Statistical Analysis Plan

Study Title: Effect of Local Application of Hyaluronic Acid on Wound Healing after Crown Lengthening Procedure in Smokers: A Randomized Controlled Clinical Trial, Double Blind

Protocol No.: E-20-4826

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List of Abbreviations

Abbreviation	Full Term
BoP	Bleeding on Probing
CI	Confidence Interval
CL	Crown Lengthening
CONSORT	Consolidated Standards of Reporting Trials
GI	Gingival Index
HA	Hyaluronic Acid
ICH-GCP	International Council for Harmonisation - Good Clinical Practice
IRB	Institutional Review Board
IQR	Interquartile Range
ITT	Intent-to-Treat
MAR	Missing At Random
Mm	Millimeter
PI	Plaque Index
PP	Per-Protocol
PPD	Periodontal Probing Depth
SAP	Statistical Analysis Plan
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
VAS	Visual Analogue Scale

1. Introduction

This document outlines the statistical analysis plan (SAP) for a randomized controlled clinical trial investigating the effect of locally applied hyaluronic acid (HA) on post-surgical healing in smokers undergoing osseous crown lengthening procedures. It aims to ensure transparency and consistency in data handling and analysis and to reduce the potential for bias during interpretation. The SAP has been developed before the database lock and final analysis to maintain scientific rigor.

2. Study Objectives

2.1. Primary Objective

The primary objective of this study is to assess whether the application of cross-linked hyaluronic acid at the surgical site improves wound healing outcomes in smokers. Healing will be measured using the Landry Healing Index at two- and six-weeks following crown lengthening surgery.

2.2. Secondary Objectives

This study also aims to determine if the use of HA has additional clinical benefits, including:

- Reduced post-operative pain, as reported by patients using the Visual Analogue Scale (VAS)
- Improvement in clinical periodontal parameters, such as periodontal probing depth (PPD), bleeding on probing (BoP), plaque index (PI), and gingival index (GI),
- Radiographic evidence of bone level stability or change

These outcomes are intended to support clinical decision-making about the use of HA in periodontal surgeries among smokers.

3. Study Design

This is a randomized, double-blind, controlled clinical trial involving 30 adult smokers who require crown lengthening. Participants are randomly assigned to either a test group, which receives hyaluronic acid during surgery, or a control group, which receives a saline rinse. The study is conducted at the Department of Periodontics, King Saud University. The follow-up duration is six weeks, with assessments at baseline, two weeks, and six weeks post-surgery. Blinding is maintained for both the patients and the outcome assessors.

4. Analysis Population

- Intent-to-Treat (ITT) Population: This includes all participants who are randomized and have at least one follow-up measurement. This analysis preserves randomization and reflects real-world clinical practice.
- Per-Protocol (PP) Population: This subset includes participants who adhered strictly to the study protocol without major deviations and completed all follow-up assessments. It helps evaluate treatment efficacy under ideal conditions.
- Safety Population: All participants who received any surgical intervention will be considered for safety monitoring and adverse event assessment.

5. Variables and Outcomes

5.1. Primary Outcome

Landry Healing Index: A clinical index used to assess soft tissue healing based on tissue color, bleeding, granulation, and epithelialization. This will be scored on a 5-point ordinal scale by the blinded examiner at follow-up visits.

5.2. Secondary Outcomes

- Pain (VAS): Self-reported pain will be measured on a scale from 0 (no pain) to 10 (worst possible pain).
- PPD: The depth of periodontal pockets in millimeters, measured at six sites per tooth using a standardized probe.
- BoP: Presence of bleeding at probing sites, reported as a percentage of affected sites.
- PI: The proportion of tooth surfaces showing plaque, measured with a disclosing solution.
- GI: Gingival health status based on color, consistency, and bleeding on probing, using Loe and Silness criteria.
- Radiographic Bone Level: Assessment of bone height changes through standardized vertical bitewing radiographs taken at baseline and at the 6-week visit.

6. Statistical Methods

6.1. General Approach

All statistical analyses will be performed using IBM SPSS Version 24.0. A p-value of less than 0.05 will be considered statistically significant. All variables will be analyzed using both descriptive and inferential statistics. Data will be examined for completeness, consistency, and distribution before analysis.

2.1. Descriptive Analysis

Demographic characteristics (e.g., age, gender) and baseline clinical parameters will be summarized:

- Continuous variables will be reported as mean ± standard deviation or median and interquartile range, depending on data distribution.
- Categorical variables will be presented as frequencies and percentages.

2.2. Comparative Analysis

Outcome	Test	Type of Data	Timepoints
Healing Index	Mann-Whitney U Test	Ordinal	Week 2 and Week 6
Pain (VAS)	Student's t-test or Mann-Whitney U	Continuous	Week 2 and Week 6
PPD	Repeated Measures ANOVA or Mixed Model	Continuous	Baseline, Week 2, and Week 6
BoP and PI	Chi-square test or Fisher's Exact	Categorical	Baseline, Week 2, and Week 6
GI	Mann-Whitney U or Wilcoxon Signed-Rank	Ordinal	Baseline, Week 2, and Week 6
Radiographs	Descriptive only or McNemar (if binary rating used)	Categorical	Baseline and Week 6

Abbreviations: BoP, Bleeding on probing; GI, Gingival index; PI, Plaque index; PPD, Periodontal Probing Depth.

