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RESEARCH ARTICLE

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Mouth rinses efficacy on salivary SARS-CoV-2 viral load: A randomized clinical trial

Manar M. Alzahrani[1] | Shatha Bamashmous[2] | Hanaa Alkharobi[3] | Abdullah Alghamdi[4] | Rahaf H. Alharbi[5] | Ahmed M. Hassan[6] | Manar Darwish[5] | Abdullah Bukhari[7] | Ahmad Bakur Mahmoud[8] | Mohamed A. Alfaleh[5,9] | Ahmed A. Mirza[10] | Adel M. Abuzenadah[10] | Turki S. Abujamel[5,10] | Anwar M. Hashem[5,11]

[1]Department of Oral and Maxillofacial Prosthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

[2]Department of Periodontology, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

[3]Department of Oral Biology, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia

[4]Ministry of Health, Jeddah, Saudi Arabia

[5]Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[6]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

[7]Department of Medicine, Faculty of Medicine, Imam Mohammed Ibn Saud Islamic University, Riyadh, Saudi Arabia

[8]College of Applied Medical Sciences, Taibah University, Almadinah Almunwarah, Saudi Arabia

[9]Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

[10]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia

[11]Department of Medical Microbiology and Parasitology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence

Manar M. Alzahrani, Department of Oral and Maxillofacial Prosthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia.
Email: mmalzhrani@kau.edu.sa

Turki S. Abujamel, Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.
Email: tabujamel@kau.edu.sa

Anwar M. Hashem, Department of Medical Microbiology and Parasitology, Faculty of Medicine, Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.
Email: amhashem@kau.edu.sa

Funding information

King Abdulaziz University

Abstract

Considering the global trend to confine the COVID-19 pandemic by applying various preventive health measures, preprocedural mouth rinsing has been proposed to mitigate the transmission risk of SARS-CoV-2 in dental clinics. The study aimed to investigate the effect of different mouth rinses on salivary viral load in COVID-19 patients. This study was a single-center, randomized, double-blind, six-parallel-group, placebo-controlled clinical trial that investigated the effect of four mouth rinses (1% povidone-iodine, 1.5% hydrogen peroxide, 0.075% cetylpyridinium chloride, and 80 ppm hypochlorous acid) on salivary SARS-CoV-2 viral load relative to the distilled water and no-rinse control groups. The viral load was measured by quantitative reverse transcription PCR (RT-qPCR) at baseline and 5, 30, and 60 min post rinsing. The viral load pattern within each mouth rinse group showed a reduction overtime; however, this reduction was only statistically significant in the hydrogen peroxide group. Further, a significant reduction in the viral load was observed between povidone-iodine, hydrogen peroxide, and cetylpyridinium

chloride compared to the no-rinse group at 60 min, indicating their late antiviral potential. Interestingly, a similar statistically significant reduction was also observed in the distilled water control group compared to the no-rinse group at 60 min, proposing mechanical washing of the viral particles through the rinsing procedure. Therefore, results suggest using preprocedural mouth rinses, particularly hydrogen peroxide, as a risk-mitigation step before dental procedures, along with strict adherence to other infection control measures.

KEYWORDS
coronavirus, COVID-19, cetylpyridinium chloride, hydrogen peroxide, mouthwashes, povidone-iodine, saliva, SARS-CoV-2, viral load

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a single-stranded lipid-enveloped RNA virus, transmitted via direct inhalation of infected respiratory droplets or indirect contact with contaminated surfaces or fomites.[1] COVID-19 has drastically disrupted the routine dental care system; once the World Health Organization declared COVID-19 a pandemic on March 11, 2020, in which dental clinics completely closed doors for several months.[2] The American Dental Association (ADA) recommended limiting dental practice activities to emergency treatments and refraining from elective procedures.[3] Gradually, work in dental clinics has returned with updated infection prevention recommendations and restrictions on some key dental procedures, which negatively impacted the workflow patterns in dental practices worldwide.[4] Due to the lack of effective treatment for COVID-19, most recommendations are based on preventing viral transmission.[5]

COVID-19 transmission through aerosols and salivary droplets has become a concern in dentistry.[6] Angiotensin-converting enzyme 2 (ACE2), which represents the main entry site for coronavirus-expressing cells such as epithelial cells found in the tongue and salivary glands, are SARS-CoV-2 favorable reservoirs that regularly shed the virus in saliva.[7,8] SARS-CoV-2 RNA had been detected in the saliva of 91.7% of COVID-19-positive patients.[9] Aerosol-generating procedures during different periodontics, restorative, and prosthodontics procedures generate droplets and splatters carrying the SARS-CoV-2 virus, which may contaminate nearby operatory surfaces, and remain suspended in the air for several hours, facilitating the spread of the infection.[10] Ott et al.[11] reported that SARS-CoV-2 RNA in saliva was stable at room temperature for prolonged times (about 25 days). The risk of bidirectional spread of SARS-CoV-2 infection between patients and dentists necessitates practicing meticulous preventive strategies to reduce the risk of transmission, including using preprocedural mouth rinsing with potential antiviral activity.[12]

Preprocedural mouth rinsing has been widely used before routine dental procedures to reduce the number of oral microorganisms and the risk of pathogen transmission.[5] The virucidal activity of numerous mouth rinses against different viruses, such as herpes simplex virus,[13] influenza,[14] and the middle east respiratory syndrome-coronavirus (MERS-CoV), has been suggested by in vitro studies.[15,16] Consequently, preprocedural rinsing has been recommended as a preventive measure to mitigate SARS-CoV-2 transmissions during the COVID-19 pandemic by ADA and the Centers for Disease Control and Prevention (CDC).[17,18] However, evidence from in vitro and clinical studies is limited and contradictory regarding the antiviral potential of different mouth rinses against SARS-CoV2.[19–27] The limited number of clinical studies and the equivocal results mandate conducting more clinical trials to evaluate the effectiveness of mouth rinses against salivary SARS-CoV-2.

