STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title: Evaluation of the Effects of Dry Needling and Botulinum Toxin Applications on VAS Scores and Muscle Thickness in the Masseter and Temporalis Muscles of Patients Diagnosed With Bruxism

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1. STUDY DESCRIPTION AND OBJECTIVES

The aim of this randomized, prospective, comparative, single-blind clinical study is to evaluate and compare the effects of Dry Needling (DN), PraBotulinumtoxinA, and AbobotulinumtoxinA on Masseter and Anterior Temporal muscle thicknesses and pain intensity in patients diagnosed with bruxism.

2. ETHICAL APPROVAL

Ethical approval was obtained from the Recep Tayyip Erdogan University Faculty of Medicine Clinical Research Ethics Committee (Date: 08.01.2026, Decision No: 2026/19). The study was conducted in accordance with the principles of the Declaration of Helsinki.

3. ELIGIBILITY CRITERIA

- **Inclusion Criteria:** * Patients aged 18–65 years diagnosed with "Probable Bruxism".
    - ASA I or ASA II physical status.
    - Initial pain intensity (VAS score) >= 8.
- **Exclusion Criteria:** * Allergy to botulinum toxin, silver, or gold.
    - Pregnancy, neuromuscular diseases, or bleeding disorders.
    - Previous TMJ surgery or current use of other treatments.

4. STUDY DESIGN AND INTERVENTIONS

Participants were randomly assigned to three groups:

1. **Group 1 (Dry Needling):** Three sessions of dry needling applied at 7-day intervals to trigger points.
2. **Group 2 (PraBotulinumtoxinA - Nabota):** Total 50U BT-A (15U per masseter, 10U per temporal muscle).
3. **Group 3 (AbobotulinumtoxinA - Dysport):** Total ≈167U BT-A (≈50U per masseter, ≈33U per temporal muscle).

5. OUTCOME MEASURES

- **Primary Outcome:** Change in pain intensity measured by Visual Analog Scale (VAS) at baseline, 1st month, and 3rd month.
- **Secondary Outcome:** Change in muscle thickness (Masseter and Anterior Temporal) measured via Ultrasonography (USG) at baseline and 3rd month.

6. STATISTICAL ANALYSIS PLAN

- **Sample Size Calculation:** Power analysis was performed using G*Power (ver. 3.1.9.7). With a power of 0.80, effect size of 0.5, and alpha of 0.05, a minimum of 14 patients per group was required. A total of 69 patients were included to ensure robustness.
- **Statistical Methods:**
    - Data normality was tested using Kolmogorov-Smirnov, Skewness, and Kurtosis tests.
    - One-Way ANOVA and Post-hoc Duncan tests were used for inter-group comparisons.
    - Paired samples t-test was used for intra-group comparisons.
    - Repeated Measures ANOVA was used to analyze changes over time.
    - All analyses were performed using IBM SPSS Statistics 26.0, and the statistical significance level was set at $\mathbf{p < 0.05}$.