Reduction in SARS-CoV-2 Oral Viral Load with Prophylactic Mouth Rinse


1Department of Oral and Maxillofacial Surgery, Augusta University, Georgia, United States
2Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3Department of Oral and Maxillofacial Surgery, School of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran
4Department of Otolaryngology, Masih Daneshvari Hospital, Tehran, Iran
5Health Sciences Research Center, Health Sciences & Technology Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran
6Nutrition Health Research Center, Health Sciences & Technology Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran
7Center of Excellence for Occupational Health, Research Center for Health Sciences, School of Public Health, Hamadan University of Medical Sciences, Hamadan, Iran
8Department of Public Health, School of Health, Social Determinants of Health Research Center, Health Sciences & Technology Research Institute, Hamadan University of Medical Sciences, Hamadan, Iran

Address for correspondence Mahmood Dashti, DDS, School of Dentistry, Faculty of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran 1467695845, Iran (e-mail: dashti.mahmood72@gmail.com).

Eur J Gen Dent

Abstract

Objectives The medical and health facilities are at high risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. This study tested the preprocedural prophylactic mouthwash rinses to reduce the oral viral load. The findings from this study will help the practitioners to select the best mouthwash for the patients to mitigate the risk of transmission during aerosolizing. This study aimed to evaluate the effectiveness of four commonly used types of mouthwash in reducing intraoral viral load among hospitalized coronavirus disease 2019 patients.

Materials and Methods This prospective cohort study was conducted with 116 patients referred to the Masih Daneshvari Hospital in Tehran, Hamadan University of Medical Sciences of Hamadan City, and Mashhad University of Medical Sciences. Patients were randomized into four groups with each group rinsed their mouth with 20 mL of 2% povidone-iodine, 1% hydrogen peroxide, normal saline as a control study.

Keywords

► mouthwashes
► viral load
► COVID-19
► chlorhexidine
► povidone-iodine
► hydrogen peroxide

ISSN 2320-4753.
group, or 0.12% chlorhexidine, respectively, for 20 seconds. The standard reverse transcription polymerase chain reaction method evaluated the virus load before and at 1 hour, 2 hours, and 4 hours after using the mentioned mouthwash.

**Results** Our results revealed that chlorhexidine and H$_2$O$_2$ showed the highest efficiency in reducing SARS-CoV-2 load in the oral cavity and nasopharyngeal region of patients; they increased the Ct values by 9 to 10 (before: 25.84 vs. after 32.4, $p < 0.455$) (17.333 vs. after 26.497, $p < 0.097$).

**Conclusion** Our findings suggest that chlorhexidine and H$_2$O$_2$ could be used in dental clinics to reduce the risk of transmitting the SARS-CoV-2 virus from infected individuals to dentists before dental procedures.

**Introduction**

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread worldwide and has created a significant threat to global health.\textsuperscript{1,2} It is known that SARS-CoV-2 can be detected in the nasopharynx, nasal cavity, viral shedding, and oropharynx in various clinical phases of illness and is mainly transmitted from infected individuals to others through respiratory aerosols and droplets exhaled from the mouth and nose during breathing and speaking.\textsuperscript{3–5} Dentists are at a high risk of contracting the coronavirus due to many droplets and aerosols produced during dental procedures.\textsuperscript{6} It has been reported that the virus genome is detectable in saliva\textsuperscript{7} and only in saliva in some cases.\textsuperscript{8} Previous studies showed that the virus polymerase chain could remain in the workplace for 3 to 8 hours and thus had the potential to infect other individuals at the dental clinics.\textsuperscript{9} There is currently no effective treatment for the SARS-CoV-2 infection. In this regard, nasal and oral antisepsis may be helpful to reduce the amount of active aerosolized virus particles in the oral cavity and nose, thus consequently decreasing the transmission risk of SARS-CoV-2. There are different kinds of antiseptic mouth rinses with antimicrobial properties used in different clinical situations for various therapeutic purposes.\textsuperscript{10} Benzamide peroxide hydrogen, chlorhexidine, ethanol, and povidone-iodine (PVP-I) are the most common commercially available mouthwashes with antimicrobial properties that can reduce a load of some bacteria in the oral environment. However, the ability of these mouthwashes to inactivate SARS-CoV-2 is still controversial.\textsuperscript{11} Therefore, due to the high risk of SARS-CoV-2 transmission in dental clinics and the lack of a known effective mouthwash, the current study was conducted to assess the effects of several types of mouthwash on the reduction of viral load among COVID-19 patients referred to three public hospitals in Iran.

