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COMBINED PROTOCOL FOR THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: RANDOMIZED CONTROLLED CLINICAL TRIAL

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COMBINED PROTOCOL FOR THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introduction Dentin hypersensitivity (DH) is defined as high sensitivity of the vital dentin when exposed to thermal, chemical or tactile stimuli. Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp. Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel and, in most cases, is accompanied by DH. The control of tooth sensitivity is fundamental to the successful treatment of MIH. The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of DH in patients with teeth affected by MIH.

Methods and analysis One hundred forty patients who meet the inclusion criteria will be allocated to four groups. Group 1 will be the control group (placebo). In Group 2, sensitive teeth will be sealed with Permaseal (Ultradent). In Group 3, sensitive teeth will receive low-level laser (AsGaAl) at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil). In Group 4, sensitive teeth will be treated with both LLL and Permaseal (Ultradent). DH will be evaluated 15 min after the application of the treatments and the patients will be reevaluated one week, one month, three months and six months after the treatments. This study will enable the determination of differences in the effectiveness of the proposed treatments as well as differences among the evaluation times for each proposed treatment.

Ethics and dissemination This protocol has been ethically approved by the local medical ethical committee Protocol Number: 4.020.261. Results will be submitted to international peer-reviewed journals and presented at international conferences.

Trial registration: NCT04407702

Strengths and Limitations

- This study is to evaluate the effectiveness of different protocols for the control of dentin hypersensitivity in patients with teeth affected by MIH.
- A randomised design will be used to compare different treatment protocols.
- The patients and the evaluator of the degree of sensitivity will be blinded to the allocation.
- The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products.

INTRODUCTION

Dentin hypersensitivity (DH) is defined as high sensitivity of vital dentin exposed to thermal, chemical or tactile stimuli. The exposure of the dentinal tubules causes a reduction in the pain threshold, leading to a response from nerves in the pulp characterized by a rapid, acute, intense pain associated with the mechanoreceptor hydrodynamic mechanism. The proper diagnosis is essential to the establishment of adequate treatment. [1-2]

Brännström's hydrodynamic theory is the most widely accepted for explaining mechanisms that involve the triggering of pain sensations in DH. According to this theory, sensitivity is the result of the rapid movement of the fluid contained in the interior of the dentinal tubules. When a stimulus is applied to the dentin, the fluids within the tubules move both toward the pulp and in the opposite direction, producing a mechanical deformation of the nerve fibers found in the interior of the tubules or at the pulp/dentin interface, which is transmitted to the central nervous system as a sensation of pain.[3] Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp.[4]

Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel. Clinically, MIH presents as yellowish-white or brownish demarcations larger than 1 mm that involve the permanent first molars and, occasionally, the incisors. This condition is generally accompanied by DH, frail enamel and high susceptibility to caries.[5]

Raposo et al. (2019) investigated the prevalence of DH in molars affected by MIH and concluded that hypersensitivity was significantly greater on affected teeth than unaffected teeth. The authors also found an association between mild to moderate cases and DH, which could not be proven for severe cases due to the high frequency of cavities.[6] The principal aim of the treatment of DH is to improve quality of life through the control of pain by suppressing nerve impulses or by the obliteration of the dentinal tubules.[7]

Low-level laser (LLL) has been used as treatment for DH. In a literature review, Shintome et al. concluded that both high- and low-level lasers are effective in the treatment of cervical DH. The studies reviewed also reported that treatment with laser offers greater patient comfort and enables longer lasting results in comparison to dentifrices and

desensitizing agents, as light act directly on the dental tissue, promoting morphological changes in the dentin, stimulating the pulp tissue and making treatment more lasting.[8]

The control of tooth sensitivity is a fundamental factor to the successful treatment of MIH. Therefore, this paper proposes the evaluation of the effectiveness of different protocols for the control of DH.

METHODS

Overview

The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of dentin hypersensitivity in patients with teeth affected by MIH. This protocol follows the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations displayed in Table 1.

Table 1. Schedule of enrolment, interventions, and assessments of the study

			STUDY PERIOD					
	Enrolment	Allocation	Post-allocation					Close-out
			0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:								
Eligibility screen	x							
Informed consent	x							
Allocation		x						
INTERVENTIONS:								
Control			x	x	x	x	x	x
Sealant			x	x	x	x	x	x
Low level laser			x	x	x	x	x	x
Low-level laser and sealant			x	x	x	x	x	x
ASSESSMENTS:								
Pain			x	x	x	x	x	x

* 0 = Baseline, t_1 = immediately after treatment, t_2 = 01 week after the treatment, t_3 = 01 month after the treatment, t_4 = 03 months after the treatment, t_5 = 06 months after the treatment

The study will be conducted in accordance with the ethical precepts stipulated in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee (Certificate of Presentation for Ethical Appreciation n. 31651120.7.0000.5509 / Report n. 4.020.261 – Universidade Metropolitana de Santos - UNIMES) and this protocol is registered at ClinicalTrials.gov; the registration number is NCT04407702.

Participants

Patients between between 18 and 35 years of age selected at the Clinic of the School of Dentistry of UNIMES will participate in the study. The sample size was determined based on the primary outcome of the study: final pain visual analog scale. Based on the data from[9], our initial sample size estimation was of 24 subjects per group for a significant level of 0.05 and an estimated test power of 80%. To account for the possible non-parametric distribution of the data, 15% more subjects must be added to each group.[10] Another 25% will be added to account for possible dropouts, resulting in 35 subjects per group. G*Power 3.1.9.6 was used to perform the calculations

Patient and public involvement statement

The patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group. All information will be delineated in the statement of informed consent (Resolution N° 196 of the National Board of Health, Ministry of Health, Brazil, 10/03/1996). Two copies of the statement will be signed – one for the volunteer and one of the researchers. The statement will declare the commitment on the part of the researchers to provide adequate treatment for the participants in the placebo group at the end of the four weeks of the study and to the subjects of the other groups at the end of six months if the pain symptoms have not improved. The treatment offered will be that which achieved the best result in the initial four weeks of the study. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire. The researchers will also be able to remove the participants from the study, if deemed necessary.

Experimental design

A randomized, double-blind (subject and evaluator of pain), parallel, interventional study will be conducted at the dental clinic of UNIMES. The study will be will be conducted following the CONSORT guidelines (http://www.consort-statement.org/).[11] Pain will be evaluated using the visual analog scale (VAS) after stimulation with compressed air from the triple syringe and using an exploratory probe at the time of recruitment (baseline), immediately after treatment as well as one week, one month, three months and six months after treatment.

Preclinical trial

During the first visit to the clinic, a form addressing the volunteer's medical history will be filled out. Each volunteer will then be submitted to a clinical examination for the determination of his/her oral status. Next, the inclusion and exclusion criteria will be applied to determine the eligibility of the volunteers.

Inclusion Criteria

- Age between 18 and 35 years;
- Good overall health;
- At least one tooth with MIH and DH reported in the cervical region with sensitivity equal to or greater than 4 on the VAS.

Exclusion Criteria

- Active caries or defective restorations on the tooth to be analyzed;
- Sufficient dentin loss that requires restorative treatment or periodontal surgery;
- Having undergone any professional desensitizing treatment in the previous six months;
- Having used a desensitizing paste in the previous three months;
- Use of anti-inflammatory drugs or analgesics at the time of recruitment;
- Currently pregnant or nursing.

Recruitment

Recruitment will be performed by a researcher trained to diagnosis MIH. After the volunteer reports the occurrence a tooth with hypersensitivity, the researcher will assess the sensitive

tooth using cold air from the triple syringe (2 s of compressed air at a pressure of approximately 40 psi with the syringe perpendicular to the tooth surface at a distance of approximately 0.5 cm). Neighboring teeth will be protected with cotton rolls or the examiner's fingers[12]. The volunteer will then indicate a whole number between 0 (absence of pain) to 10 (worst pain possible) on the 10-cm VAS scale that best describes his/her perception of pain. The data will be stored in appropriate files with the identification of each patient.

Allocation and blinding

The participants will be randomly assigned to three experimental groups and a control group. Randomization will be performed using opaque envelopes stratified based on the VAS scores. Briefly, the mean of the initial VAS scores will be determined for each subject and the participants will be randomly allocated to the experimental groups ensuring similar initial VAS scores among the different treatments. All teeth with hypersensitivity in each volunteer will be evaluated using the VAS and the mean will be considered for the initial score. All teeth with hypersensitivity will be treated and reassessed using the VAS. The mean will then be calculated for the final sensitivity score.

Two weeks prior to the onset of the study, the volunteers will undergo a wash-out period, in which they will only use oral hygiene products donated by the researchers. These products will be used through to the end of the study. The oral hygiene kit will contain a soft-bristle toothbrush (Professional Lab Series, Colgate Palmolive), fluoride toothpaste with no desensitizing agent (Elmex) and dental floss (Colgate). The participants will be extensively trained with regards to all procedures involved in the experiment.

All volunteers and the evaluator of the degree of sensitivity will be blinded to the allocation. The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products.

Procedures

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence regarding treatment with LLL. The neural desensitizing protocol will involve the use of LLL

(Therapy XT, DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent). The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W. The participants will be allocated to four groups according to the proposed treatments.

Control Group

Group 1 will be the control group, which will receive no treatment. The volunteers in this group will receive the same instructions as the other groups and will undergo both treatments, except that water will be used instead of the sealant and the laser device will be set to a power of 0 W. In other words, the same irradiation procedure will be performed but without the emission of light.

