Study Protocol Clinical Trial

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Efficacy of erbium-doped yttrium aluminium garnet for achieving pre-emptive dental laser analgesia in children

A study protocol for a randomized clinical trial

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Abstract

Introduction: A current non-pharmacological mean for attaining painless conservative treatment is presented by laser analgesia (LA), considered as bio-photomodulation of pulp reactivity aiming reduction of nociceptive impulse formation. Currently no consensus is reached regarding a detailed protocol with laser parameter settings for pre-emptive LA. The purpose of this study is determining the efficacy of erbium-doped yttrium aluminium garnet (Er:YAG) laser in achieving pulpal analgesia and quantifying duration and extent of any effects assessed.

Methods and analysis: The proposed study is a double-blind placebo-controlled randomized split-mouth clinical trial with 2-way repeated measures design. Eligible patients of age 10 to 12 years undergo 2 single-visit treatments, receiving LA or placebo analgesia (PA) prior to caries ablation, randomized via computer-generated, permuted-block sequence. Primary outcome measure is pain felt during treatment, reported by patient on visual-analogue scale. Secondary outcomes: changes in pulpal sensibility to electrical and cold-stimuli; patient experience during LA/PA; pain-related behavior according to Faces, Legs, Activity, Cry, Consolabilty (FLACC) scale; heart-rate dynamics. Data will be analyzed with intention-to-treat concept by Student *t* test for paired samples, $P < .05$. Pre-test on 20 subjects resulted in n=41 patients needing to be recruited.

Ethics and dissemination: This study protocol has been approved by the Committee for Scientific Research Ethics, Medical University - Plovdiv, Bulgaria (Reference number P-8604, Protocol of approval N:6/23.11.2017) and registered on a publically accessible database. This research received institutional funding from the Medical University – Plovdiv, Bulgaria under project SPD-03/2017. Findings will be reported in scientific publications and at research conferences, and in project summary papers for participants.

Trial registration: ClinicalTrials.gov (Registration number: NCT03412721).

Abbreviations: EPT = electrical pulp testing, ER:YAG = erbium-doped yttrium aluminium garnet, FLACC = Faces, Legs, Activity, Cry, Consolabilty scale, LA = laser analgesia, LASER = light amplification by stimulated emission of radiation, PA = placebo analgesia, SPIRIT = Standard Protocol Items for Randomized Trials, VAS = visual analog scale.

Keywords: erbium-doped yttrium aluminium garnet anesthesia, laser analgesia, low-level laser therapy, photobiomodulation, photomodulation

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1. Introduction

1.1. Background and rationale

Achieving local anesthesia in children is one of the critical aspects of pain management. A current non-pharmacological mean for attaining painless conservative treatment is presented by the laser analgesia (LA) method.

LA is a non-invasive, non-thermogenic bio-modulation of the dental pulp reactivity aiming for reduction of impulse formation of the pulpal nociceptors. It is hypothesized that laser pulses alter the cell membrane behavior of the pulpal nerve fibers by hyperpolarization and loss of impulse conduction, and thus an analgesic effect is achieved.[1–3]

The assessment of changes in pulp's sensory responses can be explored by pulp sensibility testing,[4] such as one delivered through thermal or electric test. The aforementioned tests could be valuable means for investigating the occurrence of any pulpal analgesic effect obtained by dental lasers.

The A-delta nociceptive nerve fibers in the pulp are able to generate a fast, sharp pain that is easily localized[5,6] such as one

from rapid temperature change,[7] achieved by application of Cold-test. Electric pulp testing (EPT) depends on ionic movement and triggers the A-delta fibers as well, due to their conduction speed and their myelin sheath.

The rationale based on the neurophysiology of the pulp, led to choosing both electric and cold testing as means for evaluating the pulpal analgesic effect of the erbium-doped yttrium aluminium garnet (Er:YAG) pulp laser. To our best knowledge, no study before has implemented the use of Cold-test to complement EPT-results in assessment of LA efficacy.

