

BMJ Open Evaluation of in-office tooth whitening treatment with violet LED: protocol for a randomised controlled clinical trial

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ABSTRACT

Introduction In-office tooth whitening treatment using violet light emitted diode (LED) (405 nm) is a novel bleaching method that causes less sensitivity while offering the same effectiveness as the gold standard (35% hydrogen peroxide, H₂O₂). This study describes a protocol for the first randomised controlled clinical trial to compare the effects of the two methods.

Methods and analysis Eighty patients will be divided into four groups: G1 violet LED; G2 violet LED +35% carbamide peroxide; G3 35% H₂O₂ and G4 violet LED +gingivoplasty. Colour will be measured at baseline, immediately after the first session and at the 15 and 180 days follow-up using the Vita Classical and the digital Easyshade V spectrophotometer (Vita, Zahnfabrik, Germany). Sensitivity after whitening will be measured using the Visual Analogue Scale at baseline and at each session in all groups and in all follow-ups. The tissue removed during gingivoplasty (G4) will be submitted to immunohistochemical analysis for the determination of inflammatory changes caused by violet LED. The Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ) will be evaluated before, as well as at established time point controls. The results will be expressed as mean and SD values. After determining the normality of the data, a one-way repeated-measures analysis of variance will be used for the comparison of data with normal distribution and the Kruskal-Wallis test will be used for data with non-normal distribution. A p<0.05 will be considered indicative of statistical significance. After determining the normality of the data, the Kruskal-Wallis test will be used for non-parametric data. Multivariate analysis of variance (MANOVA) and the Wilcoxon test will be used for comparing data from the PIDAQ.

Ethics and dissemination This protocol has been approved by the Human Research Ethics Committee of Universidade Nove de Julho (certificate: 2.034.518). The findings will be published in a peer-reviewed journal.

Trial registration number NCT03192852; Pre-results.

INTRODUCTION

Harmonious dental aesthetics is an important aspect of one's appearance and tooth whitening is considered the most popular aesthetic

Strengths and limitations of this study

- The Easyshade V spectrophotometer (Vita, Zahnfabrik, Germany) will be used to measure tooth colouration, which is an objective analysis method that minimises subjective errors.
- This is a parallel study. The patient will not be his/her own control as in a split-mouth study. We chose this design because we cannot ensure that the two sides of the arch will be compatible after using two different treatments.
- One limitation will be the difficulty in obtaining a large enough sample size due to the scarcity of patients with >A2 colour between 18 and 40 years of age.
- Finding patients with gingival tissue with a regular contour in G4 will be difficult.

treatment in dentistry.¹ Although this procedure has been widely studied,²⁻⁵ sensitivity remains a limiting factor.² Different methods have been tested with the application of hydrogen peroxide (H₂O₂) to achieve a satisfactory change in tooth colour. The major challenge is reaching a reasonable aesthetic standard while causing minimal discomfort. Some authors who have previously used the Millon Index of Personality Styles in patients, before and after tooth whitening, observed that almost all patients presented satisfactory results when submitted to tooth whitening.⁶

There are limitations to the bleaching technique that are pertinent to the tooth structure.⁷ To achieve a satisfactory colour change, a detailed evaluation of the intrinsic and/or extrinsic causes of yellowing is necessary prior to bleaching.⁸ Intrinsic causes directly affect the structure of the tooth, causing a colour change that generally does not respond well to bleaching and can only be resolved through restorative procedures.^{9 10} In contrast, teeth yellowed due to extrinsic causes respond

positively to the bleaching process, enabling more conservative aesthetic treatment.^{11 12} The limitations of tooth whitening are determined by techniques, the type of light sources chosen and knowledge regarding the limit of each tooth based on intrinsic and extrinsic factors.⁷

Researchers have studied whitening mechanisms promoted by H₂O₂ using scanning electron microscopy and have concluded that hydroxylapatite (inorganic matter) is not degraded by H₂O₂ or the hydroxyl ion, but these substances interact with the organic portion of the dentin, which suggests that they are the main components responsible for the whitening process.¹³ The interaction between the whitening agent and the dentin is a redox process capable of breaking down the large molecules of organic matter responsible for pigmentation into smaller molecules, resulting in a whiter appearance.¹⁴⁻¹⁷

Studies show that some undesirable effects can occur after dental bleaching, such as microcracks, root resorption, sensitivity¹⁸ and pulp irritation.¹⁹ The chemical agents used in bleaching procedures, although quite efficient, generally produce some degree of roughness on the dental structure²⁰ and can induce hypersensitivity due to the exposure of the dentin. This discomfort makes some patients avoid tooth bleaching.^{2 20-23} Moreover, the increase in temperature caused by the use of light sources together with the deep penetration of H₂O₂ results in an increase in pulp temperature, which causes tooth sensitivity.² Thus, tooth sensitivity is a very common side effect of bleaching, although the mechanism has not yet been fully clarified.