Sealant Group

The volunteers in Group 2 will receive treatment with sealant (Permaseal - Ultradent), which is a photopolymerizable methacrylate-based resin. The teeth to be sealed will be isolated and 35% phosphoric acid will be applied for 20 seconds, followed by rinsing and drying the dental surfaces. A thin layer of PermaSeal will be applied to the tooth surface for 5 seconds and photopolymerized for 20 seconds. The occlusion will then be evaluated. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser Group

The volunteers in Group 3 will receive irradiation with AsGaAl laser at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil) with relative isolation. A power meter (Laser Check, MMOptics, São Carlos, SP, Brazil) will be used to determine the power of the equipment before and after all treatment interventions. The power will be set to 100 mW; energy density will be 35 J/cm^2 (considering a spot size of 0.028 cm^2 of the equipment) and the dose will be 1 J per point. Irradiation will be performed on a cervical point, an apical

point and a point precisely over the lesion, totaling a dose of 3 J. Treatment will be performed in three sessions with a 72-hour interval between sessions.

During the laser treatments, both the volunteer and operator will use protective eyewear and all rules of safety will be obeyed. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser and sealant Group

The volunteers in Group 4 will receive the same irradiation administered to Group 3. During the last session, these volunteers will also receive the same sealant applied in Group 2. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Data analysis and statistical evaluation

DH will be determined by a researcher who will be blinded to the treatments. This evaluation will be performed 15 min after the application of the treatments (Mehta et al. 2014) using the same method described above for the determination of the initial degree of sensitivity. The volunteers will be reevaluated for the determination of sensitivity one week, one month, three months and six months after the treatments. These evaluations will be recorded on blank sheets of paper that will only indicate the number of the volunteer and the tooth evaluated.

The initial VAS score will be subtracted from the scores obtained one week and four weeks after treatment in all groups and the data will be submitted to statistical analysis. Descriptive statistics will involve the calculation of mean and standard deviation values. Inferential statistics will involve tests of normality and equal variance, which will determine the appropriate statistical tests. The level of significance will be set to 5%. The three-month and six-month data from the three experimental groups (not the placebo group) will be analyzed in the same way.

DISCUSSION

Despite the wide variety of available therapeutic methods, dentin hypersensitivity is still considered a chronic dental problem with an uncertain prognosis. The efficacy of treatment

(reduction in thermal and tactile sensitivity) with the use of LLL has been demonstrated in the literature. The proposed study will enable the determination of differences in the effectiveness of the proposed treatments as well as differences among the evaluation times for each treatment.

Author Contributons: Substantial contributions to the conception: APTS, EMS and SKB. Design of the work: APTS, ELM, ACCA, PVS, CMM, MLLG and SKB. Drafting the work: APTS, EMS and SKB. Revising the work: APTS, EMS, ACCA, PVS, CMM, MLLG, RAR, LJM, ACRTH, KPSF, RAMF and SKB. Final approval of the work: APTS, EMS, CMM, MLLG and SKB.

Ethics approval and consent to participate: This study was approved the Universidade Metropolitana de Santos - UNIMES- Ethics Committee - Protocol Number: 4.020.261. All participants will provide informed consent before participating in this study.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest: None declared. The authors have no conflict of interest, financial or otherwise to declare.

Availability of data and materials: Not appropriate. This paper is a protocol description and does not contain any data.

Data sharing statement: The original protocol and substantive amendments are available. These were available for the included authors and the local medical ethical committee.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Pag
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	ok 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	ok 2
	2b	All items from the World Health Organization Trial Registration Data Set	ok 6
Protocol version	3	Date and version identifier	ok 4
Funding	4	Sources and types of financial, material, and other support	NA
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	ok 11
	5b	Name and contact information for the trial sponsor	NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	NA
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	ok 4
	6b	Explanation for choice of comparators	ok 4
Objectives	7	Specific objectives or hypotheses	ok 4
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	ok 4
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	ok 5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	ok 7

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	ok	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	ok	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	ok	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	ok	9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	ok	5
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	ok	5
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	ok	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	ok	7
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	ok	8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	ok	8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	ok	8
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	ok	8
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	ok	8
Methods: Data collection, management, and analysis				

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	ok	10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	ok	6
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	ok	6
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	ok	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	ok	8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	ok	8
Methods: Monitoring				
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA	
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	ok	6
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	ok	6
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	ok	6
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	ok	6
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	ok	6
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	ok	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA	

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	ok	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	NA	
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	ok	11
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA	
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	ok	6
	31b	Authorship eligibility guidelines and any intended use of professional writers	ok	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	ok	11
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	ok	6
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA	

∗It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked anddated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons“Attribution-NonCommercial-NoDerivs 3.0 Unported”license.NA=not applicable.

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COMBINED PROTOCOL FOR THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introduction Dentin hypersensitivity (DH) is defined as high sensitivity of the vital dentin when exposed to thermal, chemical or tactile stimuli. Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp. Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel and, in most cases, is accompanied by DH. The control of tooth sensitivity is fundamental to the successful treatment of MIH. The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of DH in patients with teeth affected by MIH.

Methods and analysis One hundred and forty patients who meet the inclusion criteria will be allocated to four groups. Group 1 will be the control group (placebo). In Group 2, sensitive teeth will be sealed with Permaseal (Ultradent). In Group 3, sensitive teeth will receive low-level laser (AsGaAl) at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil). In Group 4, sensitive teeth will be treated with both LLL and Permaseal (Ultradent). DH will be evaluated 15 min after the application of the treatments and the patients will be reevaluated one week, one month, three months and six months after the treatments. The primary outcome of this study is change in pain/sensitivity, when evaluated through a Visual Analog Scale (VAS), to determine the effectiveness of the proposed treatments, as well as differences among the evaluation times for each proposed treatment.

Ethics and dissemination This protocol has been ethically approved by the local medical ethical committee Protocol Number: 4.020.261. Results will be submitted to international peer-reviewed journals and presented at international conferences.

Trial registration: NCT04407702

Strengths and Limitations

- The randomized design that will be used to compare different treatment protocols is a strength.
- Another strength lies in the fact that the patients and the evaluator of the degree of sensitivity will be blinded to the allocation.
- The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products, what is a limitation.
- The fact that the treatments are very different among themselves is believed to be another strength, seeing as different results are expected to be achieved.
- Besides the evaluation of the treatment separately, the evaluation of their combined use is another strength.

INTRODUCTION

Dentin hypersensitivity (DH) is defined as high sensitivity of vital dentin exposed to thermal, chemical or tactile stimuli. The exposure of the dentinal tubules causes a reduction in the pain threshold, leading to a response from nerves in the pulp characterized by a rapid, acute, intense pain associated with the mechanoreceptor hydrodynamic mechanism. The proper diagnosis is essential to the establishment of adequate treatment. [1-2]

Brännström's hydrodynamic theory is the most widely accepted for explaining mechanisms that involve the triggering of pain sensations in DH. According to this theory, sensitivity is the result of the rapid movement of the fluid contained in the interior of the dentinal tubules. When a stimulus is applied to the dentin, the fluids within the tubules move both toward the pulp and in the opposite direction, producing a mechanical deformation of the nerve fibers found in the interior of the tubules or at the pulp/dentin interface, which is transmitted to the central nervous system as a sensation of pain.[3] Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp.[4]

Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel. Clinically, MIH presents as yellowish-white or brownish demarcations larger than 1 mm that involve the permanent first molars and, occasionally, the incisors. This condition is generally accompanied by DH, frail enamel and high susceptibility to caries.[5]

Raposo et al. (2019) investigated the prevalence of DH in molars affected by MIH and concluded that hypersensitivity was significantly greater on affected teeth than unaffected teeth. The authors also found an association between mild to moderate cases and DH, which could not be proven for severe cases due to the high frequency of cavities.[6] The principal aim of the treatment of DH is to improve quality of life through the control of pain by suppressing nerve impulses or by the obliteration of the dentinal tubules.[7]

It is believed that the exacerbated sensitivity that the teeth affected by MIH may present can be explained by the high porosity of the affected area, which allows microorganisms to penetrate the enamel and reach the dentinal tubules, causing a subclinical inflammatory reaction of the pulp cells.[8,9]

There is not a defined protocol in the literature for the treatment of DH, but some alternatives have been proposed, such as: fluoride varnishes, occlusal sealants, arginine/calcium carbonate products and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).[7,10-12] However, it is important to emphasize that we do not have enough evidence and therefore it cannot be said that one treatment is more effective than the other.[13]

Fragelli *et al.* (2017) followed the adhesion resistance of resin sealants on first molars affected by MIH for 18 months and concluded that there was no significant difference in durability between affected and unaffected molars, what suggests that they may be a good option for treatment.[14]

Low-level laser (LLL) has been used as treatment for DH. In a literature review, Shintome et al. concluded that both high- and low-level lasers are effective in the treatment of cervical DH. The reviewed studies also reported that treatment with laser offers greater patient comfort and enables longer lasting results in comparison to dentifrices and desensitizing agents, as light acts directly on the dental tissue, promoting morphological changes in the dentin, stimulating the pulp tissue and making treatment more lasting.[15]

The control of tooth sensitivity is a fundamental factor to the successful treatment of MIH. Therefore, this paper proposes the evaluation of the effectiveness of different protocols for the control of DH.

METHODS

Overview

The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of dentin hypersensitivity in patients with teeth affected by MIH. This protocol follows the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations displayed in Table 1.

Table 1. Schedule of enrolment, interventions, and assessments of the study.