Therefore, this study aimed to compare the efficacy of four commercially available mouth rinses povidone-iodine (PVP-I), hydrogen peroxide (H_2O_2), cetylpyridinium chloride (CPC), hypochlorous acid (HOCl) on the salivary SARS-CoV-2 viral load at four-time points (baseline (*T*0) and 5 (*T*1), 30 (*T*2), and 60 min (*T*3) post rinsing) relative to two control groups (distilled water and no-rinse) in a cohort of positive COVID-19 patients. The no-rinse group was added as a second control to investigate the possible mechanical washing effect of the mouth rinsing procedure.[28,29] The findings of this study would show whether any of these mouth rinses could potentially reduce the salivary SARS-CoV-2 viral load of nonhospitalized symptomatic patients who posed a contagious risk of disease transmission and could therefore suggest its potential use in the dental setting. The first null hypothesis was that salivary viral load would not change significantly within studied mouth rinse groups overtime compared to baseline viral load. The second null hypothesis was that salivary viral load would not be statistically significantly different between studied mouth rinse groups overtime.

2 | METHODS

2.1 | Trial design

The study design was a single-center, randomized, double-blind, six-parallel-group, placebo-controlled trial. The Ethical Committee of the

Directorate Health Affairs, Ministry of Health, Jeddah, Saudi Arabia approved the study protocol (H-02-J-002; 1384). The study was performed according to the Ministry of Health relevant guidelines/regulations and registered on ClinicalTrials.gov (22/01/2021; Identifier: NCT04721457).

2.2 | Participants

The study was conducted at the Tetamman primary health care center, specifically allocated to provide healthcare support to nonhospitalized COVID-19 patients (Jeddah, Saudi Arabia), between January and March 2021. Patients with positive nasopharyngeal or throat swab results based on RT-PCR for SARS-CoV-2 were recruited and signed written informed consent. Inclusion criteria were adults (≥18 years old), required no hospitalization, presented within 7 days of symptoms and within 2 days of positive nasopharyngeal or throat swabs, able to gargle and expectorate, and did not use mouth rinse 24 h before saliva collection. Patients were excluded from the study if pregnant and lactating, on established antiviral, corticosteroid, antimicrobial, or immunosuppressive medications, on lithium therapy, have active uncontrolled thyroid conditions, on current radioactive iodine therapy, radiotherapy, or chemotherapy, or allergic to components of mouth rinses.

2.3 | Sample size

A priori power analysis was conducted to determine the number of patients in each group using the power program (G* Power software; Christian-Albrechts-Universität Kiel).[30] A total sample size of 66 patients (11 patients per group) was calculated using an alpha of 0.05 and a power of 0.8 to estimate an effect size of 0.20 or less between and within groups to verify a 20% reduction in the salivary viral load of SARS-CoV-2 based on previous studies.[22,26] The study recruited 15 patients per group (90 in total) to overcome possible patient drop-out or undetectable SARS-CoV-2 viral load at baseline.

2.4 | Randomization, allocation concealment, and blinding

This study adopted a simple randomization method. An independent researcher performed randomization using online software generating three-digit allocation numbers (GraphPad PRISM 9.0; GraphPad Software),[31] and concealed the allocated mouth rinses in opaque sealed envelopes. Each sealed envelope contained a 15 ml sterile amber test tube filled with the assigned mouth rinse, a 120 ml sterile empty specimen container for expectoration of the mouth rinse, four identical empty 50 ml sterile test tubes for collecting saliva samples, each labeled with the allocated number and time point (*T*0, *T*1, *T*2, or *T*3), and a biohazard bag. Field researchers enrolled the patients and assigned the sealed envelopes sequentially following the allocation numbers sequence. Patients, field researchers, and research teams who performed the viral load quantification were blinded to the allocated mouth rinses.

2.5 | Intervention

A total of four mouth rinses and two control groups (distilled water [Water for Injections BP; Pharmaceutical Solutions Industry] and no-rinse) were randomly assigned to the enrolled patients ($n = 90$), with 15 patients in each group. The studied mouth rinses were: 1% povidone-iodine (PVP-I) (Betadine Mouthwash/Gargle; Avrio Health LP), 1.5% hydrogen peroxide (H_2O_2) (Peroxyl; Colgate-Palmolive), 0.075% cetylpyridinium chloride (CPC) (Colgate Total; Colgate-Palmolive), 80 ppm hypochlorous acid (HOCl) (Clinisept Dental Mouthwash; Clinical Health Technologies) and all used in full commercial concentrations with no further dilution. The study design is shown in Figure 1.

Patients completed an electronic questionnaire regarding demographic characteristics (age, sex), smoking habits, presence of comorbidities, and common COVID-19 symptoms. The patients abstained from eating, drinking, smoking, and brushing their teeth for 1 h before sample collection and during the entire collection procedure. Detailed instructions on rinsing and saliva collection procedures were given to enrolled patients.

At least 2 ml of unstimulated saliva was collected using the passive drool technique (saliva is pooled in the mouth while the head is tilted forward, then drooled directly into the tube).[32] A total of four saliva samples were collected from each patient, one at baseline (*T*0). After that, patients were requested to vigorously rinse with 15 ml of the assigned mouth rinse for 30 s. Then, three saliva samples were collected at 5 (*T*1), 30 (*T*2), and 60 min (*T*3) post rinsing. Saliva samples were kept at 4°C and transported on ice to the lab for subsequent analysis.