Although tooth whitening has been studied for decades, postbleaching sensitivity continues to be discussed in the current literature,²⁴⁻³⁰ as considerable discomfort to patients is the second major effect after treatment.^{30 31} Some authors^{30 31} compared the efficacy of H₂O₂ at 6% and 35% associated with light emitted diode (LED)/LASER in the office. Applied in two sessions, with a 7-day interval, they evaluated the colour change (ΔE) using the CieLab values obtained by the spectrophotometer. They also assessed sensitivity (duration and intensity). The H₂O₂ 35% group had a higher value of ΔE , being more effective than H₂O₂ 6%. However, the intensity and duration of sensitivity were lower for the H₂O₂ 6% group.³¹

Researchers evaluating whether 35% H₂O₂ gel is capable of causing cell damage even with the gingival tissue and lips protected have concluded that no change in cellular DNA occurs.³² The same authors report that whitening, as evaluated using a spectroelectromagnetic device (Easys-hade, Vita), can be achieved, but with an increase in sensitivity in most patients submitted to the in-office bleaching method.³² Studies have also demonstrated that bleaching agents can cause pulp damage.³³

Violet LED (405–410 nm) constitutes an advance in tooth whitening procedures and can be used with or without a chemical agent. Its small wavelength and high vibration frequency interact with pigment molecules in the dentin, as the wavelength coincides with the peak

absorbance of these molecules, selectively breaking them down into smaller molecules. The molecules are photoreceptive and therefore highly reactive to light.^{2 34 35} These molecules are excited in the presence of violet light, which weakens their chemical bonds, causing breakages that result in a whiter structure.³⁴ Due to the possibility of use without a bleaching gel, the physical interaction between the light and dental structure occurs in a selective, less invasive manner, thereby diminishing the side effect of sensitivity. This enables the use of this technique on patients who fear the development of postbleaching sensitivity.³⁴ Violet LED can also be used to potentiate the results of a bleaching gel at different concentrations, as demonstrated through colourimetric analysis and spectrometry.¹ Moreover, violet LED is within the spectroelectromagnetic band capable of interacting biologically without causing molecular damage.³⁴

Violet light, unlike blue LED, presents emission of photons that propagate with a smaller wavelength and greater frequency; consequently, it shows the physical characteristic of less penetrability of the dental tissue and greater energy on surfaces. This feature is advantageous as it is capable of promoting breakage of the large pigment chains with lower heating.^{2 34 36}

The lower penetrability of violet light leads to less molecular alteration of dental tissue in depth level, preserving the insulating and protective characteristics of the pulp.³⁶ According to this author, the wavelength of 400 nm, in human stomach mucosa, shows lower penetration than other wavelengths of the visible light spectrum.³⁷ The depth of light penetration is directly proportional to its wavelength, so the shorter the wavelength, the less penetration. Blue LED has a greater wavelength than violet. Blue LED was reported decades ago, by several authors, as being safe for dental whitening. It is estimated that violet LED will be less penetrating than blue in enamel and dentin because of its shorter wavelength, although there are no studies in the literature as of yet. This may be one of the reasons for the decrease in sensitivity after bleaching.^{2 36}

Violet LED was released on the market less than 3 years ago and studies on its effectiveness are scarce. Therefore, randomised clinical trials are needed. The authors of an *in vitro* study evaluated the degree of whitening using LED (405 nm) among other light sources and a control group. Groups were treated with carbamide peroxide (CP) at 10%, 16% and 30% and H₂O₂ at 25% and 38%. The higher concentration of H₂O₂ gel achieved the best effect on the hydroxyapatite samples.¹ However, the authors report that violet LED leads to an excellent aesthetic outcome in short application sessions, which preserves the enamel and dentin.²

In a case report, the authors describe in-office tooth whitening with violet LED (405–410 nm) in a 14-year-old patient with a large pulp chamber and incomplete root formation, which could contribute further to the occurrence of tooth sensitivity. However, no adverse effects were found, and incisor colour went from A3 to A1

of the VITA Classical Shade Guide (Vita, Zahnfabrik, Germany).³⁸

Considering the need for further knowledge regarding this tooth whitening modality, the present study outlines the protocol for a randomised clinical trial for the evaluation of in-office whitening of vital human teeth using violet LED (405 nm).

