Short-term Efficacy of Chlorhexidine and Povidone-Iodine Mouth Rinses in Reducing Salivary SARS-CoV-2 Viral Load: A Randomized Controlled Clinical Trial

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1. Background and Rationale

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is primarily transmitted through respiratory droplets and aerosols. Dental procedures pose a significant transmission risk due to close proximity to the oral cavity and aerosol generation. Saliva has been identified as a potential reservoir for SARS-CoV-2. Therefore, reducing viral load in saliva prior to dental treatment may reduce the risk of cross-infection.

Several antiseptic mouth rinses have been proposed as preprocedural interventions to reduce viral load in the oral cavity. This study evaluates and compares the short-term effects of chlorhexidine (in both rinse and lozenge forms) and povidone-iodine mouth rinse on salivary viral load in confirmed COVID-19 patients.

2. Objectives

Primary Objective

- To evaluate the short-term efficacy of different mouth rinses and lozenges (chlorhexidine rinse, chlorhexidine lozenges, and povidone iodine rinse) in reducing salivary SARS-CoV-2 viral load.

Secondary Objective

- To compare each intervention with saline as a control.
- To assess changes in viral load using Ct values of E and S genes via RT-PCR.

Material and methods:

3. Study Design

- This study is a **four-arm, open-label, randomized controlled clinical trial** designed to evaluate the short-term efficacy of three oral antiseptic interventions—chlorhexidine digluconate mouth rinse, chlorhexidine digluconate lozenges, and povidone-iodine (PVP-

I) mouth rinse—compared to a saline control in reducing salivary SARS-CoV-2 viral load in COVID-19-positive patients.

- **Type of Study:** Open-label, randomized controlled clinical trial
- **Study Setting:** King Fahad General Hospital and Al-Hamra Community Health Center, Jeddah, Saudi Arabia
- **Registration:** ClinicalTrials.gov (NCT04941131)
- **Ethical Approval:** Jeddah Health Affairs (#1485)

Study Design

Randomization and Blinding

- **Randomization:** Participants will be allocated using a simple 1:1:1:1 randomization schedule generated via computer-based random number sequencing.
- **Allocation Concealment:** Allocation will be performed using sealed opaque envelopes.
- **Blinding:** This is an open-label study due to the obvious physical and functional differences between interventions (rinse vs. lozenge), which preclude participant and clinician blinding. However, the **laboratory personnel and statisticians will be blinded** to group allocation.

4. Participants

Inclusion Criteria:

- Adults aged ≥18 years
- Positive nasopharyngeal RT-PCR for SARS-CoV-2
- Ability to provide informed consent

Exclusion Criteria:

- Known allergy to study substances (chlorhexidine or povidone-iodine)
- Pregnancy

- Prior COVID-19 treatment
- Severe oral lesions that contraindicate rinse use

5. Interventions

Participants were randomized into four arms:

1. **Chlorhexidine Mouth Rinse (CHX):**

   10 mL undiluted solution, used for 30 seconds.

2. **Chlorhexidine Lozenges (CHX Lozenges):**

   2 mg lozenge to dissolve slowly in the mouth.

3. **Povidone Iodine Mouth Rinse (PVP-I):**

   10 mL undiluted solution, used for 30 seconds.

4. **Control (Saline):**

   10 mL normal saline rinse, used for 30 seconds.

All participants were instructed to refrain from eating, drinking, or oral hygiene procedures for at least 30 minutes before the intervention.

6. Sample Collection and Laboratory Testing

- **Saliva Collection:** Passive drool technique; 3 mL of saliva collected before and after intervention (5 minutes apart).
- **Analysis Method:**

  Real-time RT-PCR using LabGun COVID-19 Kit (Biosewoom, Korea).

  - Targets: E gene and S gene
  - Measurement: Threshold cycle (Ct) values

7. Sample Size Calculation

- **Software:** G*Power 3.1
- **Effect size:** 1.1
- **Power:** 80%
- **Alpha:** 0.05
- **Required Sample:** 15 participants per group (Total N = 60)

Sample Size Justification:

- Based on an expected moderate-to-large effect size (Cohen's f = 0.4), α = 0.05, power = 0.80, and four groups:
    - **Required sample = 60 participants (15 per group)**
    - Power calculation was performed using **G*Power 3.1**.
- To account for 10% attrition, **a total of 66 participants will be recruited.**

8. Statistical Analysis

Statistical Analysis

Software:

- Data will be analyzed using **IBM SPSS Statistics version 25** and **R (v4.3 or later)** for confirmatory and exploratory analyses.

Analysis Population:

- **Intention-to-treat (ITT):** All randomized participants will be included in the primary analysis, with imputation for missing data.

Primary Outcome Analysis:

- **Primary Endpoint:**
    - Change in **Ct value** of SARS-CoV-2 E and S genes from baseline to post-intervention (ΔCt).

- **Between-Group Comparison:**
    - **One-way ANOVA** to assess differences in mean ΔCt across all four groups.
    - **Post-hoc Bonferroni test** for pairwise comparisons.
    - If assumptions of ANOVA are violated, **Kruskal–Wallis test** with **Dunn's test** for post-hoc comparisons will be used.
- **Within-Group Comparison:**
    - **Paired t-test** to compare pre- and post-intervention Ct values within each group.
    - **Wilcoxon signed-rank test** will be used if normality is not met.

Assumptions and Validation:

- **Normality:** Assessed using **Shapiro-Wilk test** and QQ plots.
- **Homogeneity of variance:** Evaluated using **Levene's test**.
- **Effect Size Reporting:** Partial η^2 for ANOVA, Cohen's d for t-tests.

Missing Data Handling:

- For the ITT analysis, missing outcome data will be handled using **multiple imputation** (MI) under the assumption of missing at random (MAR).
- Sensitivity analyses using **last observation carried forward (LOCF)** will be conducted to assess the robustness of findings.

9. Expected Outcomes

- Povidone-iodine rinse will demonstrate the highest reduction in viral load.
- Chlorhexidine lozenges may show moderate efficacy.
- Chlorhexidine rinse may show variable outcomes.
- Control (saline) is expected to have no significant effect.

10. Ethical Considerations

- All participants provided informed written consent.
- Ethical approval was secured from local ethics board.
- All data were anonymized and securely stored.
- Participants had the right to withdraw at any time without consequences.

11. Timeline

Phase	Duration
Ethical Approval	Completed
Participant Recruitment	2 months
Data Collection	2 months
Laboratory Analysis	Concurrent
Data Analysis & Manuscript	1 month

12. Dissemination Plan

The study results will be disseminated through:

- Peer-reviewed publication
- Scientific conferences
- Institutional and ministry health updates

References

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