			STUDY PERIOD					
	Enrolment	Allocation	Post-allocation					Close-out
			0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:								
Eligibility screen	x							
Informed consent	x							
Allocation		x						
INTERVENTIONS:								
Control			x	x	x	x	x	x
Sealant			x	x	x	x	x	x
Low level laser			x	x	x	x	x	x
Low-level laser and sealant			x	x	x	x	x	x
ASSESSMENTS:								
Pain			x	x	x	x	x	x

* 0 = Baseline, t_1 = immediately after treatment, t_2 = 01 week after the treatment, t_3 = 01 month after the treatment, t_4 = 03 months after the treatment, t_5 = 06 months after the treatment

The study will be conducted in accordance with the ethical precepts stipulated in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee (Certificate of Presentation for Ethical Appreciation n. 31651120.7.0000.5509 / Report n. 4.020.261 – Universidade Metropolitana de Santos - UNIMES) and this protocol is registered at ClinicalTrials.gov; the registration number is NCT04407702 and it was first posted on 05/25/2020 and last updated on 11/25/2020. The patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group. All information will be delineated in the statement of informed consent (Resolution N° 196 of the National Board of Health, Ministry of Health, Brazil, 10/03/1996). Two copies of the statement will be signed – one for the volunteer and one of the researchers. The statement will declare the commitment on the part of the researchers to provide adequate treatment for the participants in the placebo group at the end of the study and to the subjects of the other groups at the end of six months,

if the pain symptoms have not improved. The treatment offered, in this case, will be that which achieved the best result in the initial four weeks of the study. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire. The researchers will also be able to remove the participants from the study, if deemed necessary.

Participants

Patients between between 18 and 35 years of age selected at the Clinic of the School of Dentistry of UNIMES (Santos, SP, Brazil) will participate in the study. Data collection will also take place in this same location. The sample size was determined based on the primary outcome of the study: final pain visual analog scale. Based on the data from[16], our initial sample size estimation was of 24 subjects per group for a significant level of 0.05 and an estimated test power of 80%. To account for the possible non-parametric distribution of the data, 15% more subjects must be added to each group.[17] Another 25% will be added to account for possible dropouts, resulting in 35 subjects per group. G*Power 3.1.9.6 was used to perform the calculations.

Patient and public involvement statement

Patients will not be involved in the conceptualization of the study design, nor will they conduct it. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it.

Experimental design

A randomized, double-blind (subject and evaluator of pain), parallel, interventional study will be conducted at the dental clinic of UNIMES. The study will be conducted following the CONSORT guidelines (http://www.consort-statement.org/).[18] Pain will be evaluated using the visual analog scale (VAS) after stimulation with compressed air from the triple syringe and using an exploratory probe at the time of recruitment (baseline), immediately after treatment as well as one week, one month, three months and six months after treatment. The study will follow the flowchart presented in Figure 1.

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

Preclinical trial

During the first visit to the clinic, a form addressing the volunteer's medical history will be filled out. Each volunteer will then be submitted to a clinical examination for the determination of his/her oral status. Next, the inclusion and exclusion criteria will be applied to determine the eligibility of the volunteers.

Inclusion Criteria

- Age between 18 and 35 years;
- Good overall health;
- At least one tooth with MIH and DH reported in the cervical region with sensitivity equal to or greater than 4 on the VAS.

Exclusion Criteria

- Active caries or defective restorations on the tooth to be analyzed;
- Sufficient dentin loss that requires restorative treatment or periodontal surgery;
- Having undergone any professional desensitizing treatment in the previous six months;
- Having used a desensitizing paste in the previous three months;
- Use of anti-inflammatory drugs or analgesics at the time of recruitment;
- Currently pregnant or nursing.

Recruitment

Recruitment will be made through announcements on the clinic (pictures and publications). An initial evaluation will be performed by a researcher trained to diagnosis MIH. After the volunteer reports the occurrence a tooth with hypersensitivity, the researcher will assess the sensitive tooth using cold air from the triple syringe (2 s of compressed air at a pressure of approximately 40 psi with the syringe perpendicular to the tooth surface at a distance of approximately 0.5 cm). Neighboring teeth will be protected with cotton rolls or the examiner's fingers[19]. The volunteer will then indicate a whole number between 0 (absence of pain) to 10 (worst pain possible) on the 10-cm VAS scale that best describes his/her

perception of pain. The data will be stored in appropriate files with the identification of each patient. The possible decrease in sensitivity will, probably, increase adherence to the protocol.

Allocation and blinding

The participants will be randomly assigned to three experimental groups and a control group. For the random distribution of volunteers, randomization will be carried out by drawing lots using the website www.randomizer.org. Briefly, the mean of the initial VAS scores will be determined for each subject and the participants will be randomly allocated to the experimental groups ensuring similar initial VAS scores among the different treatments. All teeth with hypersensitivity in each volunteer will be evaluated using the VAS and the mean will be considered for the initial score. All teeth with hypersensitivity will be treated and reassessed using the VAS. The mean will then be calculated for the final sensitivity score.

Two weeks prior to the onset of the study, the volunteers will undergo a wash-out period, in which they will only use oral hygiene products donated by the researchers. These products will be used through to the end of the study. The oral hygiene kit will contain a soft-bristle toothbrush (Professional Lab Series, Colgate Palmolive), fluoride toothpaste with no desensitizing agent (Elmex) and dental floss (Colgate). The participants will be extensively trained with regards to all procedures involved in the experiment.

All volunteers and the evaluator of the degree of sensitivity will be blinded to the allocation, they will not know to which group they belong. Unblinding in not permissible. The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products. A researcher who will not be involved in the application of treatments and has no conflicts of interest will be responsible for generating the allocation sequence and assigning patients to treatments.

Recruitment is intended to start on January, 2021, the primary completion anticipated date is August 30, 2021, and the anticipated study completion date is December 10, 2021.

Procedures

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence

regarding treatment with LLL. The neural desensitizing protocol will involve the use of LLL (Therapy XT, DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent). The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W. The participants will be allocated to four groups according to the proposed treatments. If, by request, participants decide to withdraw from the study, they will suffer no harm. However, improvement is sensitivity and its follow-ups should encourage patients to keep coming. Data from those who choose to discontinue will not be used for analysis. No adverse effects are expected from any of the treatments.

Control Group

Group 1 will be the control group, which will receive no treatment. The volunteers in this group will receive the same instructions as the other groups and will undergo both treatments, except that water will be used instead of the sealant and the laser device will be set to a power of 0 W. In other words, the same irradiation procedure will be performed but without the emission of light.

Sealant Group

The volunteers in Group 2 will receive treatment with sealant (Permaseal - Ultradent), which is a photopolymerizable methacrylate-based resin. The teeth to be sealed will be isolated and 35% phosphoric acid will be applied for 20 seconds, followed by rinsing and drying the dental surfaces. A thin layer of PermaSeal will be applied to the tooth surface for 5 seconds and photopolymerized for 20 seconds. The occlusion will then be evaluated. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser Group

The volunteers in Group 3 will receive irradiation with AsGaAl laser at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil) with relative isolation. A power meter (Laser Check, MMOptics, São Carlos, SP, Brazil) will be used to determine the power of the

equipment before and after all treatment interventions. The power will be set to 100 mW; energy density will be 35 J/cm^2 (considering a spot size of 0.028 cm^2 of the equipment) and the dose will be 1 J per point. Irradiation will be performed on a cervical point, an apical point and a point precisely over the lesion, totaling a dose of 3 J. Treatment will be performed in three sessions with a 72-hour interval between sessions.

During the laser treatments, both the volunteer and operator will use protective eyewear and all rules of safety will be obeyed. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser and sealant Group

The volunteers in Group 4 will receive the same irradiation administered to Group 3. During the last session, these volunteers will also receive the same sealant applied in Group 2. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Data analysis and statistical evaluation

DH will be determined by a researcher who will be blinded to the treatments. This evaluation will be performed 15 min after the application of the treatments (Mehta et al. 2014) using the same method described above for the determination of the initial degree of sensitivity. The volunteers will be reevaluated for the determination of sensitivity one week, one month, three months and six months after the treatments. These evaluations will be recorded on blank sheets of paper that will only indicate the number of the volunteer and the tooth evaluated. The initial VAS score will be subtracted from the scores obtained one week and four weeks after treatment in all groups and the data will be submitted to statistical analysis. The change in sensitivity evaluated through VAS in the different groups and periods is the primary outcome of the study, as it is the main objective of the treatments. Descriptive statistics will involve the calculation of mean and standard deviation values. Inferential statistics will involve tests of normality and equal variance, which will determine the appropriate statistical tests. The level of significance will be set to 5%. The three-month and six-month data from the three experimental groups (not the placebo group) will be analyzed in the same way.

Author Contributons: Substantial contributions to the conception: APTS, EMS and SKB. Design of the work: APTS, EMS, ACA, PVS, CMM, MLLG and SKB. Drafting the work: APTS, EMS and SKB. Revising the work: APTS, EMS, ACA, PVS, CMM, MLLG, RAR, LJM, ACRTH, KPSF, RAMF and SKB. Final approval of the work: APTS, EMS, CMM, MLLG and SKB. In case stopping the trial is necessary, these authors will have access to these interim results and make the final decision to terminate the trial.

Ethics approval and consent to participate: This study was approved the Universidade Metropolitana de Santos - UNIMES- Ethics Committee - Protocol Number: 4.020.261. All participants will provide informed consent before participating in this study. Any changes to this protocol will also be reported to this same committee.

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Competing interest: None declared. The authors have no conflict of interest, financial or otherwise to declare.

Availability of data and materials: This paper is a protocol description and does not contain any data to this point. Future data will be available, as described in the Data sharing statement below.

Data sharing statement: The datasets (Excel spreadsheets) generated from this protocol will be available with the corresponding author (Sandra Kalil Bussadori – sandra.skb@gmail.com) at a reasonable request. However, reuse of this data will not be allowed for people who are not authors of this paper.