METHODS

A randomised, controlled, single-centre, parallel-group, clinical trial was designed based on the Consolidated Standards of Reporting Trials statement and is registered with www.clinicaltrials.gov. Participants will be recruited from the dental clinics of Universidade Nove de Julho (UNINOVE) by a researcher who is not otherwise involved in the study (MMP).

Hypothesis of the study

Null hypothesis: The in-office vital tooth whitening treatment protocol with violet LED (405 nm) does not cause colourimetric changes compared with the gold standard 35% H₂O₂ at 6 months after bleaching.

Experimental hypothesis: The in-office vital tooth whitening treatment protocol with violet LED (405 nm) causes colourimetric changes compared with the gold standard 35% H₂O₂ at 6 months after bleaching

Calculation of sample size

Using the G* Power software V.3.1.9.2, the sample size was calculated using analysis of covariance (ANCOVA) F tests with fixed effects, main effects and interactions, since the groups will begin the study with different colour tones, which will influence the final response of each patient. We based our sample size calculation on Bernardon *et al* study, which compares the mean±SD of ΔE differences of four distinct groups.

The effect size was determined using the following formula:

$$d = \frac{\text{largest} - \text{smallest}}{\left(\frac{\sigma}{\sqrt{n}}\right)^2} = \frac{10.82 - 8.41}{\left(\frac{3.62}{\sqrt{4}}\right)^2} = 0.74$$

The effect size is as a standardised index that is independent of sample size and quantifies the magnitude of the difference between populations or the relationship between explanatory and response variables. The largest and smallest mean values as well as the SD (σ) were based on the literature.⁵ Considering a 5% acceptable rate of error and 95% test power, 11 patients per group will be necessary to detect differences in tooth whitening (ΔE).

We believe it will be difficult to maintain adherence of patients in this study. The protocol has repeated sessions (four sessions). As seen in previous studies,³⁹ once teeth have reached a pleasing colour, some patients are not interested in continuing during the follow-up period, just to verify colour stability after bleaching. Therefore, we believe it will be necessary to

include more patients in this study. According to our original sample size calculation, only 11 patients were necessary for our study. However, we have included nine more patients in each group to compensate for this drop-out factor.

Thus, the decision was made to increase the sample to 20 patients per group (total: 80 participants) to compensate for possible drop-outs during the course of the study.

Once we have reached n=10 patients per group in our study, we will recalculate the sample size based on our own results. We expect there will be a need to increase sample size to find a difference between the groups as there are no controlled clinical studies on this subject (violet LED).

Patient and public involvement statement

The patients in the four groups will receive standard information about the steps of research, but they will not be involved in the recruitment and conduct of the study. Data collection will begin on receipt of a favourable opinion and after signing of the informed consent form by the participants and/or their guardians.

Inclusion and exclusion criteria

Male and female patients between 18 and 40 years of age with good systemic and oral health and aesthetic complaints of teeth 13–23 and 43–34 as well as patients with a desire to alter their dental aesthetics and with an indication for gingivoplasty (G4) will be invited to participate in the study. The following will be the exclusion criteria: tooth hypersensitivity, tooth wear on the incisal and/or occlusal faces, untreated caries, pulp abnormalities, dental anomalies, tooth fracture, restorations on the vestibular surface of teeth 13–23 and 43–34, having undergone a tooth whitening procedure in the previous 2 years, use of a fixed orthopaedic appliance, tooth discolouration due to intrinsic factors, smokers, pregnant or lactating women, a history of allergy to CP or H₂O₂, prosthetics on maxillary and/or mandibular anterior teeth or premolars and chronic use of an anti-inflammatory agent or analgesic.

Randomisation of patients and blinded

An internal researcher (ACRTH), not directly involved with treatment and evaluations, will perform randomisation through www.randomizer.com of the participants in groups 1 (n=20), 2 (n=20) and 3 (n=20). Randomisation will be by block (1:1:1). Patients will be randomised into three intervention groups (G1, G2 and G3). Five blocks of 12 different treatments will be formed. The researcher (ACRTH) will prepare and identify opaque envelopes (1–60) that will contain information as to which group the participant will be allocated. The envelopes will remain sealed until the onset of the treatments. The only researcher who will know the treatment performed will be the researcher responsible for the application of bleaching (REMC). These data will be revealed after statistical analysis.

