Study Protocol and Statistical Analysis Plan

Official title: The effect of Er,Cr:YSGG laser-assisted topical anesthetic on oral mucosal anesthesia

NCT number: NCT07169032

Document date: 12/25/2023

This study is a randomized controlled clinical trial with a single-blind design. The protocol has been approved by the Medical Ethics Review Committee of Jinan University (Approval No.: JNUKY-2023-0149). All participants will provide written informed consent before the study begins.

1. **Study Participants**

The study will be conducted in an outpatient setting, with a total of 20 participants (10 males and 10 females). Participants will be recruited through online advertisements and from among outpatient clinic visitors. All participants will receive free oral health examinations and related treatments as compensation.

1.1 Inclusion Criteria

- Aged 20–45 years;
- Able to understand and follow verbal and written instructions.

1.2 Exclusion Criteria

- Severe dental anxiety;
- Allergy to local anesthetics;
- Presence of oral mucosal lesions;
- History of cardiovascular disease;
- History of diabetes;
- Pregnant or lactating women;
- History of severe psychiatric disorders;
- Hearing or speech impairments.

2. Group Allocation

This is a single-blind randomized controlled trial. After providing informed consent, participants will be stratified by gender. Three sites in the mandibular anterior

vestibular groove will be selected and randomly assigned to one of the following three interventions:

- Experimental group: Irradiation with Er,Cr:YSGG water laser (wavelength 2780 nm, power 1.0 W) for 1 minute, followed by application of 2% lidocaine gel;
- Control group: Irradiation with placebo laser (0.1 W) for 1 minute, followed by application of 2% lidocaine gel;
- Blank group: Irradiation with placebo laser (0.1 W) for 1 minute, followed by application of petroleum jelly.

All procedures will be performed by the same operator and assistant. Participants will be blinded to group assignment.

3. Materials and Equipment

3.1 Experimental Equipment

3.1.1 Water Laser Device

Device: Er,Cr:YSGG Waterlase (Model MDX300), manufacturer: BOOLASE (USA), registration number: National Medical Device Approval 20153241177. The laser wavelength is 2780 nm and is suitable for various dental procedures. Two modes are predefined:

- Mode 1a (active laser): Power 1.0 W, water 15%, air 35%, frequency 30 Hz, H mode (soft tissue mode);
- Mode 1b (placebo laser): Power 0.1 W, other parameters same as Mode 1a.

All personnel must wear laser protective glasses during operation. The handpiece must be moved continuously to avoid localized heat accumulation.

3.1.2 Electrocardiogram (ECG) Monitor

Device: Baolaite M7000 Multi-parameter Monitor (Guangdong Medical Device Approval 2012 No. 2210328), used for continuous monitoring of heart rate, blood pressure, and oxygen saturation. The cuff is placed on the upper arm, and the oxygen sensor is attached to the index finger. Data will be recorded throughout the procedure.

3.1.3 Visual Analog Scale (VAS)

A 10 cm linear VAS will be used to assess pain intensity, ranging from "no pain" (0) to "worst pain imaginable" (10). Participants will slide the indicator to reflect their subjective pain level, and the operator will record the corresponding value.

3.2 Experimental Drugs and Materials

- Topical anesthetic: 2% lidocaine gel (Gandermal, Registration No.: H20150426);
- Placebo: Petroleum jelly;
- Other materials: 27G injection needle (Fukada), periodontal probe (Hu-Friedy), mouth prop (Ivoclar), dental mirror, sterile cotton swabs, timer, etc.

4. Outcome Measures

Primary outcome measures include:

- Penetration depth and VAS score at the moment of "sensation of foreign body";
- Penetration depth and VAS score at the moment of "pain";
- Heart rate at each needle insertion and the increase in heart rate (heart rate at pain moment – baseline heart rate).
- Penetration depth is calculated based on the exposed length of the needle (penetration depth = 30 mm – length outside the mucosa).

5. Study Procedure

5.1 Preparation Phase

- Participants will rest for 30 minutes after signing consent to stabilize vital signs;

- Baseline heart rate will be recorded. Laser protective glasses and ECG monitoring devices will be worn;
- The operator will preset the laser device parameters.

5.2 Experimental Procedure

- Three sites in the maxillary anterior vestibular groove with intervals >20 mm will be selected;
- Interventions will be applied in random order (laser irradiation and subsequent drug application);
- Penetration tests will be performed at 10, 20, 30, 40, and 50 minutes after treatment. Penetration depth, VAS score, and heart rate will be recorded at the moments of "sensation" and "pain";
- Adverse reactions (redness, pain, bruising, etc.) will be assessed immediately after the procedure and at 24-hour follow-up.

6. Statistical Analysis

- Data will be analyzed using SPSS 27.0. Normally distributed continuous variables will be expressed as mean ± standard deviation ($\bar{x} \pm s$);
- Repeated measures ANOVA will be used to compare differences across time points and groups. If the sphericity assumption is violated, multivariate tests (Roy's Max Root) will be applied;
- Pairwise comparisons will be performed using Bonferroni correction with significance level $\alpha = 0.05$;
- A p-value < 0.05 will be considered statistically significant