The pulp sensibility testing, along with the assessment of subjective and objective pain sensation during treatment should help estimate the clinical adequacy of the LA method by finding out if pain-free operative treatment can subsequently be performed.

1.2. Objectives

The intention of the technique of "pre-emptive LA" is to reduce sensation in that small percentage of patients who may experience unpleasant sensations during caries removal. We hypothesized that when operating at low level densities, the laser energy leads to loss of nociceptive impulse formation by coinciding with the natural resonance frequency (15–20 Hz)[8] of cell membranes of nerve fibers in the dental pulp, leading to an analgesic effect.

The aim of this study is efficacy approbation of a modified protocol for LA with Er:YAG for achieving pulpal analgesia in pediatric patients and quantification of the duration and extent of any effects assessed.

The main objectives are to compare pain felt during treatment in laser and placebo analgesia (PA) control group and to register the reactivity of the pulp towards cold and electrical stimuli before and after inducing laser or PA. The second objectives are to evaluate latency of any analgesic effect, patient experience during analgesic or placebo procedure, as well as heart rate dynamics and need for additional anesthesia during treatment.

1.3. Trial design

The trial to be conducted is a double-blind controlled clinical crossover experimental study with 2-way repeated measures design. Figure 1 summarizes the enrollment, intervention, and assessment schedule, all of which are in accordance with the Standard Protocol Items for Randomized Trials (SPIRIT) recommendations,[9] shown in Table 1. Patients and outcomes assessor are blinded for the study. Experimental group in this study consists of permanent upper jaw first molars, receiving pre-emptive LA prior to laser caries ablation, whereas control group consists of contralateral permanent upper jaw first molars with similar defects of the same patient, receiving placebo-analgesia prior to laser treatment.

The required number of patients is calculated on the basis of a micro-sample at an accepted level of significance ($P < .05$) and a maximum permissible error $\alpha < 0.05$ and $\beta < 0.2$. Intention-to-treat concept is chosen as statistical approach for data analysis.

2. Methods and analyses

2.1. Study setting

The setting of this trial is the Department of Pediatric Dentistry at the Faculty of Dental Medicine, Medical University of Plovdiv, Bulgaria.

2.2. Eligibility

2.2.1. Inclusion criteria.

1. Participants in the study are children 10 to 12 years old, compliant with the cognitive development of the child and the requirement for full root development for diagnostics with electrical pulp testing.
2. Children, identified as positive or definitely positive through Frankl behavioral rating scale.
3. Children who are not considered medically compromised or medically complex patients. The absence of disease is confirmed by anamnestic interview with a parent or a care-giver of the child and excludes general acute or chronic disease, cognitive impairment.
4. Patients, requiring conservative treatment of occlusal or foramen caecum caries on 2 first permanent upper jaw molars without prior restorations or dental sealants. Lesions are to be classified as moderate caries by the International caries detection and assessment system (ICDAS) with code 03 or 04, which do not present spontaneous unprovoked pain, percussion or palpation pain or other symptoms, indicating of pulpal or periodontal pathology. Included are carious lesions only on vital teeth, involving up to half of the dentine thickness.
5. Obtained informed consent from parents or gave-givers to participate in the study, in which procedures are explained in appropriate manner (see supplementary data file S1—"Informed consent" and supplementary data file S2—"Information leaflet").

2.2.2. Exclusion criteria.

1. Patients who are undergoing therapy with neurological, sedative, analgesic, and/or anti-inflammatory drugs 7 days prior to treatment.
2. Children, who are first time ever dental patients.
3. Patients who are undergoing treatment or have been treated 6 months prior to inclusion with remineralizing agents.
4. Excluded are first molars which are affected by hypoplasia or hypomineralization.