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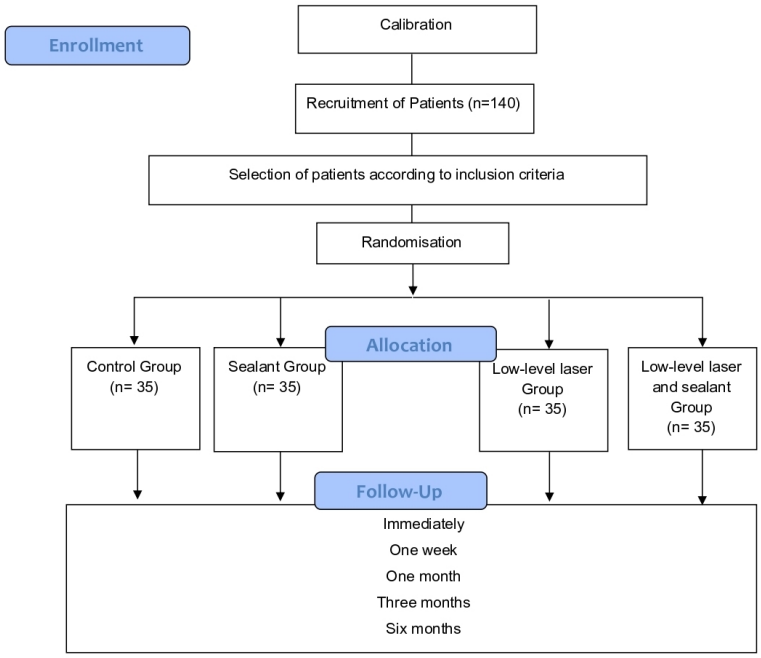

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

107x139mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description		Pag
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	ok	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	ok	2
	2b	All items from the World Health Organization Trial Registration Data Set	ok	6
Protocol version	3	Date and version identifier	ok	6
Funding	4	Sources and types of financial, material, and other support		NA
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	ok	12
	5b	Name and contact information for the trial sponsor		NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Ok	12
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Ok	12
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	ok	4
	6b	Explanation for choice of comparators	ok	4/5
Objectives	7	Specific objectives or hypotheses	ok	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	ok	5
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	ok	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	ok	8/9

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	ok	10/11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	ok	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	ok	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	ok	9/10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	ok	12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	ok	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	ok	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	ok	9
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	ok	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	ok	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	ok	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	ok	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	ok	10
Methods: Data collection, management, and analysis				

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	ok	12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	ok	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	ok	13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	ok	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)		NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)		10
Methods: Monitoring				
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed		12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	ok	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	ok	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	ok	7
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	ok	2/6/12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	ok	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	ok	6/12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable		NA

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	ok	13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	ok	13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	ok	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Ok	7
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	ok	7
	31b	Authorship eligibility guidelines and any intended use of professional writers	ok	12
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	ok	13
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates		NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA	

∗It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked anddated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons"Attribution-NonCommercial-NoDerivs 3.0 Unported"license.NA=not applicable.

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COMBINED PROTOCOL FOR THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: RANDOMIZED CONTROLLED CLINICAL TRIAL

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COMBINED PROTOCOL FOR THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introduction Dentin hypersensitivity (DH) is defined as high sensitivity of the vital dentin when exposed to thermal, chemical or tactile stimuli. Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp. Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel and, in most cases, is accompanied by DH. The control of tooth sensitivity is fundamental to the successful treatment of MIH. The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of DH in patients with teeth affected by MIH.

Methods and analysis One hundred and forty patients who meet the inclusion criteria will be allocated to four groups. Group 1 will be the control group (placebo). In Group 2, sensitive teeth will be sealed with Permaseal (Ultradent). In Group 3, sensitive teeth will receive low-level laser (AsGaAl) at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil). In Group 4, sensitive teeth will be treated with both LLL and Permaseal (Ultradent). DH will be evaluated 15 min after the application of the treatments and the patients will be reevaluated one week, one month, three months and six months after the treatments. The primary outcome of this study is change in pain/sensitivity, when evaluated through a Visual Analog Scale (VAS), to determine the effectiveness of the proposed treatments, as well as differences among the evaluation times for each proposed treatment.

Ethics and dissemination This protocol has been ethically approved by the local medical ethical committee Protocol Number: 4.020.261. Results will be submitted to international peer-reviewed journals and presented at international conferences.

Trial registration: NCT04407702

Strengths and Limitations

- The randomized design that will be used to compare different treatment protocols is a strength.
- Another strength lies in the fact that the patients and the evaluator of the degree of sensitivity will be blinded to the allocation.
- The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products, which is a limitation.
- Another strength lies in the fact that we are comparing a more conventional treatment (sealant), with a more current therapy (LLL), bringing a sense of innovation to the study.
- Besides the evaluation of the treatment separately, the evaluation of their combined use is another strength.

INTRODUCTION

Dentin hypersensitivity (DH) is defined as high sensitivity of vital dentin exposed to thermal, chemical or tactile stimuli. The exposure of the dentinal tubules causes a reduction in the pain threshold, leading to a response from nerves in the pulp characterized by a rapid, acute, intense pain associated with the mechanoreceptor hydrodynamic mechanism. The proper diagnosis is essential to the establishment of adequate treatment. [1-2]

Brännström's hydrodynamic theory is the most widely accepted for explaining mechanisms that involve the triggering of pain sensations in DH. According to this theory, sensitivity is the result of the rapid movement of the fluid contained in the interior of the dentinal tubules. When a stimulus is applied to the dentin, the fluids within the tubules move both toward the pulp and in the opposite direction, producing a mechanical deformation of the nerve fibers found in the interior of the tubules or at the pulp/dentin interface, which is transmitted to the central nervous system as a sensation of pain.[3] Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp.[4]

Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel. Clinically, MIH presents as yellowish-white or brownish demarcations larger than 1 mm that involve the permanent first molars and, occasionally, the incisors. This condition is generally accompanied by DH, frail enamel and high susceptibility to caries.[5]

Raposo et al. (2019) investigated the prevalence of DH in molars affected by MIH and concluded that hypersensitivity was significantly greater on affected teeth than unaffected teeth. The authors also found an association between mild to moderate cases and DH, which could not be proven for severe cases due to the high frequency of cavities.[6] The principal aim of the treatment of DH is to improve quality of life through the control of pain by suppressing nerve impulses or by the obliteration of the dentinal tubules.[7]

It is believed that the exacerbated sensitivity that the teeth affected by MIH may present can be explained by the high porosity of the affected area, which allows microorganisms to penetrate the enamel and reach the dentinal tubules, causing a subclinical inflammatory reaction of the pulp cells.[8,9]

There is not a defined protocol in the literature for the treatment of DH, but some alternatives have been proposed, such as: fluoride varnishes, occlusal sealants, arginine/calcium carbonate products and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).[7,10-12] However, it is important to emphasize that we do not have enough evidence and therefore it cannot be said that one treatment is more effective than the other.[13]

Fragelli *et al.* (2017) followed the adhesion resistance of resin sealants on first molars affected by MIH for 18 months and concluded that there was no significant difference in durability between affected and unaffected molars, what suggests that they may be a good option for treatment.[14]

Low-level laser (LLL) has been used as treatment for DH. In a literature review, Shintome et al. concluded that both high- and low-level lasers are effective in the treatment of cervical DH. The reviewed studies also reported that treatment with laser offers greater patient comfort and enables longer lasting results in comparison to dentifrices and desensitizing agents, as light acts directly on the dental tissue, promoting morphological changes in the dentin, stimulating the pulp tissue and making treatment more lasting.[15]

The control of tooth sensitivity is a fundamental factor to the successful treatment of MIH. Therefore, this paper proposes the evaluation of the effectiveness of different protocols for the control of DH.

METHODS

Overview

The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of dentin hypersensitivity in patients with teeth affected by MIH. This protocol follows the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations displayed in Table 1.

Table 1. Schedule of enrolment, interventions, and assessments of the study.

			STUDY PERIOD					
	Enrolment	Allocation	Post-allocation					Close-out
			0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:								
Eligibility screen	x							
Informed consent	x							
Allocation		x						
INTERVENTIONS:								
Control			x	x	x	x	x	x
Sealant			x	x	x	x	x	x
Low level laser			x	x	x	x	x	x
Low-level laser and sealant			x	x	x	x	x	x
ASSESSMENTS:								
Pain			x	x	x	x	x	x

* 0 = Baseline, t_1 = immediately after treatment, t_2 = 01 week after the treatment, t_3 = 01 month after the treatment, t_4 = 03 months after the treatment, t_5 = 06 months after the treatment

The study will be conducted in accordance with the ethical precepts stipulated in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee (Certificate of Presentation for Ethical Appreciation n. 31651120.7.0000.5509 / Report n. 4.020.261 – Universidade Metropolitana de Santos - UNIMES) and this protocol is registered at ClinicalTrials.gov; the registration number is NCT04407702 and it was first posted on 05/25/2020 and last updated on 11/25/2020. The patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group. All information will be delineated in the statement of informed consent (Resolution N° 196 of the National Board of Health, Ministry of Health, Brazil, 10/03/1996) and the explanations in order to obtain this consent will be made by the same researcher that will apply the treatments. Two copies of the statement will be signed – one for the volunteer and one of the researchers. This consent form has been submitted as a Supplementary File. The statement will declare the commitment

on the part of the researchers to provide adequate treatment for the participants in the placebo group at the end of the study and to the subjects of the other groups at the end of six months, if the pain symptoms have not improved. The treatment offered, in this case, will be that which achieved the best result in the initial four weeks of the study. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire. The researchers will also be able to remove the participants from the study, if deemed necessary.

Participants

Patients between between 18 and 35 years of age selected at the Clinic of the School of Dentistry of UNIMES (Santos, SP, Brazil) will participate in the study. Data collection will also take place in this same location. The sample size was determined based on the primary outcome of the study: final pain visual analog scale. Based on the data from[16], our initial sample size estimation was of 24 subjects per group for a significant level of 0.05 and an estimated test power of 80%. To account for the possible non-parametric distribution of the data, 15% more subjects must be added to each group.[17] Another 25% will be added to account for possible dropouts, resulting in 35 subjects per group. G*Power 3.1.9.6 was used to perform the calculations.

