Official Title:

Metagenomic Analysis and Post-operative Pain Assessment of Infected Root Canals Using Different Irrigation Protocols: A Randomized Controlled Clinical Trial

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Study Protocol

1. Aim & Objectives

The study compares bacterial reduction and postoperative pain in root canals using four irrigation protocols: ultrasonic activation, laser activation, negative pressure irrigation, and mechanical activation. It also evaluates bacterial changes with metagenomic analysis and patient-reported pain levels.

2. Methodology

This randomized clinical trial involves 40 adult patients divided into four groups. Pre- and post-irrigation bacterial samples are collected and analyzed using metagenomic sequencing to assess changes in microbial composition.

Eligibility Criteria

Inclusion Criteria:

- 1. Adult patients aged 18–60 years requiring root canal treatment for teeth with necrotic pulp and periapical radiolucency.
- 2. Patients in good general health.

Exclusion Criteria:

- 1. Patients with open apices or severe periodontal disease.
- 2. Patients currently on antibiotics or allergic to materials used in the study.
- 3. Pregnant or breastfeeding women.

3. Clinical Procedures

Procedures include aseptic isolation with a rubber dam, access cavity preparation, and standard instrumentation with rotary NiTi files. Four irrigation protocols are used: Ultrasonic Activation (NaOCl activated by ultrasonic device), Laser Activation (Laser-activated NaOCl with methylene blue), Easy Clean (Mechanical activation), and PulpSucker (Negative pressure suction).

4. Evaluation

Postoperative pain is evaluated using the Visual Analogue Scale (VAS) at 6 hours, 24 hours, 48 hours, and 7 days. Bacterial reduction is assessed via Next-Generation Sequencing (NGS).

5. Statistical Plan

Sample size calculations

Sample size calculation using G*Power software indicates a total sample of 48 (including 20% dropout) to achieve 95% power. Data will be analyzed using two-way repeated measures ANOVA or corresponding nonparametric tests.

Current research was performed to compare between four different treatment groups (A1, A2, A3, A4); Ultrasonic activation, Laser activation, Easy clean device, PulpSucker device at two different sampling time (S1, S2); Two-way repeated measure analysis of variance is proposed (ANOVA). A minimum total sample size of 32 samples will be sufficient to detect the effect size of 0.40, at a power (1-β=0.95) of 95% at a significance probability level of $p<0.05$, and a partial eta squared of 0.14. Applying a dropout of 20% to compensate for losses of samples, a total sample size of 48 will be applied in current study, according to sample size calculations each treatment group (A1, A2, A3, A4) would be represented by 6 samples as shown in Tables 1 and 2. (Santos Nogueira et al., 2021). The sample size was calculated according to G*Power software version 3.1.9.6 (Cohen, 1988; Faul et al., 2007; 2013).

Statistical analyses

The statistical analysis will be performed for comparison between different four different treatment groups (A1, A2, A3, A4); Ultrasonic activation, Laser activation, Easy clean device, PulpSucker device at two different sampling time (S1, S2).

The data will be collected, checked, revised, and organized in tables and figures using Microsoft Excel 2016. Data will be subjected to outliers' detections and normality statistical test to detect whether the data are parametric or nonparametric.

Data will be analyzed for descriptive statistically both graphical and numerical description. Inferential statistics for evaluating and comparing between four different treatment groups (A1, A2, A3, A4); Ultrasonic activation, Laser activation, Easy clean device, PulpSucker device at two different sampling time (S1, S2), will be performed by two-way repeated measures analysis of variance (ANOVA) or corresponding nonparametric analyses at significance levels of 0.05. ANOVA will be followed by Tukey's HSD multiple range tests (DMRTs) to compare between treatment groups or corresponding posthoc for nonparametric data. Difference between four treatment groups at each sampling timepoint will be performed by one way analysis of variance (ANOVA) or Kruskal-Wallis for nonparametric data at 0.05 level. The difference between two sampling timepoint will be performed using Paired samples t-test. Data analyses will be carried out using computer software Statistical Package for Social Science SPSS (IBM-SPSS ver. 30.0 for Mac OS) (Knapp, 2017).