2.2.3. Interventions. Dental Er:YAG laser (LiteTouch, Light Instruments LTD) will be used as means to attain analgesia and caries removal. Chosen protocol parameters are modified based on previously conducted studies.[8,10,11]

Laser analgesic protocol: Water mist spray set to "maximum," non-contact handpiece with sapphire tip. Tip-to-tissue distance 10 mm from the tooth neck, achieved by using a spacer. Energy is delivered to the enamel above the gingival margin adjacent to the cemento-enamel junction (perpendicularly towards the dental pulp) on each of the 4 line angles of the tooth for 30 seconds, moving the laser handpiece in a sweeping action. Pulse energy—0.2 W/10 Hz/20 mJ. Follows increase of energy and repetition of protocol—0.6 W/15 Hz/40 mJ. Total duration of LA-induction—240 seconds.

Placebo analgesic protocol: No pulse energy applied. Moving the laser handpiece in a sweeping motion repeating actions to imitate LA placement.

Application parameters during caries ablation: Hard tissue preconditioning: 1.5 W/15 Hz/100 mJ for 1 minute; Enamel removal—3 W/15 Hz/200 mJ; Dentin removal—2 W/10 Hz/200 mJ. Smear layer removal: 2 W/10 Hz/200 mJ. Loss of tooth structure was restored with esthetic composite.

	STUDY PERIOD					
	Enrolment	Allocation	Post-allocation			
TIMEPOINT**	$-t_1$	0	t_1	t_2	t_3	t_4
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS:						
Laser analgesia				X		
Placebo analgesia				X		
ASSESSMENTS:						
Dental Fear			X			
Pain during treatment					X	
EPT			X		X	
Cold-test			X		X	
Patient experience				X		
Pain-related behavior					X	
Heart rate			◆—	—	—	—◆
Need for additional anesthesia					X	

** Post-allocation timeframe: t_1 – before start of treatment; t_2 – during laser or placebo analgesia; t_3 – during treatment; t_4 – end of treatment, before leaving the dental chair.

Figure 1. Schedule of enrollment, interventions, and assessments of treatments.

2.2.4. Clinical protocol:. First visit:

1. Parents or care-givers are informed about laser technique and get acquainted with the nature of the research being conducted in to prepare their children for the dental treatment. The parent or care-giver signs informed consent.
2. Patients are asked to complete Children's Fear Survey Schedule–Dental Subscale (CFSS-DS) questionnaire.
3. Pulse-oximeter is connected to patient's index finger. Start of heart rate monitoring and recording—7 minutes prior treatment. Time frame: until end of treatment.
4. Blind for chosen method investigator evaluates the initial reactivity of the pulp with EPT 5 minutes prior laser or PA. Four minutes before applying chosen method same investigator performs Cold-test with propane-butane gas, applied on a cotton pad on the tooth. Patient is asked to evaluate pain perception on VAS.
5. The chosen method, placebo or LA, is applied.
6. Patient experience during LA/PA is evaluated by a patient questionnaire immediately after the procedure. Patient is asked to answer 4 questions with possible answers "yes" and "no": "Did you feel pain when we put your tooth to sleep?";

Table 1

SPIRIT 2013 checklist: recommended items to address in a clinical trial protocol and related documents*.

Section/item	Item no	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	10
Funding	4	Sources and types of financial, material, and other support	2
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	2
	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	2
	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)	n/a
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	2
	6b	Explanation for choice of comparators	3
Objectives	7	Specific objectives or hypotheses	3
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	4
Methods: participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centers and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7
Participant timeline	13	Time schedule of enrollment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
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	8
Recruitment	15	Strategies for achieving adequate participant enrollment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9

(*continued*)

Table 1

(continued).

Section/item	Item no	Description	Addressed on page number
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enroll participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
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 (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., 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	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	n/a
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistical methods	20a	Statistical methods for analyzing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	10
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomized analysis), and any statistical methods to handle missing data (e.g., 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	10
	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	n/a
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	n/a
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	10
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	11
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	11
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	13

(*continued*)

Table 1
(continued).

Section/item	Item no	Description	Addressed on page number
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	n/a
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	n/a
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
	31b	Authorship eligibility guidelines and any intended use of professional writers	11
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	S1–S3
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	n/a

* 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 and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

"Were you frightened when we put your tooth to sleep?";
"Do you feel any pain now that your tooth is put to sleep?";
"Do you feel any tingling sensation now that your tooth is put to sleep?".