No randomisation will be performed for G4 (n=20), since all patients will have an indication for gingivoplasty, along with the other aforementioned inclusion criteria.

Calibration of clinical examiner

The calibration exercise will consist of measuring the colour of the middle third of the vestibular face from canine to canine of the maxillary and mandibular arches in the same manner as will be performed in the experimental groups in 10 patients who will not participate in the main study. These patients will be evaluated by two examiners (AECGDdS and MMP) for a

subjective evaluation of colour using Classical VITA and digital with the Easyshade V spectrophotometer on two occasions with a 10-day interval between evaluations. The Kappa statistic will be calculated for the determination of interexaminer and intraexaminer agreement, which will be considered adequate if the coefficients are higher than 0.80.

Experimental groups and study design and enrolment

The patients will be enrolled (REMC) to four groups (n=20), with a different treatments performed in each group (figure 1).

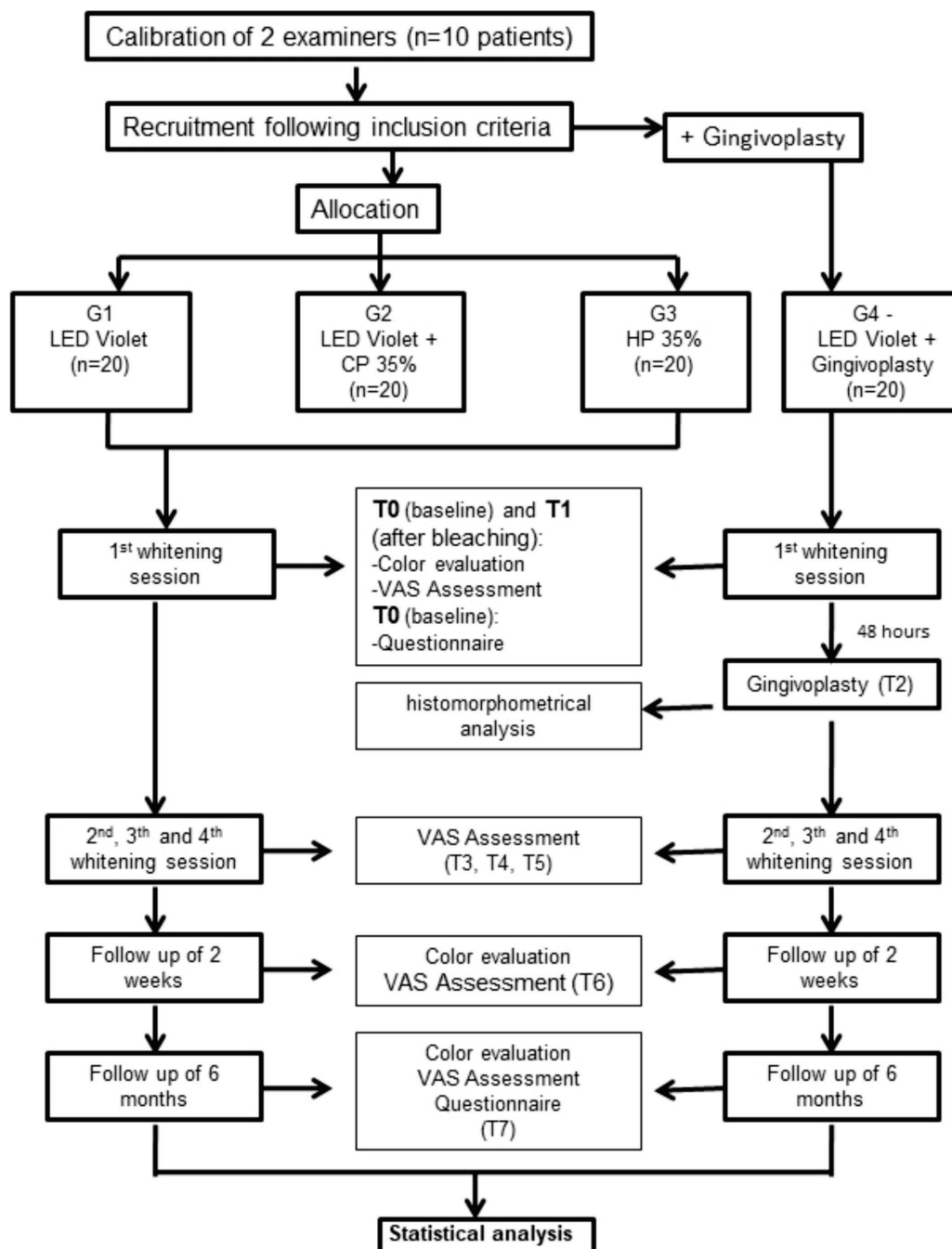


Figure 1 Study flow chart. CP, carbamide peroxide; HP, H₂O₂; VAS, Visual Analogue Scale.