2.6 | Primary outcome

This study investigated the effect of four mouth rinses (PVP-I, H_2O_2, CPC, and HOCl) on salivary SARS-CoV-2 viral load relative to the distilled water and no-rinse control groups. The viral load in saliva was measured by quantitative reverse transcription PCR (RT-qPCR) assays at baseline (*T*0) and 5 (*T*1), 30 (*T*2), and 60 min (*T*3) post rinsing.

2.7 | RNA extraction and quantification

Saliva samples were analyzed at King Fahad Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. RNA was extracted from saliva samples within 24 h upon collection, using the automated Maelstrom 9600 with TANBead (OptiPure Viral Auto Plate; Taiwan Advanced Nanotech) following the manufacturer's instructions.

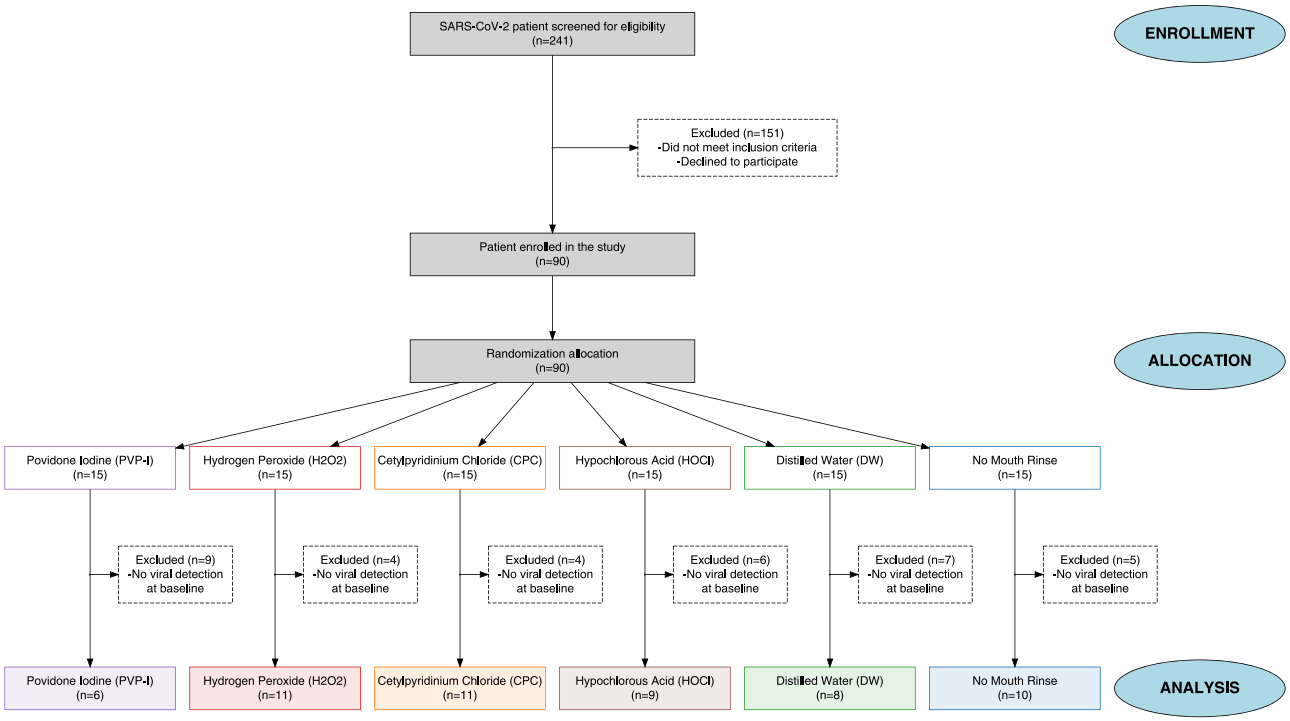

FIGURE 1 Flowchart of the patient selection in the study. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Briefly, 310 µl of each sample was added in combination with 10 µl of Proteinase K in each well. The plate was then inserted into the automated nucleic acid platform, and the purified RNA was eluted in a 50 µl elution buffer. RNA concentrations were determined using the Qubit fluorometer and Qubit RNA High Sensitivity Assay Kit (Thermo Fisher Scientific). RNA was then stored at −80°C until further processing.

2.8 | SARS-CoV-2 specific quantitative RT-qPCR

RNA was analyzed by one-step RT-qPCR using QuantiFast Probe RT-PCR Kit (Qiagen) and the QuantStudio 5 System (Applied Biosystems; Thermo Fisher Scientific). Primer pairs of the CDC (EUA 200001) N2-gene (Forward: 5′-TTACAAACATTGGCCGCAAA-3′ and Reverse 5′-GCGCG ACATTCCGAAGAA-3′) and probe (5′-FAM-ACAATTTGCCCCCAGCGC TTCAG-BHQ1-3′) were used. For every sample, 50 ng of RNA was subjected to reverse transcription performed at 50°C for 10 min. Then the amplification was initiated by a denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation for 10 s at 95°C, and combined annealing and extension for 30 s at 60°C.

For quantifications, copy numbers were calculated based on the standard curve method for absolute quantification. Ten-fold serial dilutions of viral RNA of SARS-CoV-2 were used to construct the standard curve, and nuclease-free water was used as a negative (non-template) control.[33,34] The reaction was considered positive if the cycle threshold (Ct) was <36 cycles.