7. Laser ablation of the carious lesion begins. No rotary instruments or other excavation techniques are to be applied.
8. Five minutes after the analgesic procedure same investigator evaluates the sensibility of the pulp with EPT and 6 minutes after performs Cold-test on same tooth. Patient is asked to evaluate pain perception after Cold-test on the VAS pain scale.
9. At the 20th minute after LA/PA, EPT testing is performed. At the 21st minute a Cold-test is applied and the pain perception is registered on the VAS.
10. Outcomes assessor monitors the patient during treatment and registers pain related behavior using Faces, Legs, Activity, Cry, Consolability (FLACC) Behavioral Pain Rating Scale.[12]
11. Patients who complete the procedure without the need for additional anesthesia, immediately after placement of restoration are asked to use the combined VAS scale to quantify the level of pain felt during treatment. Patients who request that the procedure is terminated to administer an anesthetic injection, are asked to rate their level of pain on VAS, immediately following termination.

Second Visit:

Application of PA/LA prior to treatment of adjacent first molar of upper jaw, according to treatment allocation sequence number. Reactivity of the pulp to cold and electric stimulation, as well as the subjective sensation after manipulation by the aforementioned methods are reported.

2.3. Outcomes

2.3.1. Primary outcome measures. Primary outcome measure to be assessed in this study is pain felt during the treatment, reported by the patient on a visual analogue scale (VAS) at the end of the treatment session.

2.3.2. Secondary outcome measures. Assessment of following secondary outcomes will be performed: changes in pulpal sensibility to electrical stimuli before and after LA/PA, evaluated by EPT (time frame: 25 minutes); changes in pulpal sensibility to cold stimulation before and after laser/PA by Cold-test—self-reported pain by the patient on VAS (Time Frame: 25 minutes); patient experience during LA/PA, evaluated by questionnaire; pain-related behavior during treatment, evaluated using the FLACC scale; heart-rate dynamics during the experiment, registered via pulse oximeter; need for additional local anesthesia infiltration.

2.3.3. Participant timeline. Each eligible patient undergoes 2 single-visit treatments of the 2 carious first upper jaw molars, receiving LA when undergoing treatment of 1 tooth and PA at the other visit during treatment of the homologous contralateral tooth. The 2 manipulations are performed by 1 operator, performing treatment of caries and restorative procedure. Outcomes are registered by the primary investigator in a clinical file (see supplementary data file S3 - "Clinical file"). A 7 to 21 day interval is allowed between one procedure and the other. The protocol to be used in the first procedure is randomly selected using a computer-generated list linked to sequence of enrollment in the trial.

2.3.4. Sample size calculation. Given the lack of comparable research and the unknown population standard deviation we conducted a pretest with 20 subjects and considered the behavior of this subgroup as population estimate. To estimate sample size for the primary outcome—pain felt during laser ablation of moderate carious lesion, according to the VAS scale—we applied a *t* test for paired groups (G* Power software version 3.1),[13] since we have 2 groups (first upper molar in right and left quadrant) on the same patient.

The effect size was determined using the formula

$$ES = \frac{\text{Control} - \text{Treated}}{\text{SDpooled}} = \frac{2.33 - 0.33}{3.25} = 0.62$$

Where SD is the pooled standard deviation—an average of the standard deviations of the experimental and control groups. The error was set at 5% and the power test at 95%. According to the calculation, a sample of 37 patients will be necessary to detect differences in pain. Since drop-outs are unavoidable when collecting follow-up data, this number needs to be adjusted for the estimated drop-out rate. During the pre-test, we had a drop-out rate of 5% for collecting the follow-up data and if we anticipated a higher drop-out rate for this study, we conservatively allowed for a 10% drop-out rate. Adjusting the sample size for this drop-out rate results in a sample of 41 patients needing to be recruited.