Procedures common to all groups

Patient histories will be taken from all participants and both clinical and periapical radiography will be performed. Periapical radiographs will be performed in the upper and lower anterior region to ensure that the involved teeth are healthy and free of restorations. The following steps will be taken in each whitening session: prophylaxis with a Robson brush, pumice stone and water for the removal of dental biofilm; initial and final digital photographs; a lip protector (Fórmula & Ação, São Paulo, SP, Brazil) will be used to moisten the lips prior to the placement of the lip bumper (Arcflex FGM Produtos Odontológicos, Joinville, SC, Brazil); gum protection will be achieved with the Top Dam resin barrier (FGM) on dry gingival tissue in both arches from the second premolar on the right side to the second premolar on the left side; cotton rolls will be used to preserve periodontal health; the Top Dam will be photoactivated for 20s for every three teeth using the Gnatus Optilight Max resin polymerisation device (1200 mW/cm², Gnatus, SP, Brazil). Care will be taken not to cause the drying out of the dental structure. Gingival protection will be described separately for group 4 due to its particularities. In groups 1, 2 and 4, both the patient and researcher will use protective eyewear with orange-coloured lenses, following the manufacturer's recommendations (Bright Max Whitening, MMO, São Carlos, SP, Brazil). Colourless lenses will be used in group 3. The gingival barrier will be removed after the whitening procedure and neutral, colourless fluoride will be applied (Flúor Care foam) for 1 min in all participants.

Evaluation of outcomes

Colour will be determined (AECGdS and DAPJ) using the Vita Classical visual scale and the Easyshade V digital spectrophotometer on the middle third of the vestibular face of the teeth submitted to the whitening procedures (teeth 13–23 and 43–53). Determinations will be made on four occasions: (T0) baseline; (T1) immediately after the first

Table 1 Parameters of violet LED

Parameters	Violet LED
Wavelength	400 nm±10 nm
Irradiance	112 mW/cm ²
Radiant power	1.2 W
LEDs	4
Target area	10.7 cm ²
Total energy/session	1440 J
Exposure duration	20 cycles–60s application/30s standby
Total radiant exposure (H)	134 J/cm ²
Radiant exposure (H)/60s	6.8 J/cm ²
Anatomical location	Vestibular surface
No of sessions	4

whitening session; (T6) 2-week follow-up (after the fourth whitening session) and (T7) 180-day follow-up after the fourth whitening session. For colour evaluation, ΔE will be used as described.³¹ The ΔE will be calculated using the following formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. To obtain L*, a* and b* values from the CIELab System for each tooth, L* indicates the brightness, a* and b* represent hue. The a* axis represents saturation in the red–green and b* axis is saturation in the blue–yellow.

Postoperative pain will be assessed using the Visual Analogue Scale (VAS), which consists of a 100 mm line with '0' printed at one end (representing absence of pain) and '100' printed at the other end (representing unbearable pain). Each patient will be instructed to mark a vertical dash on the point of the line that best corresponds to pain intensity at the time of evaluation. The measurements will be made by the same operator³⁹ for all sections (T0, T1, T3, T4, T5, T6, T7), before and after each whitening session, as well as at the 2-week and 180-day follow-up evaluation.

TIME POINT	STUDY PERIOD								
	Enrolment	Allocation	Post-allocation					Close-out	
			Bleaching	Gingivoplasty	Bleaching			Follow-up 2 weeks	Follow-up 6 month
	T-1	0	T1	T2	T3	T4	T5	T6	T7
ENROLMENT:									
Calibration of clinical examiner	x								
Informed consent	x								
Eligibility screen	x								
Allocation		x							
INTERVENTIONS:									
G1 – Violet LED			x		x	x	x		
G2 – Violet LED +CP35%			x		x	x	x		
G3 – HP 35%			x		x	x	x		
G4 – Violet LED + gingivoplasty			x	x	x	x	x		
ASSESSMENTS:									
Measurement collar		x	x					x	x
Sensibility		x	x		x	x	x	x	x
Psychological Impact of dental Aesthetics Questionnaire		x							x

Figure 2 Summarising time points of protocol. CP, carbamide peroxide; HP, H₂O₂.