**Statement of the problem:** Dentists are at a high risk of contracting COVID-19 due to the nature of their work; it is hard for dentists to serve their patients when dentists are wearing protective gear like masks. Aerosols come from the mouth of patients and get into contact with dentists as well as contaminating working areas where dentists frequently touch. The virus remains active for up to 8 hours outside the human body. The risk of SARS-CoV-2 transmission from patients to dentists is higher when the viral load in patients’ mouths is very high than when the viral load is low.

**Justification:** Some mouthwashes, such as povidone, hydrogen peroxide, and chlorhexidine, have been determined to lower the viral load in the mouth of patients with COVID-19. When used to clean the mouth, these mouthwashes reduce the amount of virus in the mouth of COVID-19 patients, consequently reducing the risk of transmitting the virus to dentists treating them. These mouthwashes can be used between one to 4 hours before their dentist starts with the treatment.

**Objectives:** The aim of this study was to determine the efficiency of the four commonly used types of mouthwash that reduce intraoral viral load among COVID-19 patients referred to the dedicated COVID-19 public hospitals at Hamadan University of Medical Sciences in Hamadan city, Shahid Beheshti University of Medical Sciences, and Mashhad University of Medical Sciences in Iran, with the supervision of Augusta University of Georgia, USA.

**Hypothesis:** Mouthwashes reduce viral load in the mouth of COVID-19 patients.

**Research in Context**

**Evidence before this study:** We reviewed the literature on PubMed, Science Direct, and Google Scholar Databases. We also searched World Health Organization, Centers for Disease Control and Prevention, and Americans with Disabilities Act websites and various journals for articles published between January 1, 2020, and November 30, 2020. Until now, the effects of various mouthwashes have been tested on the viral load of COVID-19 patients. These studies were designed and conducted due to the lack of a comparative clinical trial investigating the effect of different solutions on reducing intraoral viral load among COVID-19 patients.

**The added value of this study:** Our observations show that chlorhexidine and benzamide peroxide were the most effective mouthwashes for reducing viral load in the mouth of confirmed COVID-19 patients and normal saline for reducing SARS-CoV-2 load in the oral cavity and nasopharyngeal region of the patients. The study’s findings can be a road map for other dental institutions and dental care settings to continue their activities during the COVID-19 pandemic.

**Implications of all the available evidence:** This research can help manage COVID-19 patients to allow dental
institutions and dental care settings to continue their activities safely during the COVID-19 pandemic.

Materials and Methods

Study Population

Patients and Informed Consent
A cohort study was conducted on 59 patients with age range 18 to 70 years referred to the hospitals of Hamadan University, Iran, 69 patients referred to Masih Daneshvari Hospital, and 8 patients referred to Mashhad University of Medical Science Hospital. The illness of these patients was confirmed according to diagnostic criteria, including laboratory tests (complete blood count, erythrocyte sedimentation rate, C-reactive protein, D-dimer, ...), reverse transcription polymerase chain reaction (RT-PCR) test, radiographs, and computed tomography (CT) scans of the lungs. Patients enrolled in the study should have confirmed COVID-19. They should have a previous consistent history of using PVP-I 2%, hydrogen peroxide 1%, normal saline, or 0.2% acetyl pyridinium chloride to clean their mouth and should continue using the disinfectants regularly as they are recovering from COVID-19. Patients were excluded if they had unstable clinical conditions, mechanical ventilation, low level of consciousness, pregnant or breastfeeding thyroid disorders, and various oral diseases. Before being included in the study, all patients who met the inclusion criteria were given a consent form to participate in the study. The study's objective and possible benefits and side effects of using mouthwashes were explained to each participant. The protocol of this study was approved by Hamadan University of Medical Sciences ethical code: IR.UMSHA.REC.1398.235 and IRCT code: 20170117032025N7.