2.9 | Statistical analyses

All statistical analyses were performed, and plots were produced using statistical software (R 4.1.1; R Foundation for Statistical Computing).[35] Continuous clinical and demographic variables were reported in means and SDs, while categorical variables were reported as frequencies and percentages. Comparisons of the different groups concerning categorical demographic variables were performed using the χ^2 test. Mean differences for continuous demographic variables between the different groups were determined using Kruskal–Wallis tests as normality assumption was violated. Salivary SARS-CoV-2 viral load comparisons between the different mouth rinse groups over time and over-time changes within each group were performed using linear mixed models with patients as random effects and implemented in the "lme4" package version 1.1.27.1.[36] For multiple comparisons, p values for all tests were adjusted using Benjamini and Hochberg's false discovery rate (FDR of 5%) correction,[37] and statistical significance was determined when adjusted p values were <0.05. Post hoc corrected pairwise comparisons were performed using the "emmeans" package version 1.7.1.1 in R software.[38] Associations between viral load with demographic and clinical variables were assessed with Spearman's correlations. Percent reduction in the viral load (copies/ml) was calculated at each time point versus baseline. Statistical significance was considered when p values were less than 0.05.

3 | RESULTS

3.1 | Study population

Out of 241 screened patients with positive nasopharyngeal or throat swabs for SARS-CoV-2, 90 patients met the inclusion criteria and were randomly and equally assigned to one of the six study groups. Saliva samples from 55 patients with detectable SARS-CoV-2 RNA at baseline based on RT-qPCR were included in the statistical analysis (Figure 1). The number of patients in the different mouth rinse groups comprised 6 patients in PVP-I, 11 in H_2O_2, 11 in CPC, 9 in HOCl, 8 in distilled water, and 10 in the no-rinse group. All patients completed the study with no reported adverse events.

The demographic characteristics of the study population in the different groups are summarized in Table 1. The mean age of all patients was 37.18 ± 10.93 (range: 18–65 years); the majority were male (76.4%). Predominant symptoms reported by patients in the study included fever, fatigue, headache, dry cough, and the loss of taste and smell sensation. The time between the onset of symptoms and enrollment in the study ranged from 1 to 6 days with a median of 2 days. About 69.1% of patients were smokers, and 14.5% with systemic conditions including diabetes, hypertension, and heart diseases. There were no statistically significant differences between the different mouth rinse groups regarding demographic variables (Table 1).

3.2 | Viral load analysis

Salivary SARS-CoV-2 viral load data (copies/ml) for the different groups over time are shown in Figure 2. The total number of analyzed saliva samples was 220, of which 6 had no detectable SARS-CoV-2 RNA and were excluded and 214 saliva samples were included in the final analysis. Interindividual variability in baseline viral loads was evident; salivary viral load ranged between 10^7 and 10^{12} copies/ml with a mean of 10^9 copies/ml (Table 2). There was no statistically significant difference in salivary viral load at baseline between different study groups. A higher viral load value at baseline was associated only with older age groups ($r = 0.267$, $p = 0.04843$).

Overall, there was a reduction in the salivary SARS-CoV-2 viral load over time within the different mouth rinse groups; however, only the H_2O_2 group showed a significant reduction at all three-time points (*T*1, *T*2, and *T*3) compared to baseline viral load ($p = 0.0478$, $p = 0.0402$, and $p = 0.0485$, respectively) (Figure 2A). Similarly, the distilled water control group showed a decrease in viral load across time points compared to baseline but was not statistically significant

TABLE 1 Description of the study population

Characteristics	PVP-I *n* = 6	CPC *n* = 11	H_2O_2 *n* = 11	HOCl *n* = 8	DW *n* = 8	No-rinse *n* = 10	*p* value
Age, years (mean ± SD)	41.83 ± 9.54	32.64 ± 11.22	34.36 ± 9.09	42.67 ± 13.53	36.62 ± 12.05	38 ± 8.89	0.37
Symptoms onset, days (median, range)	2 (2–5)	2 (1–4)	2 (1–6)	2 (1–4)	2.5 (1–5)	2 (1–4)	0.92
Smoking, *n* (%)	5 (83.3)	7 (63.6)	7 (63.6)	5 (55.6)	7 (87.5)	7 (70)	0.71
Male, *n* (%)	5 (83.3)	8 (72.7)	7 (63.6)	9 (100)	4 (50)	9 (90)	0.15
Female, *n* (%)	1 (16.7)	3 (27.3)	4 (36.4)	0 (0)	4 (50)	1 (10)	
Comorbodities, *n* (%)	1 (16.7)	0 (0)	2 (18.2)	2 (22.2)	1 (12.5)	2 (20)	0.75
COVID-19-related symptoms, *n* (%)							
Fever	2 (33.3)	7 (63.6)	4 (36.4)	5 (62.5)	4 (50)	7 (70)	
Chills	2 (33.3)	6 (54.5)	2 (18.2)	4 (50)	2 (25)	6 (60)	
Runny nose	2 (33.3)	4 (36.4)	3 (27.3)	2 (25)	2 (25)	1 (10)	
Fatigue	3 (50)	5 (45.5)	7 (63.6)	6 (75)	7 (87.5)	8 (80)	
Sore throat	3 (50)	4 (36.4)	5 (45.5)	5 (62.5)	3 (37.5)	3 (30)	
Dry cough	4 (66.7)	9 (81.8)	7 (63.6)	6 (75)	3 (37.5)	6 (60)	
Headache	6 (100)	5 (45.5)	7 (63.6)	2 (25)	5 (62.5)	7 (70)	
Loss of taste sensation	4 (66.7)	5 (45.5)	2 (18.2)	3 (37.5)	2 (25)	5 (50)	
Loss of smell sensation	4 (66.7)	6 (54.5)	3 (27.3)	4 (50)	1 (12.5)	5 (50)	
Diarrhea	3 (50)	4 (36.4)	3 (27.3)	2 (25)	0 (0)	1 (10)	

Note: 1% povidone-iodine (PVP-I), 1.5% hydrogen peroxide (H_2O_2); 0.075% cetylpyridinium chloride (CPC), hypochlorous acid (HOCl), distilled water (DW) (control) or no-rinse group (control).

p Values were calculated using the χ^2 test for categorical variables and the Kruskal–Wallis test for continuous data (significance level $p < 0.05$).