2.4. Recruitment

The clinical trial is currently recruiting participants. Patient recruitment started in October 2018. Estimated study completion date is May 1, 2019. The enrollment capacity was estimated to be 6 patients/mo.

2.5. Participating centers

Eligible patients are selected from the visitors of the pediatric dental clinic of the Department of Pediatric Dentistry, Faculty of Dental Medicine—Medical University of Plovdiv, Bulgaria and treated in the laser dental office of the aforementioned.

2.6. Assignment of interventions

2.6.1. Sequence generation. A computer-generated, permuted-block randomization sequence for allocation of first procedure is to be prepared. Patients are to be randomized to treatment allocation according to number of enrollment in the trial. In this split-mouth randomized controlled trial (RCT), every patient will receive both procedures. The patient will be randomized to receive at the first visit, placebo analgesic procedure and, at the second visit, laser pre-emptive analgesia or at the first visit—LA and at second one—placebo procedure.

2.6.2. Allocation concealment mechanism and implementation. Randomization is based on treatment allocation sequence number. First procedure (PA—1; LA—2) is linked to number of enrollment in the study in Microsoft Excel table. Allocation sequence will be generated before start of the patient enrollment by the statistician. The operator will obtain each randomization allocation via a sequentially-numbered opaque sealed envelope prior to treatment, enabling the sequence to be concealed until the intervention is assigned. The outcomes assessor will not be aware of chosen treatment. Patients will be enrolled by the primary investigator.

2.6.3. Blinding. The current study is designed as a double-blind trial. Patients as well as outcomes assessor are blinded for the study. The operator will get acquainted with procedure to be performed prior to treatment session. The clinicians involved in this study as operator and outcomes assessor are selected to be the only ones performing the manipulations in order to prevent bias.

2.6.4. Data collection, confidentiality, storage, and monitoring of study documents. According to the regulations of the Personal Data Protection Act, the collected paper forms will be stored in a secure manner in the Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University - Plovdiv, Bulgaria. Outcomes will be transferred electronically by the primary investigator after transmission of paper forms. Clinical research files will be stored in a locked, secure office. Data will be electronically stored on a double password-protected computer. Only the primary investigator and the statistician will have access to the final data set. The trial will be monitored by the research monitoring officer of Medical University—Plovdiv, verifying that the study is conducted in accordance with the Good Clinical Practice guidelines.

3. Statistical methods

The unit of analysis will be the tooth for the split-mouth RCT (2 first permanent molars belonging to the same dental arch treated per patient). The data will be recorded and analyzed using SPSS 20.0. All data will be analyzed using an intention-to-treat analysis.[14] Descriptive statistics will be calculated. Discrete variables will be summarized by frequencies or proportions. Continuous variables will be reported as means and standard errors or medians and range (depending on the distribution of the variables). Data will be checked for baseline differences between the treatment arms. If baseline differences do occur for any of the variables, they will be added to subsequent models to compensate for those differences using an analysis of covariance approach.

We will compare pain mean scores according to the visual analogue scale (VAS), containing numerical symbols. We will report the mean differences between groups and the associated 95% CIs. For the split-mouth RCT, with each patient being his or her own control, our statistical analysis will take into account the paired nature of data and the results will be analyzed by Student *t* test for paired samples.

4. Ethics and dissemination

The clinical trial will be carried out in line with the principles of the Declaration of Helsinki and according to the Clinical Trials Directive 2001/20/EC of the European Parliament on the approximation of the laws, regulations, and administrative provisions of the Member States relating to the implementation of Good Clinical Practices in the conduct of clinical trials on medicinal products for human use.

4.1. Research ethics approval

This study has been approved by the Committee for Scientific Research Ethics, Medical University—Plovdiv, Bulgaria (Reference number P-8604, Protocol amendment number: 01, Protocol of approval N:6/23.11.2017) and registered on a publically accessible database ClinicalTrials.gov (Registration number: NCT03412721). The protocol of the study, as well as written information leaflets and informed consent documents are approved by the Ethics committee. Should there be any changes in the aforementioned, they will be consulted with the committee.