Patient and public involvement statement

Patients will not be involved in the conceptualization of the study design, nor will they conduct it. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it.

Experimental design

A randomized, double-blind (subject and evaluator of pain), parallel, interventional study will be conducted at the dental clinic of UNIMES. The study will be conducted following the CONSORT guidelines (http://www.consort-statement.org/).[18] Pain will be evaluated using the visual analog scale (VAS) after stimulation with compressed air from the triple syringe and using an exploratory probe at the time of recruitment (baseline), immediately after treatment as well as one week, one month, three months and six months after treatment. The study will follow the flowchart presented in Figure 1.

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

Preclinical trial

During the first visit to the clinic, a form addressing the volunteer's medical history will be filled out. Each volunteer will then be submitted to a clinical examination for the determination of his/her oral status. Next, the inclusion and exclusion criteria will be applied to determine the eligibility of the volunteers.

Inclusion Criteria

- Age between 18 and 35 years;
- Good overall health;
- At least one tooth with MIH and DH reported in the cervical region with sensitivity equal to or greater than 4 on the VAS.

Exclusion Criteria

- Active caries or defective restorations on the tooth to be analyzed;
- Sufficient dentin loss that requires restorative treatment or periodontal surgery;
- Having undergone any professional desensitizing treatment in the previous six months;
- Having used a desensitizing paste in the previous three months;
- Use of anti-inflammatory drugs or analgesics at the time of recruitment;
- Currently pregnant or nursing.

Recruitment

Recruitment will be made through announcements on the clinic (pictures and publications). An initial evaluation will be performed by a researcher trained to diagnosis MIH. After the volunteer reports the occurrence a tooth with hypersensitivity, the researcher will assess the sensitive tooth using cold air from the triple syringe (2 s of compressed air at a pressure of approximately 40 psi with the syringe perpendicular to the tooth surface at a distance of approximately 0.5 cm). Neighboring teeth will be protected with cotton rolls or the

examiner's fingers[19]. The volunteer will then indicate a whole number between 0 (absence of pain) to 10 (worst pain possible) on the 10-cm VAS scale that best describes his/her perception of pain. The data will be stored in appropriate files with the identification of each patient. The possible decrease in sensitivity will, probably, increase adherence to the protocol.

Allocation and blinding

The participants will be randomly assigned to three experimental groups and a control group. For the random distribution of volunteers, randomization will be carried out by drawing lots using the website www.randomizer.org. Briefly, the mean of the initial VAS scores will be determined for each subject and the participants will be randomly allocated to the experimental groups ensuring similar initial VAS scores among the different treatments. All teeth with hypersensitivity in each volunteer will be evaluated using the VAS and the mean will be considered for the initial score. All teeth with hypersensitivity will be treated and reassessed using the VAS. The mean will then be calculated for the final sensitivity score.

Two weeks prior to the onset of the study, the volunteers will undergo a wash-out period, in which they will only use oral hygiene products donated by the researchers. These products will be used through to the end of the study. The oral hygiene kit will contain a soft-bristle toothbrush (Professional Lab Series, Colgate Palmolive), fluoride toothpaste with no desensitizing agent (Elmex) and dental floss (Colgate). The participants will be extensively trained with regards to all procedures involved in the experiment.

All volunteers and the evaluator of the degree of sensitivity will be blinded to the allocation, they will not know to which group they belong. Unblinding in not permissible. The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products. A researcher who will not be involved in the application of treatments and has no conflicts of interest will be responsible for generating the allocation sequence and assigning patients to treatments.

Recruitment is intended to start on January, 2021, the primary completion anticipated date is August 30, 2021, and the anticipated study completion date is December 10, 2021.

Procedures

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence regarding treatment with LLL. The neural desensitizing protocol will involve the use of LLL (Therapy XT, DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent). The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W. The participants will be allocated to four groups according to the proposed treatments. If, by request, participants decide to withdraw from the study, they will suffer no harm. However, improvement is sensitivity and its follow-ups should encourage patients to keep coming. Data from those who choose to discontinue will not be used for analysis. No adverse effects are expected from any of the treatments.

Control Group

Group 1 will be the control group, which will receive no treatment. The volunteers in this group will receive the same instructions as the other groups and will undergo both treatments, except that water will be used instead of the sealant and the laser device will be set to a power of 0 W. In other words, the same irradiation procedure will be performed but without the emission of light.

Sealant Group

The volunteers in Group 2 will receive treatment with sealant (Permaseal - Ultradent), which is a photopolymerizable methacrylate-based resin. The teeth to be sealed will be isolated and 35% phosphoric acid will be applied for 20 seconds, followed by rinsing and drying the dental surfaces. A thin layer of PermaSeal will be applied to the tooth surface for 5 seconds and photopolymerized for 20 seconds. The occlusion will then be evaluated. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser Group

The volunteers in Group 3 will receive irradiation with AsGaAl laser at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil) with relative isolation. A power meter (Laser Check, MMOptics, São Carlos, SP, Brazil) will be used to determine the power of the equipment before and after all treatment interventions. The power will be set to 100 mW; energy density will be 35 J/cm^2 (considering a spot size of 0.028 cm^2 of the equipment) and the dose will be 1 J per point. Irradiation will be performed on a cervical point, an apical point and a point precisely over the lesion, totaling a dose of 3 J. Treatment will be performed in three sessions with a 72-hour interval between sessions.

During the laser treatments, both the volunteer and operator will use protective eyewear and all rules of safety will be obeyed. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser and sealant Group

The volunteers in Group 4 will receive the same irradiation administered to Group 3. During the last session, these volunteers will also receive the same sealant applied in Group 2. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Data analysis and statistical evaluation

DH will be determined by a researcher who will be blinded to the treatments. This evaluation will be performed 15 min after the application of the treatments (Mehta et al. 2014) using the same method described above for the determination of the initial degree of sensitivity. The volunteers will be reevaluated for the determination of sensitivity one week, one month, three months and six months after the treatments. These evaluations will be recorded on blank sheets of paper that will only indicate the number of the volunteer and the tooth evaluated.

The initial VAS score will be subtracted from the scores obtained one week and four weeks after treatment in all groups and the data will be submitted to statistical analysis. The change in sensitivity evaluated through VAS in the different groups and periods is the primary outcome of the study, as it is the main objective of the treatments. Descriptive statistics will

involve the calculation of mean and standard deviation values. Inferential statistics will involve tests of normality and equal variance, which will determine the appropriate statistical tests. The level of significance will be set to 5%. The three-month and six-month data from the three experimental groups (not the placebo group) will be analyzed in the same way.

Author Contributons: Substantial contributions to the conception: APTS, EMS and SKB. Design of the work: APTS, EMS, ACA, PVS, CMM, MLLG and SKB. Drafting the work: APTS, EMS and SKB. Revising the work: APTS, EMS, ACA, PVS, CMM, MLLG, RAR, LJM, ACRTH, KPSF, RAMF and SKB. Final approval of the work: APTS, EMS, CMM, MLLG and SKB. In case stopping the trial is necessary, these authors will have access to these interim results and make the final decision to terminate the trial.

Ethics approval and consent to participate: This study was approved the Universidade Metropolitana de Santos - UNIMES- Ethics Committee - Protocol Number: 4.020.261. All participants will provide informed consent before participating in this study. Any changes to this protocol will also be reported to this same committee.

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Competing interest: None declared. The authors have no conflict of interest, financial or otherwise to declare.

Availability of data and materials: This paper is a protocol description and does not contain any data to this point. Future data will be available, as described in the Data sharing statement below.

Data sharing statement: The datasets (Excel spreadsheets) generated from this protocol will be available with the corresponding author (Sandra Kalil Bussadori – sandra.skb@gmail.com) at a reasonable request. However, reuse of this data will not be allowed for people who are not authors of this paper.

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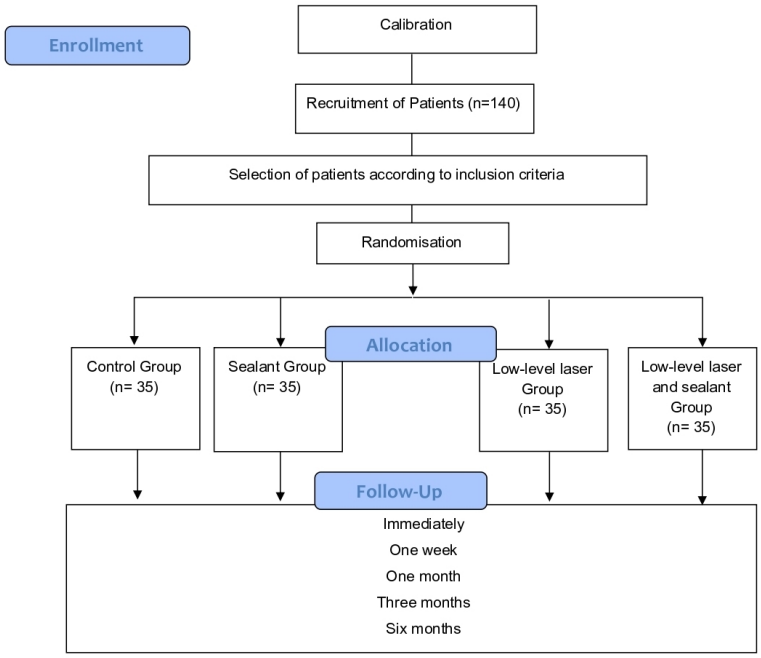

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

107x139mm (300 x 300 DPI)

Universidade Metropolitana de Santos - UNIMES

RESEARCH ETHICS COMMITTEE

FREE AND CLARIFIED CONSENT

I - DATA IDENTIFYING THE SUBJECT OF THE RESEARCH OR LEGAL RESPONSIBLE

1. PATIENT'S NAME:

DOCUMENT Nº: GENDER: .M ☐ F ☐

BIRTH DATE (dd / mm / yyyy):