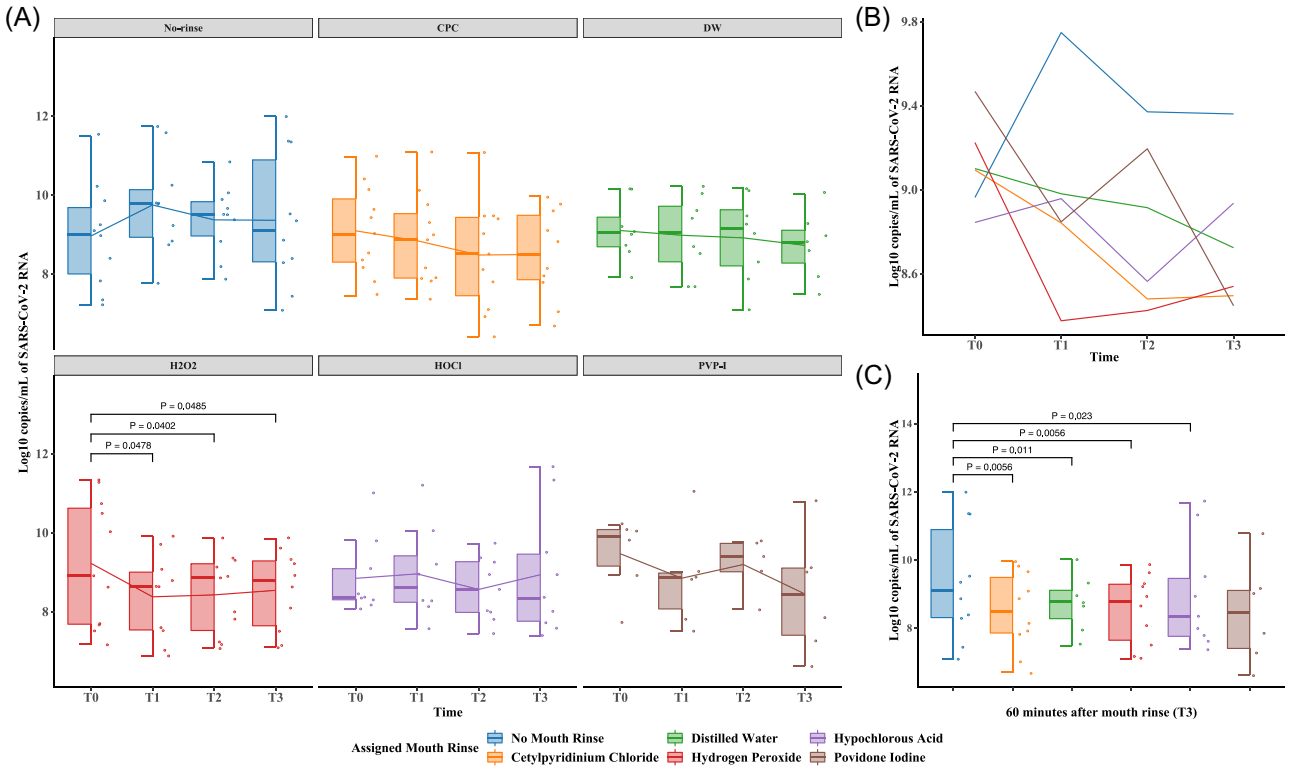

FIGURE 2 Salivary SARS-CoV-2 viral loads for the different groups over time. (A) Temporal changes in the salivary viral load (copies/ml) stratified by the different study groups across time points. *T0* for baseline and *T1*, *T2*, and *T3* correspond to 5, 30, and 60 min post rinsing, respectively. One percent povidone-iodine (PVP-I) ($n = 6$), 1.5% hydrogen peroxide (H_2O_2) ($n = 11$), 0.075% cetylpyridinium chloride (CPC) ($n = 11$), 80 ppm hypochlorous acid (HOCl) ($n = 9$), distilled water (DW) (control) ($n = 8$), or no-rinse group (control) ($n = 10$). Boxes represent data and medians ± interquartile ranges (IQR); whiskers and outliers >1.5 IQR below the 25th and above 75th percentile. *Y*-axis log10-scaled. Significance level $p < 0.05$. (B) Trend lines represent mean values of salivary viral loads (copies/ml) in the different groups at the four different time points. *T0* for baseline and *T2*, *T3*, and *T4* correspond to 5, 30, and 60 min after the mouth rinse, respectively. *Y*-axis log10-scaled. (C) Comparison of salivary SARS-CoV-2 loads between different groups at 60 min (T3) shows that H_2O_2, CPC, PVP-I, and DW were significantly different from the no-rinse group. *Y*-axis log10-scaled. *p* Values were obtained using linear mixed models with FDR adjustments (significance level $p < 0.05$). Salivary viral loads (copies/ml) for all different groups over time

TABLE 2 Mean ± SD salivary SARS-CoV-2 load (copies/ml) in the different mouth rinse groups, at baseline, 5, 30, and 60 min after rinsing. Salivary SARS-CoV-2 viral load (copies/ml) in the different study groups, at baseline (*T0*), 5 (*T1*), 30 (*T2*), and 60 (*T3*) min after rinsing