The degree of psychosocial impact tooth colour has on the patient will be determined before and after bleaching, as well as at the time point controls. This evaluation will be performed for all participants in all groups using the Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ), which has been validated for the Portuguese language (figure 2).

Composition of experimental groups

Bleaching efficacy (groups 1–3)

Whitening in G1, G2 and G4 will be performed using a violet light system (BMW, MMOptics) (table 1). The active tip of the device will be positioned at a 90° angle in relation to the incisors in both arches as close as possible to the dental surface. The device is preprogrammed by the manufacturer to operate intermittently in cycles of 60s (on) and 30s (off).³⁶ The lower and upper arches will be irradiated simultaneously. Twenty cycles will be performed, corresponding to 20 min of light and 10 min of rest. Thus, total session length will be 30 min.

In group 1 (n=20), whitening will be performed exclusively with violet LED (405 nm) at a frequency of one session per week for 4 weeks, with the gingival dam placed prior to the 20 cycles of light application. Colour will be measured at T0 (baseline), T1, T6 and T7. Pain will be evaluated (using VAS) before and after each whitening session as well as at the 2-week and 180-day follow-up evaluations (T0, T1, T3, T4, T5, T6 and T7). Neutral, colourless fluoride (Flúor Care foam) will be applied for 1 min at the end of each session.

In group 2 (n=20), whitening will be performed with transparent 35% CP (Whiteform, Fórmula & Ação, São Paulo, SP, Brazil) combined with violet LED (Bright Max Whitening, MMO) at a frequency of one session per week for 4 weeks. After prophylaxis and gingival protection (Top Dam) as described above, a 1–2 mm layer of CP gel will be applied to the vestibular surface to teeth 15–25 and 45–35, followed by 20 cycles of violet LED. The gel will then be removed with abundant water and the gingival protection will be removed. Colour will be measured at T0 (baseline), T1, T6 and T7. Pain will be evaluated (using VAS) before and after each whitening session as well as at the 2-week and 180-day follow-up evaluations (T0, T1, T3, T4, T5, T6 and T7). Neutral, colourless fluoride will be applied for 1 min at the end of each session.

Group 3 (n=20) will be the positive control, that is, the gold standard described in various studies.^{5 30 31 40} In this group, whitening will be performed with 35% H₂O₂ gel (Whiteness H₂O₂ 35%) as described elsewhere³ and following the manufacturer's instructions. The gingival dam will be placed over the gums at teeth 15–25 and 45–35. Phase 1 (peroxide) will be mixed with phase 2 (thickener) at a respective proportion of 3:1 drops for each tooth. The gel will remain on the tooth surface for 15 min and will be changed twice in the same session (three applications). To potentiate the effect, the gel will be moved around with a microapplicator to enhance the release of oxygen bubbles and enable better contact

between the gel and teeth. According to the manufacturer, the bleaching process should be completed in three sessions with a 7-day interval between sessions. However, to standardise the number of sessions in relation to groups 1 and 2, a fourth session will be held with a placebo gel of the same colour and consistency from the same manufacturer and applied in the same manner as the H₂O₂ gel (Whiteness H₂O₂). After the bleaching process, the gel will be removed with abundant water and the gingival protection will be removed. Colour will be measured at T0 (baseline), T1, T6 and T7. Pain will be evaluated (using VAS) before and after each whitening session, as well as at the 2-week and 180-day follow-up evaluations (T0, T1, T3, T4, T5, T6 and T7). Neutral, colourless fluoride (Flúor Care foam) will be applied for 1 min at the end of each session.

Histology, morphology and immunohistochemical analysis (group 4)