ADDRESS:

NEIGHBORHOOD: CITY

ZIP CODE: PHONE: DDD

2. LEGAL RESPONSIBLE

NATURE (degree of kinship)

DOCUMENT Nº: GENDER: .M ☐ F ☐

BIRTH DATE (dd / mm / yyyy):

ADDRESS:

NEIGHBORHOOD: CITY

ZIP CODE: PHONE: DDD

II - DATA ON SCIENTIFIC RESEARCH

1. RESEARCH PROTOCOL TITLE: Associative Protocol in Dentin Hypersensitivity pain control in patients with IMH: Randomized controlled clinical trial

2. RESEARCHER: Ana Paula Taboada Sobral

POSITION / FUNCTION: Volunteer researcher

REGIONAL COUNCIL REGISTRATION No. 76.693

UNIMES UNIT: **Faculty of Dentistry -** Av. Conselheiro Nébias, 536 - Encruzilhada, Santos - SP, 11045-002-Brazil

3. RESEARCH RISK ASSESSMENT:

WITHOUT RISK x MINIMUM RISK ☐ AVERAGE RISK ☐

LOW RISK ☐ GREATER RISK ☐

(probability that the individual will suffer some damage as an immediate or late consequence of the study)

4.RESEARCH DURATION: 14 months

III - REGISTRATION OF THE RESEARCHER'S EXPLANATIONS TO THE PATIENT OR ITS LEGAL REPRESENTATIVE ON RESEARCH CONSIGNING:

1. **Justification and objectives of the research:**

The control of tooth sensitivity is fundamental to the successful treatment of Molar Incisor Hypomineralization. Therefore, the present study aims to evaluate the effectiveness of different protocols in the control of Dentin Hypersensitivity (MIH)

The objective of this work will be to evaluate, by means of a randomized and controlled clinical study, the effectiveness of different protocols for the control of dentinal hypersensitivity in patients with teeth affected by MIHI. The neural desensitization protocol will involve the use of low-power laser (Therapy XT -DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent).

2. **Procedures that will be used and purposes, including identification of procedures that are experimental:**

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence regarding treatment with low-power laser. The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W.

Participants will be divided into the proposed treatments.

Group 1. Control

Group 2. Permaseal (Sealant Group)

Group 3. Low-power laser

Group 4. Low power laser + Permaseal (Sealant Group)

Patients should return after 1 week, 1 month, 3 months and 6 months after the end of the last session to be assessed according to the visual analogue pain scale.

3. **Expected discomforts and risks:** Volunteers will not be at risk during the procedures.
4. **Benefits that can be obtained:** Treatment of dentin hypersensitivity, reducing painful sensitivity.
5. **Alternative procedures that may be beneficial to the individual:** Alternative methods will not be used.

IV - CLARIFICATIONS GIVEN BY THE RESEARCHER ABOUT GUARANTEES OF THE SUBJECT OF THE RESEARCH CONSIGNING:

1. Access, at any time, to information on procedures, risks and benefits related to research, including to resolve any doubts.

2.The patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group.

3. The researchers will be provide adequate treatment for the participants in the placebo group at the end of the study and to the subjects of the other groups at the end of six months, if the pain symptoms have not improved. The treatment offered, in this case, will be that which achieved the best result in the initial four weeks of the study.

4. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire.

5.The researchers will also be able to remove the participants from the study, if deemed necessary.

6. Safeguarding confidentiality, secrecy and privacy.

7. Availability of assistance, for possible damage to health, resulting from the research.

8 Feasibility of indemnification for possible damage to health resulting from the research.

V. INFORMATION OF NAMES, ADDRESSES AND PHONES OF RESPONSIBLE FOR MONITORING THE RESEARCH, FOR CONTACT IN CASE OF CLINICAL INTERCURRENCES AND ADVERSE REACTIONS.

Profa. Dra. Ana Paula Taboada Sobral

Contacts: (11) 98447-4570 / anapaula@taboada.com.br

Faculty of Dentistry UNIMES - Av. Conselheiro Nébias, 536 - Encruzilhada, Santos - SP-CEP: 11045-002- Brasil

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VI - COMPLEMENTARY OBSERVATIONS:

Not applicable.

VII - POST-CLARED CONSENT

I declare that, after being properly clarified by the researcher and having understood what was explained to me, I consent to participate in this Research Protocol.

Santos, ____/_____/_____.

_____ Signature of the research subject or legal guardian

_____ Signature of the researcher (Legible name or stamp)

BMJ Open

THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: PROTOCOL FOR A RANDOMIZED CONTROLLED CLINICAL TRIAL

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Primary Subject Heading:	Dentistry and oral medicine
Secondary Subject Heading:	Dentistry and oral medicine, Evidence based practice
Keywords:	PAIN MANAGEMENT, ORAL MEDICINE, Laser therapy < DERMATOLOGY

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THE CONTROL OF PAIN DUE TO DENTIN HYPERSENSITIVITY IN INDIVIDUALS WITH MOLAR-INCISOR HYPOMINERALIZATION: PROTOCOL FOR A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introduction Dentin hypersensitivity (DH) is defined as high sensitivity of the vital dentin when exposed to thermal, chemical or tactile stimuli. Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp. Molar-incisor hypomineralization (MIH) is a qualitative abnormality of a genetic origin that affects tooth enamel and, in most cases, is accompanied by DH. The control of tooth sensitivity is fundamental to the successful treatment of MIH. The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of DH in patients with teeth affected by MIH.

Methods and analysis One hundred and forty patients who meet the inclusion criteria will be allocated to four groups. Group 1 will be the control group (placebo). In Group 2, sensitive teeth will be sealed with Permaseal (Ultradent). In Group 3, sensitive teeth will receive low-level laser (AsGaAl) at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil). In Group 4, sensitive teeth will be treated with both LLL and Permaseal (Ultradent). DH will be evaluated 15 minutes after the application of the treatments and the patients will be reevaluated one week, one month, three months and six months after the treatments. The primary outcome of this study is change in pain/sensitivity, when evaluated through a Visual Analog Scale (VAS), to determine the effectiveness of the proposed treatments, as well as differences among the evaluation times for each proposed treatment.

Ethics and dissemination This protocol has been ethically approved by the local medical ethical committee Protocol Number: 4.020.261. Results will be submitted to international peer-reviewed journals and presented at international conferences.

Trial registration: NCT04407702

Strengths and Limitations

- The randomized design that will be used to compare different treatment protocols.
- The patients and the evaluator of the degree of sensitivity will be blinded to the allocation.
- We are comparing a more conventional treatment (sealant), with a more current therapy (LLL), bringing a sense of innovation to the study.
- Besides the evaluation of the treatment separately, the evaluation of their combined use is another strength.
- The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products, which is a limitation.

INTRODUCTION

Dentin hypersensitivity (DH) is defined as high sensitivity of vital dentin exposed to thermal, chemical or tactile stimuli. The exposure of the dentinal tubules causes a reduction in the pain threshold, leading to a response from nerves in the pulp characterized by a rapid, acute, intense pain associated with the mechanoreceptor hydrodynamic mechanism. The proper diagnosis is essential to the establishment of adequate treatment.[1-2]

Brännström's hydrodynamic theory is the most widely accepted for explaining mechanisms that involve the triggering of pain sensations in DH. According to this theory, sensitivity is the result of the rapid movement of the fluid contained in the interior of the dentinal tubules. When a stimulus is applied to the dentin, the fluids within the tubules move both toward the pulp and in the opposite direction, producing a mechanical deformation of the nerve fibers found in the interior of the tubules or at the pulp/dentin interface, which is transmitted to the central nervous system as a sensation of pain.[3] Two mechanisms are required for the occurrence of DH: 1) the dentin must be exposed and 2) the dentinal tubules must be open and connected to the pulp.[4]

Molar-incisor hypomineralization (MIH) is a qualitative abnormality of genetic origin that affects tooth enamel. Clinically, MIH presents itself as yellowish-white or brownish demarcations larger than 1 mm that involve the permanent first molars and, occasionally, the incisors. This condition is generally accompanied by DH, frail enamel and high susceptibility to caries.[5]

Raposo *et al.* (2019) investigated the prevalence of DH in molars affected by MIH and concluded that hypersensitivity was significantly greater on affected teeth than on unaffected teeth. The authors also found an association between mild to moderate cases and DH, which could not be proven for severe cases due to the high frequency of cavities.[6] The principal aim of the treatment of DH is to improve quality of life through the control of pain by suppressing nerve impulses or by the obliteration of the dentinal tubules.[7]

It is believed that the exacerbated sensitivity that the teeth affected by MIH may present can be explained by the high porosity of the affected area, which allows microorganisms to penetrate the enamel and reach the dentinal tubules, causing a subclinical inflammatory reaction of the pulp cells.[8,9]

There is not a defined protocol in the literature for the treatment of DH, but some alternatives have been proposed, such as: fluoride varnishes, occlusal sealants, arginine/calcium carbonate products and Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP).[7,10-12] However, it is important to emphasize that we do not have enough evidence and therefore it cannot be said that one treatment is more effective than the other.[13]

Fragelli *et al.* (2017) followed the adhesion resistance of resin sealants on first molars affected by MIH for 18 months and concluded that there was no significant difference in durability between affected and unaffected molars, what suggests that they may be a good option for treatment.[14]

Low-level laser (LLL) has been used as treatment for DH. In a literature review, Shintome et al. concluded that both high- and low-level lasers are effective in the treatment of cervical DH. The reviewed studies also reported that treatment with laser offers greater patient comfort and enables longer lasting results in comparison to dentifrices and desensitizing agents, as light acts directly on the dental tissue, promoting morphological changes in the dentin, stimulating the pulp tissue and making treatment more lasting.[15]

The control of tooth sensitivity is a fundamental factor to the successful treatment of MIH. Therefore, this paper proposes the evaluation of the effectiveness of different protocols for the control of DH.