	Total (*n*)		PVP-I	H_2O_2	CPC	HOCl	DW	No-rinse
T0	55	Mean	7.65E + 09	4.64E + 10	1.27E + 10	1.23E + 10	4.16E + 09	3.42E + 10
		SD	6.35E + 09	8.11E + 10	2.76E + 10	3.37E + 10	6.25E + 09	9.87E + 10
T1	53	Mean	1.89E + 10	1.59E + 09*	1.34E + 10	2.17E + 10	4.38E + 09	9.63E + 10
		SD	4.52E + 10	2.76E + 09	3.72E + 10	5.58E + 10	6.14E + 09	1.97E + 11
T2	54	Mean	2.99E + 09	1.34E + 09*	1.18E + 10	1.21E + 09	4.22E + 09	1.02E + 10
		SD	2.59E + 09	2.18E + 09	3.57E + 10	1.74E + 09	6.04E + 09	2.10E + 10
T3	52	Mean	1.05E + 10	1.63E + 09*	2.18E + 09	7.32E + 10	2.08E + 09	1.44E + 11
		SD	2.46E + 10	2.31E + 09	3.27E + 09	1.60E + 11	3.82E + 09	3.08E + 11

Note: 1% povidone-iodine (PVP-I), 1.5% hydrogen peroxide (H_2O_2), 0.075% cetylpyridinium chloride (CPC), hypochlorous acid (HOCl), distilled water (DW) (control), or no-rinse group (control).

The asterisk indicates a significant difference compared to baseline (*T0*) ($p < 0.05$, linear mixed models with FDR adjustments).

(*T*0 vs. *T*1 $p = 0.9933$, *T*0 vs. *T*2 $p = 0.9995$, and *T*0 vs. *T*3 $p = 0.5913$). The viral load for the no-rinse control group showed a transient initial increase after 5 min ($p = 0.6869$) which returned to baseline level at 60 min. The reduction in viral load for the H_2O_2 group was seen immediately after rinsing at 5 min and was sustained over time at 30 and 60 min, with a median percent reduction in viral load around 81.3%, 88%, and 64.9%, respectively. However, the observed reduction in viral load for CPC (83.1%) and PVP-I (91.0%) was delayed until after 30 and 60 min, respectively. HOCl did not show a significant effect on salivary viral load overtime compared to the baseline (*T*0 vs. *T*1 $p = 0.9179$, *T*0 vs. *T*2 $p = 0.9628$, and *T*0 vs. *T*3 $p = 0.253$).

Statistical comparisons showed no significant difference between the different mouth rinse groups in the efficacy of viral load reduction at the different time points (Figure 2B). The effect of PVP-I, H_2O_2, and CPC mouth rinses on salivary viral load reduction was significant compared to the no-rinse group at 60 min ($p = 0.023$, $p = 0.0056$, and $p = 0.0056$, respectively) (Figure 2C). Interestingly, the distilled water control group also showed a significant decrease in viral load compared to the no-rinse group at 60 min ($p = 0.011$) (Figure 2C).

The median percent reduction in SARS-CoV-2 viral load for the different mouth rinses groups at 5 min ranged between 31.8% through 81.3%, which increased after 30 and 60 min of rinsing to 16.8%–88% and 52.4%–91%, respectively (Figure 3A). There were significant differences in the percent reduction after 5 min of rinsing between the different mouth rinse groups (PVP-I, H_2O_2, CPC, and HOCl) as well as the distilled water control group compared to no-rinse group, $p = 0.0262$, $p = 0.0116$, $p = 0.0116$, $p = 0.0398$, and $p = 0.0197$, respectively (Figure 3A). The percentage of patients with more than 50% decrease in viral load relative to baseline increased over time in the H_2O_2, PVP-I, and CPC groups reaching 60%, 66.7%, and 50% of patients at 60 min, respectively (Figure 3B).

4 | DISCUSSION

The COVID-19 pandemic created an exceptional circumstance where different agencies rapidly developed updated infection prevention and control guidelines to cope with the crisis.[3,18] The association between the salivary viral load and the risk of SARS-CoV-2 transmission has been demonstrated. Bhavnani et al.[39] reported that 77% of contacts that tested positive were significantly correlated with the case's salivary viral loads of more than 1×10^5 per ml (relative risk [RR] = 1.27). Preprocedural mouth rinsing using 1% H_2O_2 or 0.20% PVP-I was among the recommended interim guidelines to minimize the risk of SARS-CoV-2 transmission to the patients as well as healthcare personnel via decreasing viral load in saliva, thus reducing dental aerosols viral load.[17,40,41] Although in vitro studies showed rapid and effective virucidal activity of different mouth rinses against the SARS-CoV-2 virus, these studies are usually conducted where viruses are cultured under artificial conditions and