Group 4 (n=20) will be formed by patients with an indication for gingivoplasty, and therefore, no randomisation procedure will be used for this group. The surgically removed tissue will be submitted to histomorphometric and immunological analyses 48 hours after being irradiated with violet LED following the clinical indication of gingivoplasty (T2). Initial intraoral care, digital photography, the determination of colour, measurement of sensitivity and application of fluoride will be the same as the procedures performed in the other groups. In this group, the patients will be submitted to the first whitening session with violet LED (405 nm) as described for group 1. Gingival protection will be applied using a split-mouth design: the right side will receive partial gingival protection with the Top Dam. The part of the gingival tissue exposed to violet LED will be removed through gingivoplasty and submitted to histomorphometric analysis. The left side will receive complete gingival protection and will serve as the control. Cotton rolls will also be used to preserve the health of the adjacent tissues. At 48 hours from the first whitening session, gingivoplasty will be performed along the gingival margin. The following will be the inclusion criteria for this group: abnormal passive eruption leading to less exposure of the dental crown with no need for osteotomy; absence of an inflammatory process in the periodontal tissues and a sufficient amount of inserted gingival tissue. The area to be removed will be determined with the aid of a millimetre periodontal probe. The incision will be made with a no 15 scalpel at a 45° angle to the teeth along the demarcated area. The tissue will be removed with the aid of a curette for subsequent histomorphometric analysis.⁴¹ Whitening sessions T1, T3, T4 and T5 will be performed at 7-day intervals. Colour will be measured at T0 (baseline), T1, T6, T7. Pain will be evaluated (using VAS) before and after each whitening session, as well as at the 2-week and 180-day follow-up evaluations (T0, T1, T3, T4, T5, T6 and T7). Neutral, colourless fluoride will be applied for 1 min at the end of each session.

Groups 1 and 4 will use the same bleaching method. However, group 4 will only be used for gingival structural evaluation. Bleaching data from this group will be evaluated only to guarantee that bleaching is as effective as in group 1 (for ethical reasons).

The patients in all groups will be instructed to take the following care throughout the entire treatment process: avoid ingesting foods and beverages with dark pigments; avoid acidic foods; avoid cold foods and beverages; and not smoke (exclusion criterion).

All possible adverse effects will be recorded and qualified during dental bleaching and during the maintenance period (180-day follow-up) using questionnaires developed for this protocol. The adverse events will be reported in the results section of the manuscript and will be discussed. The Research Ethics Committee of UNINOVE will be notified of any protocol alterations and the records at www.clinicaltrials.gov will be updated.

Histology and morphological analysis

After removal, tissue samples will be fixed in 10% buffered formalin solution for 48 hours and submitted to dehydration in increasing concentrations of ethanol. The samples will be treated with xylol, embedded in paraffin (Paraplast, Oxford, St. Louis, Missouri, USA), cut to a thickness of 5 µm and stained with H&E. Slides with the tissue samples will be qualitatively and quantitatively evaluated using light microscopy (magnification: ×100).

Immunohistochemical analysis

Formalin-fixed tissue samples embedded in paraffin (thickness: 3 µm) will be mounted on silane-coated slides. The sections will be deparaffinised in decreasing concentrations of ethanol. After rinsing in phosphate buffer solution (PBS), endogenous peroxidase activity will be blocked with an H₂O₂ solution (3%) for 30 min. Heat-induced epitope retrieval will be performed by incubation with phosphate citrate buffer (P4809, Sigma-Aldrich) at 96°C, pH 6.0, for 20 min. Non-specific binding will be blocked by incubation with 10% non-fat milk diluted in PBS for 1 hour. The sections will be incubated in a humidity chamber at 4°C overnight and then incubated with a chain polymer-conjugated technology-based detection system (EnVision, DAKO) for 30 min at room temperature. The reactions will be revealed by a chromogenic substrate mixture (3,3'-diaminobenzidine; D5905, Sigma-Aldrich) and counterstained with Harris' haematoxylin.

Statistical analysis

The results will be expressed as mean and SD values. After determining the normality of the data, one-way repeated-measures analysis of variance will be used for the comparison of data and the Kruskal-Wallis test will be used for non-parametric data. Multivariate analysis of variance (MANOVA) and Wilcoxon will be used for comparing data from the PIDAQ. A $p < 0.05$ will be considered indicative of statistical significance.

DISCUSSION

Although many studies on tooth whitening are found in the literature and the effect of both photo-assisted and chemically activated whitening has been demonstrated,³ the decision was made to evaluate violet LED due to the peak absorption of the molecules in dentin responsible for pigmentation, causing a physical interaction between the light and pigments rather than a chemical reaction.² Moreover, this treatment modality does not cause sensitivity. Indeed, randomised, controlled, clinical trials for the evaluation of this method are needed. In a case report involving a 14-year-old patient with a large pulp chamber and incomplete root formation, the authors achieved whitening with violet LED (405 nm), in which the tooth colour went from A3 to A1 on the VITA Classical Shade Guide, with the absence of dental sensitivity following the whitening procedure.³⁸ Despite the effectiveness of bleaching gels, these materials can cause changes to dental structures and increase sensitivity.²⁰

H₂O₂ and CP are the most common bleaching agents used on vital teeth.⁴² To evaluate the degree of colour change with this protocol, 35% H₂O₂ will be used in the positive control group, which is the product employed in the majority of studies.³ As an agent that may or may not potentiate the whitening effect of violet LED, 35% CP will be used in group 2, since, according to the manufacturer, one of the ingredients is H₂O₂ at a concentration of approximately 10.5%.