METHODS

Overview

The aim of the proposed randomized, controlled, clinical trial is to evaluate the effectiveness of different protocols for the control of dentin hypersensitivity in patients with teeth affected by MIH. This protocol follows the SPIRIT (Standard Protocol Items for Randomized Trials) recommendations, as displayed in Table 1.

Table 1. Schedule of enrolment, interventions, and assessments of the study.

			STUDY PERIOD					
	Enrolment	Allocation	Post-allocation					Close-out
			0	t_1	t_2	t_3	t_4	t_5
ENROLMENT:								
Eligibility screen	x							
Informed consent	x							
Allocation		x						
INTERVENTIONS:								
Control			x	x	x	x	x	x
Sealant			x	x	x	x	x	x
Low level laser			x	x	x	x	x	x
Low-level laser and sealant			x	x	x	x	x	x
ASSESSMENTS:								
Pain			x	x	x	x	x	x

* 0 = Baseline, t_1 = immediately after treatment, t_2 = 01 week after the treatment, t_3 = 01 month after the treatment, t_4 = 03 months after the treatment, t_5 = 06 months after the treatment

The study will be conducted in accordance with the ethical precepts stipulated in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee (Certificate of Presentation for Ethical Appreciation n. 31651120.7.0000.5509 / Report n. 4.020.261 – Universidade Metropolitana de Santos - UNIMES) and this protocol is registered at ClinicalTrials.gov; the registration number is NCT04407702 and it was first posted on 05/25/2020 and last updated on 11/25/2020. Patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group. All information will be delineated in the statement of informed consent (Resolution N° 196 of the National Board of Health, Ministry of Health, Brazil, 10/03/1996) and the explanations in order to obtain this consent will be made by the same researcher that will apply the treatments. Two copies of the statement will be signed – one for the volunteer and one of the researchers. This consent form has been submitted as a Supplementary File. The statement will declare the commitment

on the part of the researchers to provide adequate treatment for the participants in the placebo group at the end of the study and to the subjects of the other groups at the end of six months, if the pain symptoms have not improved. The offered treatment, in this case, will be that which achieved the best result in the initial four weeks of the study. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire. The researchers will also be able to remove the participants from the study, if deemed necessary.

Participants

Patients between between 18 and 35 years of age, selected at the Clinic of the School of Dentistry of UNIMES (Santos, SP, Brazil), will participate in the study. Data collection will also take place in this same location. The sample size was determined based on the primary outcome of the study: final pain visual analog scale. Based on the data from[16], our initial sample size estimation was of 24 subjects per group for a significant level of 0.05 and an estimated test power of 80%. To account for the possible non-parametric distribution of the data, 15% more subjects must be added to each group.[17] Another 25% will be added to account for possible dropouts, resulting in 35 subjects per group. G*Power 3.1.9.6 was used to perform the calculations.

Patient and public involvement statement

Patients will not be involved in the conceptualization of the study design, nor will they conduct it. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it.

Experimental design

A randomized, double-blind (subject and evaluator of pain), parallel, interventional study will be conducted at the dental clinic of UNIMES. The study will be conducted following the CONSORT guidelines (http://www.consort-statement.org/).[18] Pain will be evaluated using the visual analog scale (VAS) after stimulation with compressed air from the triple syringe and using an exploratory probe at the time of recruitment (baseline), immediately after treatment, as well as one week, one month, three months and six months after treatment. The study will follow the flowchart presented in Figure 1.

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

Inclusion/Exclusion criteria

During the first visit to the clinic, a form addressing the volunteer's medical history will be filled out. Each volunteer will then be submitted to a clinical examination for the determination of his/her oral status. Next, the inclusion and exclusion criteria will be applied to determine the eligibility of the volunteers.

Inclusion Criteria

- Age between 18 and 35 years;
- Good overall health;
- At least one tooth with MIH and DH reported in the cervical region with sensitivity equal to or greater than 4 on the VAS.

Exclusion Criteria

- Active caries or defective restorations on the tooth to be analyzed;
- Sufficient dentin loss that requires restorative treatment or periodontal surgery;
- Having undergone any professional desensitizing treatment in the previous six months;
- Having used a desensitizing paste in the previous three months;
- Use of anti-inflammatory drugs or analgesics at the time of recruitment;
- Currently pregnant or nursing.

Recruitment

Recruitment will be made through announcements on the clinic (pictures and publications). An initial evaluation will be performed by a researcher who is trained to diagnosis MIH. After the volunteer reports the occurrence a tooth with hypersensitivity, the researcher will assess the sensitive tooth using cold air from the triple syringe (2 seconds of compressed air at a pressure of approximately 40 psi with the syringe perpendicular to the tooth surface at a distance of approximately 0.5 cm). Neighboring teeth will be protected with cotton rolls or

the examiner's fingers[19]. The volunteer will then indicate a whole number between 0 (absence of pain) to 10 (worst pain possible) on the 10-cm VAS scale that best describes his/her perception of pain. The data will be stored in appropriate files with the identification of each patient. The possible decrease in sensitivity will, probably, increase adherence to the protocol.

Allocation and blinding

The participants will be randomly assigned to three experimental groups and a control group. For the random distribution of volunteers, randomization will be carried out by drawing lots using the website www.randomizer.org. Briefly, the mean of the initial VAS scores will be determined for each subject and the participants will be randomly allocated to the experimental groups ensuring similar initial VAS scores among the different treatments. All teeth with hypersensitivity in each volunteer will be evaluated using the VAS and the mean will be considered for the initial score. All teeth with hypersensitivity will be treated and reassessed using the VAS. The mean will then be calculated for the final sensitivity score.

Two weeks prior to the onset of the study, the volunteers will undergo a wash-out period, in which they will only use oral hygiene products donated by the researchers. These products will be used through to the end of the study. The oral hygiene kit will contain a soft-bristle toothbrush (Professional Lab Series, Colgate Palmolive), fluoride toothpaste with no desensitizing agent (Elmex) and dental floss (Colgate). The participants will be extensively trained with regards to all of the procedures involved in the experiment.

All volunteers and the evaluator of the degree of sensitivity will be blinded to the allocation, they will not know to which group the participants belong. Unblinding in not permissible. The researcher in charge of applying the products will not be blinded due to the different forms of clinical manipulation of the products. A researcher who will not be involved in the application of treatments and has no conflicts of interest will be responsible for generating the allocation sequence and assigning patients to treatments.

Recruitment is intended to start on January, 2021, the primary completion anticipated date is August 30, 2021, and the anticipated study completion date is December 10, 2021.

Procedures

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence regarding treatment with LLL. The neural desensitizing protocol will involve the use of LLL (Therapy XT, DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent). The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W. The participants will be allocated to four groups according to the proposed treatments. If, by request, participants decide to withdraw from the study, they will suffer no harm. However, improvement in sensitivity and its follow-ups should encourage patients to keep coming. Data from those who choose to discontinue will not be used for analysis. No adverse effects are expected from any of the treatments.

Control Group

Group 1 will be the control group, which will receive no treatment. The volunteers in this group will receive the same instructions as the other groups and will undergo both treatments, except that water will be used instead of the sealant and the laser device will be set to a power of 0 W. In other words, the same irradiation procedure will be performed but without the emission of light.

Sealant Group

The volunteers in Group 2 will receive treatment with sealant (Permaseal - Ultradent), which is a photopolymerizable methacrylate-based resin. The teeth to be sealed will be isolated and 35% phosphoric acid will be applied for 20 seconds, followed by rinsing and drying the dental surfaces. A thin layer of PermaSeal will be applied to the tooth surface for 5 seconds and photopolymerized for 20 seconds. The occlusion will then be evaluated. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser Group

The volunteers in Group 3 will receive irradiation with AsGaAl laser at a wavelength of 780 nm (Laser XT Therapy, DMC, São Carlos, SP, Brazil) with relative isolation. A power meter (Laser Check, MMOptics, São Carlos, SP, Brazil) will be used to determine the power of the equipment before and after all treatment interventions. The power will be set to 100 mW; energy density will be 35 J/cm^2 (considering a spot size of 0.028 cm^2 of the equipment) and the dose will be 1 J per point. Irradiation will be performed on a cervical point, an apical point and a point precisely over the lesion, totaling a dose of 3 J. Treatment will be performed in three sessions with a 72-hour interval between sessions.

During the laser treatments, both the volunteer and operator will use protective eyewear and all rules of safety will be obeyed. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Low-level laser and sealant Group

The volunteers in Group 4 will receive the same irradiation administered to Group 3. During the last session, these volunteers will also receive the same sealant applied in Group 2. The volunteers will return one week, one month, three months and six months after the final treatment session for reevaluation using the VAS.

Data analysis and statistical evaluation

DH will be determined by a researcher who will be blinded to the treatments. This evaluation will be performed 15 minutes after the application of the treatments (Mehta et al. 2014) using the same method described above for the determination of the initial degree of sensitivity. The volunteers will be reevaluated for the determination of sensitivity one week, one month, three months and six months after the treatments. These evaluations will be recorded on blank sheets of paper that will only indicate the number of the volunteer and the tooth evaluated.

The initial VAS score will be subtracted from the scores obtained one week and four weeks after treatment in all groups and the data will be submitted to statistical analysis. The change in sensitivity evaluated through VAS in the different groups and periods is the primary outcome of the study, as it is the main objective of the treatments. Descriptive statistics will

involve the calculation of mean and standard deviation values. Inferential statistics will involve tests of normality and equal variance, which will determine the appropriate statistical tests. The level of significance will be set to 5%. The three-month and six-month data from the three experimental groups (not the placebo group) will be analyzed in the same way.