Settings
This research involved Shahid Beheshti Medical University, Hamadan University of Medical Sciences, and Mashhad University of Medical Sciences. Augusta University served as the lead site, indicating that all required approvals are already obtained or will be obtained at each site prior to project implementation. This is a randomized, controlled clinical trial to assess the efficacy of three different standards of care mouthwash solutions relative to normal saline as a control group study in reducing the SARS-CoV-2 virus load in the mouth of COVID-19 patients over the course of 4 hours, following the mouth rinse compared to the viral load prerinse. As one of the adaptable flexible and amiable specimen options, saliva offers extraordinary focal points for far-reaching screening techniques due to its noninvasive properties, cost-effectiveness, great perseverance, and the negligible chance of cross-infection.12

Swabs were taken every 2 hours consecutively for 4 hours after the initial cycle threshold (Ct) measurement, and the viral load was determined by PCR.

Participants
One hundred sixteen patients (40 per group, including 20% dropout rate in Betadine mouthwash due to aspiration experience in hospitalized patients).

PCR Analysis and PCR CT Values

Viral RNA extraction was performed using a high pure viral nucleic acid kit (Roche, Switzerland) following the manufacturer’s protocol. According to the manufacturer’s instructions, PCR was performed using the extracted nucleic acid and a real-time COVID-19 commercial kit (Pishtaz, Iran). Briefly, a reaction included 15 mL of 9 U of the enzyme, 1 mL of primer and probe mix (RdRp/N/IC), 5 mL water, and 5 mL of extracted RNA. Then, PCR was carried out with a reverse transcription step at 50°C for 20 minutes, cDNA initial denaturation at 95°C for 3 minutes, and finally 45 cycles of 10s at 94°C and 40s at 55°C. Light Cycler 96 (LC96) PCR machine (Roche, Germany) was used to perform the amplification and to evaluate Ct values. Ct values ≤ 29 were considered strong positive reactions, Ct values of 30 to 37 indicated positive reactions, and Ct values of 38 to 40 represented weak reactions indicative of minimal amounts of target nucleic acid.

Repeated measures analysis of variance (ANOVA) was carried out to compare the difference between the means of Ct value at the different time points pre- and postadministration of mouthwashes (► Table 1).

Patients with study criteria after obtaining informed consent were randomly allocated into forth groups through balanced block randomization (block size:4): The first, second, third, and fourth groups rinsed mouth with 20 mL of 2% PVP-I

Rinse samples collected: One-hundred thirty patients x 4 (1 sample prior to rinse, 1 post-mouth rinse t = 0, 1 sample t = 2 hours, 2 sample t = 4 hours) =520. Fourteen patients dropped out from the study due to the difficulty of aspiration of Betadine. Oropharyngeal swabs = 116. (If the viral load is below detectable levels in these nasopharyngeal samples, the samples collected from the subject will be excluded from the study. Hence, we are requiring collecting samples from 116 patients). A total of 116 cases (48.3% male, 51.7% female) were randomized to receive one of the following mouthwashes.

Exclusion Criteria of the Study Population

Patients with respiratory distress (>30 breaths/min), oxygen saturation < 90 at rest under nasal oxygenation with 5 to 6 L/min, and arterial partial pressure of oxygen/fraction of inspired oxygen (FiO2) < 300 mm Hg were admitted to intensive care unit (ICU). In addition to these, patients with sepsis (Sequential Organ Failure Assessment score greater than or equal to 2), immunosuppressed patients who have symptoms of shortness of breath, fever and/or cough, and significant comorbidities (chronic kidney disease, congestive heart failure, chronic obstructive pulmonary disease, and diabetes), which may significantly worsen with concomitant COVID-19 infection, were also admitted to ICU. Mechanical ventilation indications are the presence of hypercapnic acidosis, hypoxemia despite administration of high flow nasal oxygen (FiO2 ≥ 60% and oxygen flow rate ≥ 40%), or severe dyspnea with increased work (rate) of breathing (recruitment of accessory and expiratory muscles, intercostal recession, or nasal flaring). A lung-protective ventilation strategy was applied for patients with acute respiratory distress syndrome who require mechanical ventilation.
mouthwash, 1% hydrogen peroxide mouthwash, normal saline, and cetylpyridinium chloride (CPC) 0.2%, respectively, for 20 seconds. A sample of the oropharyngeal and nasopharyngeal swab was taken from each patient in all four study groups before using mouthwash solutions to assay the virus load. Then, 30 seconds after the start of the intervention, a second and third, and fourth samples of the oropharyngeal swab were taken to determine the viral load.

All samples were transferred to the laboratory to determine the virus load by the standard RT-PCR method. To prevent damage to the prepared samples, the samples were stored at room temperature.