Some authors³¹ described H₂O₂ 6% as being able to promote dental bleaching with less pain (intensity and duration) because of the lower concentration of H₂O₂. The same is reported by others³⁰ when using H₂O₂ 35% compared with H₂O₂ 6%. It was reported that the lower concentration may bring satisfactory clinical results, with lower adverse effect (sensitivity).

Thus, we believe that 35% CP could result in less sensitivity with whitening power similar to that of 35% H₂O₂.¹ According to the manufacturer of whiteness H₂O₂ 35%, bleaching is performed in three sessions. For this group to be equal to the other groups in terms of the number of sessions, a placebo gel of the same colour and consistency from the same manufacturer will be employed in a fourth session.

We planned our study groups as a parallel design. We think that this is suitable, as we will be studying sensibility. We expect that violet LED (groups 1 and 2), causes less sensibility than 35% H₂O₂ gel (group 3). This design was chosen to avoid the possibility of the patient confusing symptoms of sensitivity between the different treatments, which could occur if both violet LED and peroxide were used together in the same arch. Also, we were concerned that differences in bleaching colour of the hemiarcs would displease patients because we expect that violet LED is not as faster/effective as peroxide 35% (control group). Additionally, we have two experimental groups (G1 and G2) with light (violet LED with gel and without gel) and the control Group (G3) with no light. The operationalisation of split mouth becomes complicated with two different

treatments (light/no light); therefore, we chose a parallel design.

Sample size is variable in bleaching studies according to recent meta-analysis.⁴³ Parallel study sample sizes vary from 20 to 25 per group.^{26 44} For split-mouth studies, sample size vary from 17 to 30 patients per group.⁴⁵

Due to the high concentration of HP, problems with gum irritation can occur.⁴ Thus, there is a need for careful isolation of the gingival tissue to avoid contact with H₂O₂ as well as protect the dentin, which could be exposed to direct penetration of the gel in the cervical region, leading to an increase in sensitivity.⁷ In group 4 (patients selected based on the indication for gingivoplasty), the only whitening agent will be violet LED and the patients will therefore be submitted only to the physical action of light.² Although the protocol described by the manufacturer of the Bright Max Whitening device (MMO) recommends the isolation with a gingival dam over the entire gum region, the proposed protocol establishes complete protection on one side and partial protection on the other side, exposing the tissue that will subsequently be surgically removed, thereby avoiding damage to the remaining gingival tissue. To determine whether violet LED causes an inflammatory reaction, the tissue surgically removed 48 hours after the first LED whitening session will be submitted to histomorphometric analysis.

Group 4 will just be used for gingival structural evaluation. Data from G4 bleaching will be evaluated only to guarantee that bleaching of this group is as effective as group 1 (for ethical reasons). In G4, we will only use data for immune histochemical analysis, not bleaching.

The evaluation of colour at baseline and throughout the treatment process as well as the determination of the stability of the colour 6 months after the end of the treatment process will be performed using both the Vita Classical visual scale and the Easyshade V digital spectrophotometer. Although there are reports in the literature of no significant difference between the two methods,⁴⁵ some researchers state the spectrophotometer enables a more reliable evaluation.⁴⁶ Colour determinations will be made at T0 (baseline), T1 and follow-up T6 and T7 on teeth 13–23 and 43–53. However, to ensure aesthetic harmony of the smile, whitening will be performed on teeth 15–25 and 45–55 for ethical reasons.

We have chosen 2-week and 180-day follow-up sessions after bleaching, because there are dehydration results in lighter teeth immediately after bleaching.⁴⁷ The shade and colour values will be measured again after 2 weeks because this period is required for rehydration and stabilisation of shade.⁴⁸ A new evaluation will be performed 6 months after bleaching to analyse the relapse, if any, in bleaching. This period is the minimum time for relapse analysis recommended by the manufacturer.⁴⁹

The expectation with the proposed study is that violet LED is as effective as the gold-standard tooth whitening procedure used in the majority of studies. Moreover, this modality has the advantage of not causing tooth sensitivity or harm to gingival tissue.

ETHICS AND DISSEMINATION

The results of this study will be presented at international conferences and published in a peer-reviewed journal. Individual patient data will be treated as confidential to protect the privacy of the participants (patient identity will not be disclosed).

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