4.2. Consent

Parents or caregivers will be given written informed consent and information leaflets by the primary investigator in person.

4.3. Confidentiality

People with direct access to the data will take all necessary precautions to maintain confidentiality. All data collected during

the study will be rendered anonymous. Only initials and inclusion number will be registered.

4.4. Dissemination policy

The results of the study will be released to dental medicine specialists and scientific community no later than 1 year after completion of the trial, through presentation at scientific conferences and publication in peer-reviewed journals. The principal investigator (EV), the scientific expert and operator (AB), and the statistician (RR) will write the first draft of the manuscript.

5. Discussion

LA is a new alternative and a traumatic method that could help improving the quality of pediatric dental care. Different possible explanations have been suggested for low-level laser action (biophotomodulation) regarding pain relief.[15] Currently no consensus is reached regarding a detailed protocol with reliable laser parameter settings for pre-emptive LA, and some research lacks necessary parameter detail, presenting challenges to repeat or reproduce.[11] Many studies nevertheless agree that to obtain pulpal analgesia, it is necessary to take advantage of low energy and power densities.[3,8,11,16,17]

The clinical adequacy of Er:YAG laser for achieving pre-emptive dental analgesia is investigated in a complex manner by pulp sensibility testing, along with assessment of subjective and objective pain during treatment of similar cases. This randomized controlled trial (RCT) trial is a well powered one-center split-mouth experimental study with two-way repeated measures design. The trial is double-blind, where patients and outcomes assessor are blinded for the study, involving only one operator, who cannot be blinded, and one primary investigator, reducing inter-individual variability from the estimates of the treatment effect. A disadvantage of this trial is the need to include positive patients with symmetrical and similar conditions, and many patients are not eligible. The following precautions are taken in account to minimize variables in results: eligible patients are selected from a narrow age group (10–12 years), classified as positive or definitely positive through Frankl behavior scale. Age of the patients is compliant with the need for full root development of teeth to be examined with EPT. Carious lesions are restricted to moderate caries diagnosed by ICDAS with code 03/04 on first upper molars without previous restorations. Focus of the trial is pain during treatment after pre-emptive laser or PA —the VAS scale is selected as means for subjective pain rating of the patient, while the FLACC scale is chosen to register pain-related behavior by the outcomes assessor.

Any alteration in the sensibility of the pulp after laser or placebo analgesic procedures is to be investigated through EPT and cold testing. The EPT is commonly used for assessing pulpal sensibility because it is quick and reproducible and does not appear to cause pulpal damage.[16] Few clinical studies have investigated the method of LA by analyzing alteration in EPT threshold, but results are contradictory.[16–19]

It is possible that the EPT does not provide an accurate measure of pulpal analgesia[16] and it may be preferable to assess the clinical adequacy of a dental analgesia by supplemental methods such as thermal stimulation. According to authors,[20] ideally, EPT should be used in conjunction with cold testing so that the results from one test will verify the findings of the other test. Cold testing causes contraction of the dentinal fluid within the dentinal tubules by creating "hydrodynamic forces" acting on the A-delta nerve fibers, leading to a sharp sensation lasting for the duration of the thermal test.[21] The rationale behind the chosen test supports our hypothesis that if any analgesic effect is attained, then the patient is expected to report lower cold-related pain VAS-scores. To our best knowledge, no study before has explored the analgesic effect of dental laser with Cold-test, complementing the EPT results.

The pulp sensibility testing, along with the assessment of subjective and objective pain sensation during treatment of similar cases should help estimate the clinical adequacy of the LA method by finding out if pain-free operative treatment can subsequently be performed.

5.1. Trial status

This trial is currently recruiting patients. Patient recruitment started in October 2018 and by November 2018 10 patients are enrolled. Pre-test on 20 subjects resulted in n=41 patients needing to be recruited. Outcome results will be updated in clinicaltrials.gov after completion of the study, estimated due May 2019.

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