Statistical Analysis
Statistical Package for Social Sciences version 24.0 (SPSS, Chicago, Illinois, United States) was used for analyzing data. Quantitative data were expressed as mean ± standard deviation. The Kolmogorov–Smirnov test was used to evaluate the distribution of the data. A chi-squared test was used to assess the relation between PCR test results and viral virus loads based on mouthwash rinse type. ANOVA test made a comparison of the mean difference of Ct values (after – before) between the studied mouthwashes. P-value < 0.05 was considered significant. Sphericity assumed tests and Greenhouse Geiser tests were carried out.

Results
Different mouthwashes were used on different COVID 19 patients to determine their effect on the viral load in the mouth. Data was taken before use and after one hour consecutively for four hours.

For data analysis, repeated measures ANOVA was used, and for comparison between groups, the Bonferroni post hoc test was used. For the normalization test of the distribution of variables, the Kolmogorov–Smirnov test was used, which was a condition for normal data.

The Box’s M test was used to test the assumption of homogeneity of the covariance matrix, which has also been observed.

Mauchly’s sphericity test examined the hypothesis of the existence of multiple correlations between dependent variables. The significance level was obtained for time and group factors, equivalent to 0.011. Thus, the sphericity hypothesis was rejected. Due to the violation of this assumption, in this situation where we had the three proposed options, consisting of the Greenhouse Grazer test, the Huynfeldt test, and the lower limit test; among them the Greenhouse Grazer test seemed to be more conservative than the other two. According to this and considering the preconditions examined in the previous step, all the necessary assumptions to perform variance analysis on frequent measures were established. Therefore, this statistical data was used to interpret the intrasubject effect results.

The results of Table 2 showed that in the case of the average CT of patients, the intergroup and intragroup effects were significant. Also, the intergroup and intragroup interactions were not significant. Comparing the averages showed us that in the post-test and follow-up stages of the group, the

<table>
<thead>
<tr>
<th>Time</th>
<th>mouthwash (mean ± SD)</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>25.89 ± 7.76</td>
<td>36.26 ± 4.87</td>
<td>34.56 ± 4.92</td>
<td>32 ± 4.74</td>
<td></td>
</tr>
<tr>
<td>Benzamide</td>
<td>24.69 ± 6.61</td>
<td>33.86 ± 5.46</td>
<td>31.23 ± 3.96</td>
<td>29.61 ± 3.33</td>
<td></td>
</tr>
<tr>
<td>Betadine</td>
<td>25.70 ± 7.62</td>
<td>30.92 ± 7.68</td>
<td>27.15 ± 6.03</td>
<td>27.54 ± 5.46</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Ct, cycle threshold; SD, standard deviation.

<table>
<thead>
<tr>
<th>Type of mouthwash (I)</th>
<th>Type of mouthwash (J)</th>
<th>Mean difference (I–J)</th>
<th>Standard error</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>Benzamide</td>
<td>2.32</td>
<td>1.14</td>
<td>0.139</td>
</tr>
<tr>
<td>Betadine</td>
<td>4.35*</td>
<td>1.52</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>Benzamide</td>
<td>Chlorhexidine</td>
<td>-2.32</td>
<td>1.14</td>
<td>0.139</td>
</tr>
<tr>
<td>Betadine</td>
<td>2.02</td>
<td>1.63</td>
<td>0.666</td>
<td></td>
</tr>
<tr>
<td>Betadine</td>
<td>Chlorhexidine</td>
<td>-4.35*</td>
<td>1.52</td>
<td>0.019</td>
</tr>
<tr>
<td>Benzamide</td>
<td>-2.02</td>
<td>1.63</td>
<td>0.666</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 Means of Ct value at the time points pre- and postadministration of mouthwashes

Table 2 Results of repeated measures variance analysis

Table 3 Pairwise comparisons of mouthwashes

European Journal of General Dentistry © 2022. The Author(s).
mouthwash and the time factors were effective, the effect size of mouthwash was 0.154%, and the effect-size of time was 0.288%. The overall result indicates that therapeutic interventions, mouthwashes, were effective on the dependent variable (patients’ CT). According to Table 3, in terms of couple comparisons, the Bonferroni post hoc test also showed the difference between the average chlorhexidine mouthwash group and the Betadine mouthwash group in its dependent variable (patients’ CT) was significant. Table 4, in terms of couple comparisons, again showed that in the chlorhexidine and the benzamide mouthwash groups, in which the difference between the average CT of patients in T1, T2, and T3 to T0 was significant, had an increasing trend. Furthermore, in the Betadine mouthwash group, despite the increase in average CT of patients from T1 to T0, it was overall decreased in T2 and T3 and was not significant.

