaraldehyde (GlumaKulzer)

Table 3. Risk of tooth sensitivity

	Absence of pain	Presence of pain	Total
Control group	6	34	40
Test group	8	32	40
Total	14	66	80

** McNemar's test (p<0.01)*

Table 4. Medians and interquartile intervals of VAS (Visual Analog Scale) scores, according to the experimental group and evaluation time

Times	GT	GC	P value[§]
5 min	0 (0 - 1) AB	0 (0 - 1) AB	0.317
10 min	0 (0 - 1) AB	0 (0 - 1) AB	0.059
15 min	0 (0 - 1) AB	0 (0 - 1) AB	0.345
20 min	0 (0 - 1) AB	0 (0 - 1) AB	0.273
25 min	0 (0 - 2) AB	1 (0 - 2) AB	0.201
30 min	0 (0 - 2) AB	1 (0 - 2) A	0.748
35 min	1 (0 –1) A	1 (0 - 2) A	0.028*
40 min	0 (0 –2) AB	1 (0 - 2) A	0.043*
45 min	0 (0 - 2) AB	1 (0 - 2) A	0.106
1 hour	1 (0 - 2) A	1 (0 - 3) A	0.201
24 hours	0 (0 - 1) AB	0 (0 - 1) B	0.068
48 hours	0 (0 - 0) B	0 (0 - 0) B	0.317
P value[€]	0.007	0.03	

*§: Wilcoxon test for comparison between groups within each evaluation time: *Statistically different (P < 0.05).*
€: Friedman test for comparison within column (intragroup), significant differences (P < 0.05) are represented by distinct uppercase letters within the same column

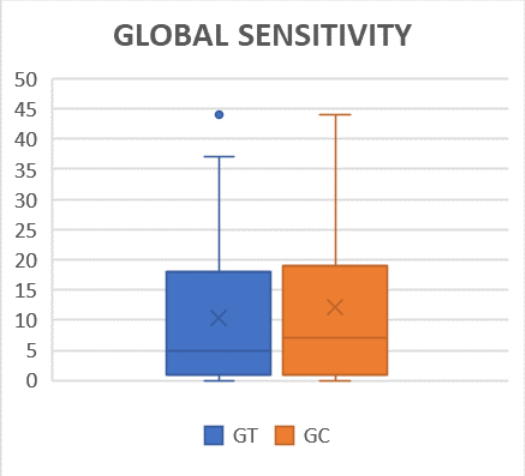

Fig. 3. Global sensitivity (GS) is the sum of sensitivity throughout treatment up to 48 hours. Statistically significant differences between treatment approaches (P = 0.0294)

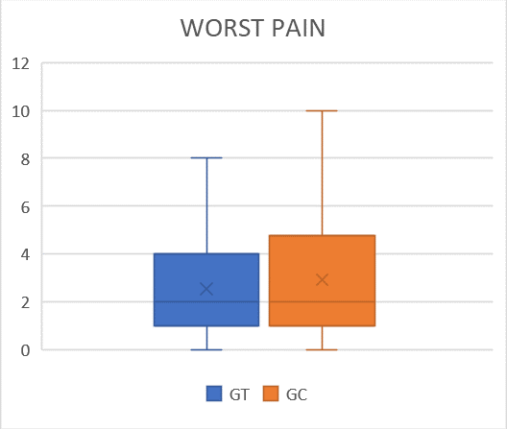

Fig. 4. Worst Pain (WP) is the most intense degree of pain felt, according to each treatment approach tested. Absence of statistically significant differences between treatment approaches (P = 0.1235)

3.4 Degree of Tooth Sensitivity

The statistical analysis of the differences between the scores of the intensity of TS intergroup (different groups) and intra-groups (different times) is described in Table 4, in which there is the absence of statistically significant differences in all comparisons (rows or columns), except for the times 35 and 40 minutes, in which there were statistically significant differences in the comparison between the scores for the test and control groups, with higher median values and quartile interval of pain scores for the control group.

The intragroup analysis revealed that in the test group statistically significant differences were found between the set of times 35, 40, 45 minutes, 1 hour, and 48 hours. While the analysis between the times for the control group showed statistically significant differences between the set of times 30, 35, 40, 45 minutes, 1 hour, and 48 hours.

3.5 Global Sensitivity (GS)

There were statistically significant differences between treatment approaches (P = 0.0294). The group treatment approach presented statistically lower values than the control group for global sensitivity (Fig. 3).

3.6 Worst Pain (WP)

There were no statistically significant differences between treatment approaches (P = 0.1235). Both groups are the same immediate global sensitivity (Fig. 4).

3.7 Bleaching Effectiveness

The data in Table 4 show the intergroup comparison of the bleaching effectiveness. There was no statistically significant difference (P > 0.05) in the bleaching effectiveness when comparing GT and GC groups.

4. CONCLUSION

A significant reduction in the color assessment level was observed in the groups, showing the desensitizers' non-interference in the bleaching result. This corroborates the study by Parreiras et al. [28], which reports that desensitizing agents do not alter the diligence of tooth whitening with 35% hydrogen peroxide. Thus, sensitizing agents allow the diffusion of the bleaching agent through enamel and dentin without impairing its diligence.

The analysis of the degree of color saturation was performed using the VITA color scale, as it is widely used in clinical research [29]. The choice of such a method is due to the fact that visual selection alone is subjective, by the simplicity of the technique and because it is well documented in the literature [30,31,32].

The protocol using an obliterating agent associated with ozonized oil proved to be a promising preventive form in the sensitivity after dental bleaching in the office [33] because it is a less invasive treatment method, without any discomfort [34]. Among the limitations of this study, we can describe the use of only one bleaching agent. Perhaps further research with bleaching agents without any desensitizing agent in their composition may contribute to the results found in the present study.

The use of an association protocol between the desensitizing agent Gluma desensitizer with ozonized oil was more efficient in the treatment of dentin hypersensitivity after dental bleaching at 35 and 40 minutes when compared to the potassium nitrate.

CONSENT

All authors confirm that they have obtained written consent, duly informed, and signed by the patient (or other authorized parties), for the publication of this case report and the inclusion of accompanying images.

ETHICAL APPROVAL

All authors affirm that they have thoroughly reviewed and obtained approval from the relevant ethics committee for all conducted experiments, thereby ensuring compliance with the ethical principles outlined in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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