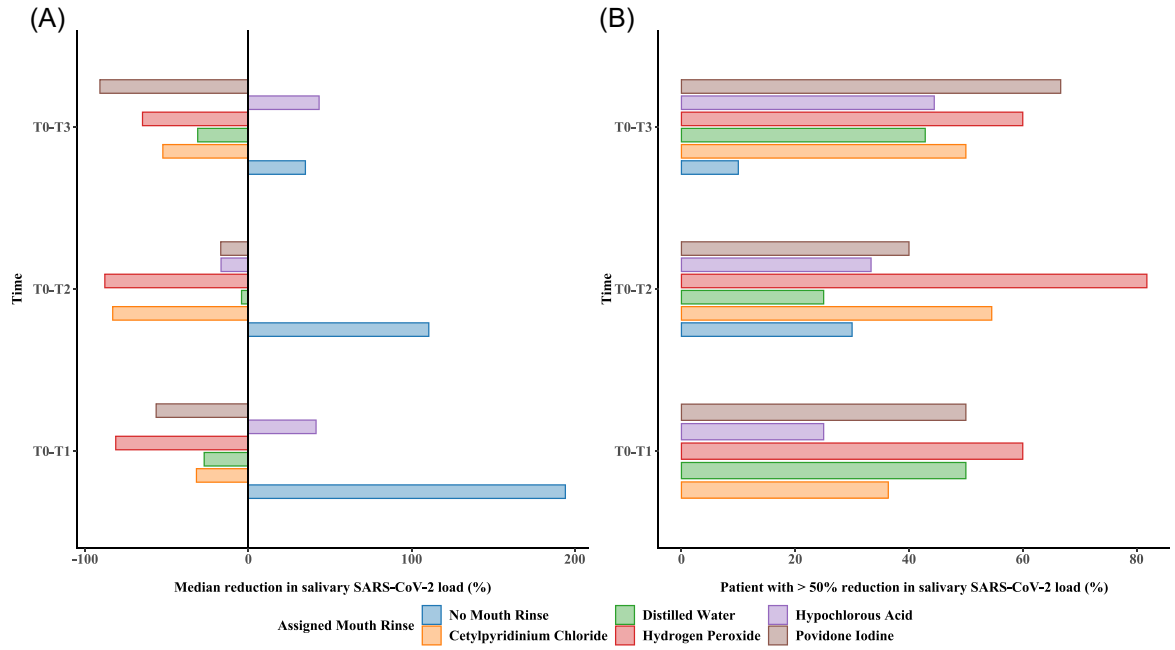

FIGURE 3 (A) Reduction of salivary SARS-CoV-2 viral load at 5 (*T*1), 30 (*T*2), and 60 (*T*3) min relative to the baseline (*T*0) in each study group; 1% povidone-iodine (PVP-I), 1.5% hydrogen peroxide (H_2O_2), 0.075% cetylpyridinium chloride (CPC), hypochlorous acid (HOCl), distilled water (control), or no-rinse group (control). Bars represent the median percent reduction from baseline at each time point. (B) Bar plots represent the percentage of individuals with >50% reduction in salivary viral load relative to baseline values at different times points in each study group (changes at 5 min: *T*0–*T*1; changes at 30 min: *T*0–*T*2; changes at 60 min: *T*0–*T*3). Reduction of salivary severe acute respiratory syndrome coronavirus 2 load.

treated directly with mouth rinses.[19,21] Thus, data obtained from vitro studies could not be extrapolated clinically without further in vivo investigation that considers human variability, including dilution of mouth rinses by saliva, deactivation by salivary glycoproteins, and the affinity of the mouth rinse to other microorganisms present in the mouth.[25] Several clinical studies emerged with conflicting results regarding the effectiveness of preprocedural mouth rinses against SARS-CoV-2.[19–27] Therefore, this study investigated the effect of four commercially available mouth rinses (PVP-I, H_2O_2, CPC, and HOCl) on salivary SARS-CoV-2 viral load overtime.

In the current study, salivary SARS-CoV-2 viral load appeared to show a reduction over time within all mouth rinse groups compared to the baseline; however, this reduction was only statistically significant for the H_2O_2 group; thereby, the first null hypothesis was only rejected for H_2O_2 group (Figure 2A). H_2O_2 is widely known for its immediate antiviral action (oxygen-free radicals disrupt the viral membrane and degrade the viral RNA), which is not expected to last long as H_2O_2 is chemically unstable.[42] In this study, H_2O_2 demonstrated an immediate reduction in the viral load after 5 min; this reduction was sustained up to 60 min post rinsing, which was not consistent with its expected low substantivity (i.e., less persistence in the mouth). Eduardo et al.[43] and Guimarães et al.[27] reported a significant reduction of the viral load in saliva immediately after rinsing with 1.5% H_2O_2 and 30 min post rinsing. However, this reduction with H_2O_2 lasted for a short-term period as viral load returned to its baseline value after 60 min.[44] Contrary findings were reported by Ferrer et al.[25] and Gottsauner et al.,[26] in which the use of 1% H_2O_2 had no statistically significant effect on salivary SARS-CoV-2 viral load. The discrepancy seen in the results between different studies could be attributed to methodological differences such as mouth rinse concentration and rinsing duration.[27,43]

The use of PVP-I, CPC, and HOCl mouth rinses resulted in a statistically insignificant reduction in salivary viral load overtime compared to baseline in this study.[19,21,25] Comparable findings have been described in other clinical studies.[22,25,27,43] Ferrer et al.[25] and Seneviratne et al.[22] found no statistically significant difference in salivary viral load after using CPC and PVP-I. On the contrary, Eduardo et al.[43] showed a significant reduction in SARS-CoV-2 viral load after rinsing with a combination of CPC + Zinc which lasted up to 60 min. On the other hand, Guimarães et al.[27] reported a significant reduction in SARS-CoV-2 viral load immediately after rinsing with 0.1% sodium hypochlorite, prepared by diluting household bleach (5%–6% NaClO) in H_2O, but not 15 and 30 min post rinsing. Although preprocedural instructions were clearly given to the patients in this study, the effect of the gargling procedure intensity cannot be eliminated. Therefore, contradictory results between studies should be interpreted with some caution, and future research is warranted considering the possible effect of factors such as the mouth rinse content and preparation and sample collection procedure on salivary SARS-CoV-2 viral load.