Author Contributons: Substantial contributions to the conception: APTS, EMS and SKB. Design of the work: APTS, EMS, ACA, PVS, CMM, MLLG and SKB. Drafting the work: APTS, EMS and SKB. Revising the work: APTS, EMS, ACA, PVS, CMM, MLLG, RAR, LJM, ACRTH, KPSF, RAMF and SKB. Final approval of the work: APTS, EMS, CMM, MLLG and SKB. In case stopping the trial is necessary, these authors will have access to the interim results and make the final decision to terminate the trial.

Ethics approval and consent to participate: This study was approved by the Universidade Metropolitana de Santos - UNIMES- Ethics Committee - Protocol Number: 4.020.261. All participants will provide informed consent before participating in this study. Any changes to this protocol will also be reported to this same committee.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interest: None declared. The authors have no conflict of interest, financial or otherwise to declare.

Availability of data and materials: This paper is a protocol description and does not contain any data to this point. Future data will be available, as described in the data sharing statement below.

Data sharing statement: The datasets (Excel spreadsheets) generated from this protocol will be available with the corresponding author (Sandra Kalil Bussadori –

sandra.skb@gmail.com) at a reasonable request. However, reuse of this data will not be allowed for people who are not authors of this paper.

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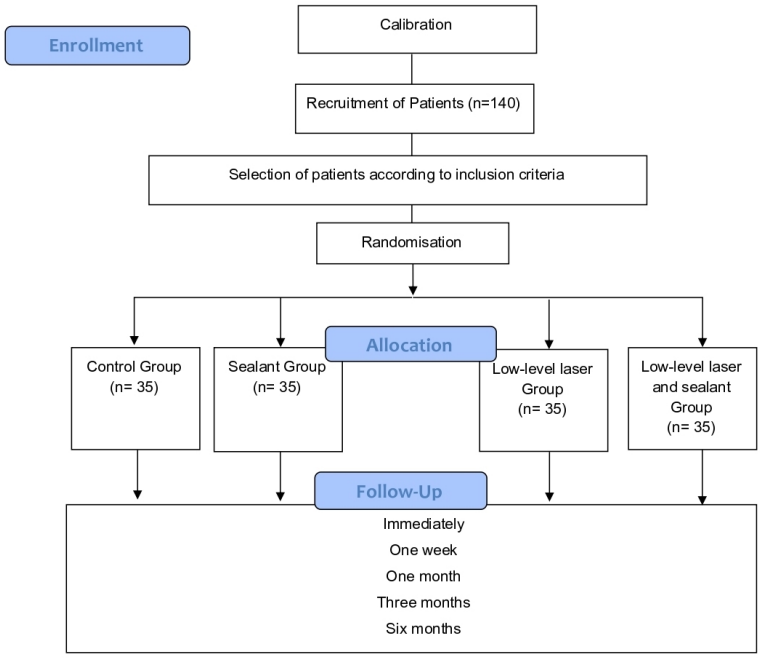

Figure 1 – Activity flowchart in accordance with Consort (Consolidated Standards of Reporting Trials).

107x139mm (300 x 300 DPI)

Universidade Metropolitana de Santos - UNIMES

RESEARCH ETHICS COMMITTEE

FREE AND CLARIFIED CONSENT

I - DATA IDENTIFYING THE SUBJECT OF THE RESEARCH OR LEGAL RESPONSIBLE

1. PATIENT'S NAME:

DOCUMENT Nº: GENDER: .M ☐ F ☐

BIRTH DATE (dd / mm / yyyy):

ADDRESS:

NEIGHBORHOOD: CITY

ZIP CODE: PHONE: DDD

2. LEGAL RESPONSIBLE

NATURE (degree of kinship)

DOCUMENT Nº: GENDER: .M ☐ F ☐

BIRTH DATE (dd / mm / yyyy):

ADDRESS:

NEIGHBORHOOD: CITY

ZIP CODE: PHONE: DDD

II - DATA ON SCIENTIFIC RESEARCH

1. RESEARCH PROTOCOL TITLE: Associative Protocol in Dentin Hypersensitivity pain control in patients with IMH: Randomized controlled clinical trial

2. RESEARCHER: Ana Paula Taboada Sobral

POSITION / FUNCTION: Volunteer researcher

REGIONAL COUNCIL REGISTRATION No. 76.693

UNIMES UNIT: **Faculty of Dentistry -** Av. Conselheiro Nébias, 536 - Encruzilhada, Santos - SP, 11045-002-Brazil

3. RESEARCH RISK ASSESSMENT:

WITHOUT RISK x MINIMUM RISK ☐ AVERAGE RISK ☐

LOW RISK ☐ GREATER RISK ☐

(probability that the individual will suffer some damage as an immediate or late consequence of the study)

4.RESEARCH DURATION: 14 months

III - REGISTRATION OF THE RESEARCHER'S EXPLANATIONS TO THE PATIENT OR ITS LEGAL REPRESENTATIVE ON RESEARCH CONSIGNING:

1. **Justification and objectives of the research:**

The control of tooth sensitivity is fundamental to the successful treatment of Molar Incisor Hypomineralization. Therefore, the present study aims to evaluate the effectiveness of different protocols in the control of Dentin Hypersensitivity (MIH)

The objective of this work will be to evaluate, by means of a randomized and controlled clinical study, the effectiveness of different protocols for the control of dentinal hypersensitivity in patients with teeth affected by MIHI. The neural desensitization protocol will involve the use of low-power laser (Therapy XT -DMC) and the obliteration protocol will involve the use of a resinous sealant (Permaseal Ultradent).

2. **Procedures that will be used and purposes, including identification of procedures that are experimental:**

The treatment sessions will be performed by a trained researcher following the manufacturer's instructions for each product and in accordance with scientific evidence regarding treatment with low-power laser. The participants in the placebo group will use only the conventional oral hygiene kit during the four weeks of the study and will receive simulated treatment involving the application of a rubber cup with no product and sham irradiation with the laser device adjusted to 0 W.

Participants will be divided into the proposed treatments.

Group 1. Control

Group 2. Permaseal (Sealant Group)

Group 3. Low-power laser

Group 4. Low power laser + Permaseal (Sealant Group)

Patients should return after 1 week, 1 month, 3 months and 6 months after the end of the last session to be assessed according to the visual analogue pain scale.

3. **Expected discomforts and risks:** Volunteers will not be at risk during the procedures.
4. **Benefits that can be obtained:** Treatment of dentin hypersensitivity, reducing painful sensitivity.
5. **Alternative procedures that may be beneficial to the individual:** Alternative methods will not be used.

IV - CLARIFICATIONS GIVEN BY THE RESEARCHER ABOUT GUARANTEES OF THE SUBJECT OF THE RESEARCH CONSIGNING:

1. Access, at any time, to information on procedures, risks and benefits related to research, including to resolve any doubts.

2.The patients will be informed of the possible risks involved in the experiment, the confidentiality of the data and the existence of a placebo group.

3. The researchers will be provide adequate treatment for the participants in the placebo group at the end of the study and to the subjects of the other groups at the end of six months, if the pain symptoms have not improved. The treatment offered, in this case, will be that which achieved the best result in the initial four weeks of the study.

4. The participants will be told that they may withdraw from the study at any time for any reason, if they so desire.

5.The researchers will also be able to remove the participants from the study, if deemed necessary.

6. Safeguarding confidentiality, secrecy and privacy.

7. Availability of assistance, for possible damage to health, resulting from the research.

8 Feasibility of indemnification for possible damage to health resulting from the research.

V. INFORMATION OF NAMES, ADDRESSES AND PHONES OF RESPONSIBLE FOR MONITORING THE RESEARCH, FOR CONTACT IN CASE OF CLINICAL INTERCURRENCES AND ADVERSE REACTIONS.

Profa. Dra. Ana Paula Taboada Sobral

Contacts: (11) 98447-4570 / anapaula@taboada.com.br

Faculty of Dentistry UNIMES - Av. Conselheiro Nébias, 536 - Encruzilhada, Santos - SP-CEP: 11045-002- Brasil

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VI - COMPLEMENTARY OBSERVATIONS:

Not applicable.

VII - POST-CLARED CONSENT

I declare that, after being properly clarified by the researcher and having understood what was explained to me, I consent to participate in this Research Protocol.

Santos, ____/_____/_____.

_____ Signature of the research subject or legal guardian

_____ Signature of the researcher (Legible name or stamp)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description		Pag
Administrative information				
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	ok	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	ok	2
	2b	All items from the World Health Organization Trial Registration Data Set	ok	6
Protocol version	3	Date and version identifier	ok	6
Funding	4	Sources and types of financial, material, and other support		NA
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	ok	12
	5b	Name and contact information for the trial sponsor		NA
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Ok	12
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Ok	12
Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	ok	4
	6b	Explanation for choice of comparators	ok	4/5
Objectives	7	Specific objectives or hypotheses	ok	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	ok	5
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	ok	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	ok	8/9

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	ok	10/11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	ok	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	ok	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	ok	9/10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	ok	12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	ok	Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	ok	7
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	ok	9
Methods: Assignment of interventions (for controlled trials)				
Allocation:				
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	ok	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	ok	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	ok	10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	ok	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	ok	10
Methods: Data collection, management, and analysis				

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	ok	12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	ok	10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	ok	13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	ok	12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)		NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)		10
Methods: Monitoring				
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed		12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	ok	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	ok	10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	ok	7
Ethics and dissemination				
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	ok	2/6/12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	ok	12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	ok	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable		NA

Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	ok	13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	ok	13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	ok	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	Ok	7
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	ok	7
	31b	Authorship eligibility guidelines and any intended use of professional writers	ok	12
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	ok	13
Appendices				
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Sup. File	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA	

∗It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked anddated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons“Attribution-NonCommercial-NoDerivs 3.0 Unported”license.NA=not applicable.