2.3. Missing Data

All data will be reviewed for completeness. The mechanism of missingness will be explored:

- If data are missing at random (MAR), multiple imputation will be considered.
- Sensitivity analysis will compare results from the complete-case and imputed datasets.

- Patients who withdraw early will be described in the Consolidated Standards of Reporting Trials (CONSORT) flow diagram and excluded from PP analysis but retained in ITT.

7. Subgroup and Sensitivity Analyses

Subgroup analyses will explore differences in primary and secondary outcomes by:

- Gender (male vs female)
- Age groups (≤35 vs >35 years)
- Tooth type (molars vs others)

A sensitivity analysis will be performed using the PP population to evaluate the robustness of the findings.

8. Interim Analysis

No interim efficacy analysis is planned due to the small sample size and short follow-up. However, descriptive summaries of patient enrolment, adherence, and safety may be reviewed periodically by the research team to ensure study integrity.

9. Quality Control and Data Management

All clinical data will be entered into a secure, password-protected database. Double data entry and validation will be employed to minimize transcription errors. The dataset will be anonymized before analysis, and all files will be regularly backed up. A final data review will be conducted before the database lock. Only locked, cleaned data will be used for the final statistical analysis.

10. Ethical Considerations

The study was reviewed and approved by the Institutional Review Board (IRB) at King Saud University (Approval Ref: 20/0416/IRB). It complies with the Declaration of Helsinki and ICH-GCP guidelines. Written informed consent was obtained from all participants before study enrollment. Data confidentiality and participant safety will be strictly maintained throughout.

11. References

Löe H, Silness J. *Periodontal Disease in Pregnancy I. Prevalence and Severity.* Acta Odontologica Scandinavica. 1963;21(6):533–551.

12. Appendices

Shell Tables

Table 1. Baseline Characteristics of Study Participants (ITT Population)

Characteristic	Test Group (HA) (n=15)	Test Group (HA) (n=15)	Total (N=30)
Age (mean ± SD), years			
Age Group, n (%)			
≤35 years			
>35 years			
Gender, n (%)			
Male			
Female			
Cigarettes/day (mean ± SD)			
Tooth type (molar/premolar/ant), n			
Periodontal Probing Depth (mm)			
Bleeding on Probing (%)			
Plaque Index (%)			
Gingival Index (mean ± SD)			

Abbreviations: HA, Hyaluronic acid; ITT, Intent-to-Treat; mm, Millimeter; SD, Standard deviation.

Table 2. Change in PPD Over Time

	Test Group (HA) (mean ± SD)	Control Group (Saline) (mean ± SD)	Between-Group p-value
Baseline			
2 Weeks			
6 Weeks			

Abbreviations: HA, Hyaluronic acid; PPD, Periodontal Probing Depth; SD, Standard deviation.

Statistical tests: Repeated Measures ANOVA or Mixed Model

Table 3. Pain Assessment by VAS Score (0–10 Scale)

	Test Group (HA) (mean ± SD)	Control Group (Saline) (mean ± SD)	Between-Group p-value
2 Weeks			
6 Weeks			

Abbreviations: HA, Hyaluronic acid; SD, Standard deviation; VAS, Visual Analogue Scale.

Statistical test: t-test or Mann–Whitney U

Table 4 Healing Index Scores (Landry Scale)

	Median (IQR) – HA Group	Median (IQR) – Control Group	p-value
2 Weeks			
6 Weeks			

Abbreviations: HA, Hyaluronic acid; IQR, Interquartile range

Statistical test: t-test or Mann–Whitney U

Table 5. Gingival Index (Löe & Silness)

	Median (IQR) – HA Group	Median (IQR) – Control Group	p-value
Baseline			
2 Weeks			
6 Weeks			

Abbreviations: HA, Hyaluronic acid; IQR, Interquartile range.

Table 6. BoP and PI (%)

	BoP – Test Group (%)	BoP – Control Group (%)	PI – Test Group (%)	PI – Control Group (%)	p-value (BoP)	p-value (PI)
Baseline						
2 Weeks						
6 Weeks						

Abbreviations: BoP, Bleeding on Probing; PI, Plaque index.

Statistical test: Chi-square or Fisher’s Exact Test

Table 7. Radiographic Assessment of Bone Level Change (Descriptive)

Participant ID	Group	Baseline Bone Level	6-Week Bone Level	Change Observed (Yes/No)

Shell Figures

Figure 1. Line Graph: Mean PPD Over Time by Treatment Group

- X-axis: Time (Baseline, 2 Weeks, 6 Weeks)
- Y-axis: Mean PPD (mm)
- Two lines: HA group vs Control group
- Error bars: Standard Deviation

Figure 2. Boxplot: Healing Index Scores at 2 and 6 Weeks

- Grouped boxplots:
    - X-axis: Group (Test vs Control)
    - Y-axis: Healing Index (1–5 scale)
    - Separate panels or colors for 2-week and 6-week timepoints

Figure 3. Bar Chart: Pain VAS Scores

- X-axis: Timepoint (2 Weeks, 6 Weeks)
- Y-axis: Mean VAS score
- Bars: HA group vs Control
- Error bars: Standard deviation or 95% CI

Figure 4. Stacked Bar Chart: Proportion of Patients with Bleeding on Probing and Plaque Presence

- X-axis: Timepoints (Baseline, 2W, 6W)
- Y-axis: % Patients
- Stacks: BoP and PI present/absent
- Separate charts for HA and Control groups