The data presented in the study reveal a significant reduction in salivary SARS-CoV-2 viral load between H_2O_2, CPC, and PVP-I groups compared to the no-rinse group at 60 min (Figure 2C), rejecting the second null hypothesis. Further, the median percent reduction in SARS-CoV-2 viral load for the H_2O_2, CPC, and PVP-I mouth rinses increased over time, reaching (64.9%, 52.4%, and 91%, respectively) at 60 min (Figure 3A). These findings can be attributed to the late antiviral potential of these mouth rinses, as time is needed to disturb the viral transmission, replication, and shedding.[8,29] Chaudhary et al. reported a similar median reduction in SARS-CoV-2 viral load for the H_2O_2 and PVP-I at 45 min ranging from 70% to 97%; however, these reductions in viral load did not differ significantly compared to the saline control group. Therefore, in conjunction with the antiviral potential of the mouth rinses, a mechanical washing effect of the viral particles due to the rinsing procedure or swallowing cannot be ignored.[43]

To eliminate the possible mechanical washing effect of the rinsing procedure on salivary viral load, two control groups were included in this study: distilled water and no-rinse groups. The distilled water control group showed a reduction pattern in viral load over time similar to the H_2O_2, CPC, and PVP-I groups; this reduction was also significant after 60 min compared to the no-rinse group, suggesting a true mechanical washing of the viral particles (Figure 2B,C). This finding indicates that the no-rinse group served as a more appropriate control than distilled water. Intriguingly, a transient increase of salivary viral load in the no-rinse group was observed after 5 min, which could be the result of the stimulation of more viral particles released from nearby oral reservoirs such as mucosa, tongue, or salivary glands.[29] Due to the possible mechanical washing effect of mouth rinses, Ferrer et al.[25] suggested conducting a viral culture experiment with saliva samples collected after mouth rinsing to further explore its virucidal potential. Gottsauner and colleagues[26,27,43] attempted to study the effect of H_2O_2 mouth rinse on viral infectivity in their samples; however, viral cultures were obtained from only one baseline sample and thus unable to test its effect. In the study, all samples which were positive for SARS-CoV-2 at baseline had detectable viral RNA by RT-qPCR-based analysis even after the use of the different mouth rinses. Therefore, further investigations are needed to confirm the effect of different mouth rinses on SARS-CoV-2 viral infectivity in saliva samples collected after their use.

The current study results show marked interindividual variability in the observed response (Table 2). The variability in viral load at baseline within different groups and the temporal variability in the no-rinse control group over time suggests variation in virus shedding.[28] Further, a higher salivary viral load at baseline was associated with increased age; a similar observation was previously reported.[25] This could be attributed to the reported age-linked changes in immune responses that may reduce microorganisms' clearance.[44,45]

Although the findings from this study shed light on the efficacy of preprocedural mouth rinses in reducing the risk of COVID-19 transmission in dental practice, several limitations of this study should be noted. In the current study, the SARS-CoV-2 viral load in saliva was measured by RT-qPCR-based analysis, which may detect noninfectious (dead) viral particles.[7] Therefore, viral culture studies of

saliva are essential to determine the infectivity of the virus after using different mouth rinses. A sample size of 15 COVID-19 patients per group based on power analysis was intended; however, 55 of 90 saliva samples had detectable SARS-CoV-2 at baseline and were included in the final analysis. Post hoc power analysis was performed and the final sample size in our study ($n = 55$) with an α of 0.05 and effect size of 0.2 resulted in a power of 72% which was considered as being adequate. Further studies with a larger sample size are needed to investigate the viral load dynamics and infectivity in response to different mouth rinses.

In summary, preprocedural mouth rinses, particularly hydrogen peroxide, continue to represent a beneficial and cost-effective measure along with other infection prevention and control strategies in reducing the risk of transmission of salivary SARS-CoV-2 before dental procedures. However, the reduction of viral load influenced by mouth rinses is neither a permanent treatment nor has a long-lasting effect. Therefore, preprocedural rinsing to reduce SARS-CoV-2 viral load in saliva should be recommended as an adjunctive measure along with meticulous protective strategies implemented in dental settings. Moreover, our study findings indicated that the mechanical washing of viral particles through the rinsing procedure may not be excluded irrespective of the mouth rinses true antiviral potential. Therefore, the effect of mouth rinses on viral viability needs to be further investigated by conducting viral culture experiments from saliva samples collected after the use of different mouth rinses.

AUTHOR CONTRIBUTIONS

Manar M. Alzahrani, Shatha Bamashmous, and Hanaa Alkharobi contributed to the conception and design of the study. Manar M. Alzahrani, Abdullah Alghamdi, and Shatha Bamashmous were involved in data acquisition. Rahaf H. Alharbi, Ahmed M. Hassan, Manar Darwish, Abdullah Bukhari, Ahmad Bakur Mahmoud, Mohamed A. Alfaleh, Adel M. Abuzenadah, Turki S. Abujamel, and Anwar M. Hashem performed the experimental work. Manar M. Alzahrani, Hanaa Alkharobi, Shatha Bamashmous, Rahaf H. Alharbi, Manar Darwish, Ahmed A. Mirza, Turki S. Abujamel, and Anwar M. Hashem contributed to data analysis and interpretation. Manar M. Alzahrani, Hanaa Alkharobi, and Shatha Bamashmous drafted the manuscript. Adel M. Abuzenadah and Anwar M. Hashem obtained the fund. All authors critically revised the manuscript, gave their final approval, and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Manar M. Alzahrani http://orcid.org/0000-0003-4639-3500
Shatha Bamashmous http://orcid.org/0000-0003-1078-5991
Anwar M. Hashem https://orcid.org/0000-0002-8471-7011

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