<table>
<thead>
<tr>
<th>Type of mouthwash</th>
<th>(I) Time</th>
<th>(J) Time</th>
<th>Mean difference (I–J)</th>
<th>Standard error</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorohexidine</td>
<td>T₀</td>
<td>T₁</td>
<td>-10.365*</td>
<td>1.602</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₂</td>
<td>-8.664*</td>
<td>1.512</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₃</td>
<td>-6.106*</td>
<td>1.371</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₀</td>
<td>10.365*</td>
<td>1.602</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
<td>1.702</td>
<td>0.836</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₃</td>
<td>4.259*</td>
<td>0.931</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₀</td>
<td>8.664*</td>
<td>1.512</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₁</td>
<td>-1.702</td>
<td>0.836</td>
<td>0.281</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₃</td>
<td>2.557*</td>
<td>0.759</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₀</td>
<td>6.106*</td>
<td>1.371</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₁</td>
<td>-4.259*</td>
<td>0.931</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₂</td>
<td>-2.557*</td>
<td>0.759</td>
<td>0.008</td>
</tr>
<tr>
<td>Benzamide</td>
<td>T₀</td>
<td>T₁</td>
<td>-9.164*</td>
<td>2.102</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₂</td>
<td>-6.534*</td>
<td>1.984</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₃</td>
<td>-4.922</td>
<td>1.799</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₀</td>
<td>9.164*</td>
<td>2.102</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
<td>2.630</td>
<td>1.097</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₃</td>
<td>4.242*</td>
<td>1.222</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₀</td>
<td>6.534*</td>
<td>1.984</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₁</td>
<td>-2.630</td>
<td>1.097</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₃</td>
<td>1.612</td>
<td>0.996</td>
<td>0.668</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₀</td>
<td>4.922</td>
<td>1.799</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₁</td>
<td>-4.242*</td>
<td>1.222</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₂</td>
<td>-1.612</td>
<td>0.996</td>
<td>0.668</td>
</tr>
<tr>
<td>Betadine</td>
<td>T₀</td>
<td>T₁</td>
<td>-5.220</td>
<td>3.153</td>
<td>0.622</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₂</td>
<td>-1.453</td>
<td>2.976</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T₀</td>
<td>T₃</td>
<td>-1.838</td>
<td>2.699</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₀</td>
<td>5.220</td>
<td>3.153</td>
<td>0.622</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₂</td>
<td>3.767</td>
<td>1.646</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>T₁</td>
<td>T₃</td>
<td>3.383</td>
<td>1.833</td>
<td>0.423</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₀</td>
<td>1.453</td>
<td>2.976</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₁</td>
<td>-3.767</td>
<td>1.646</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>T₃</td>
<td>-0.385</td>
<td>1.494</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₀</td>
<td>1.838</td>
<td>2.699</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₁</td>
<td>-3.383</td>
<td>1.833</td>
<td>0.423</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>T₂</td>
<td>0.385</td>
<td>1.494</td>
<td>1.000</td>
</tr>
</tbody>
</table>
The two types of mouthwash (chlorhexidine and Peroxide Hydrogen) can be recommended for dentists to use on their patients to reduce the possibility of transmission as the risk of transmission will remain high. They can be used for general mouth hygiene or further research on them to introduce an active ingredient that can inactivate viruses.

Repeated measures ANOVA was carried out to compare the difference between the means of Ct value at the different time points pre- and postadministration of each mouthwash. In all groups, Mauchly's test did not indicate any violation of sphericity. Normal saline used as control could not reduce the viral load; the Ct value changed from 25.2 before to 26.8 at hours after using it. The chlorhexidine mouthwash can be recommended to patients with COVID-19 by their dentists to reduce the viral load in their mouth an hour or 3 hours before the treatment. Further research should be done on improving their efficacy by looking at the active ingredient, the concentration in parts per million, or changing it.

p-Value also communicated the same results as $p < 0.045$ is more significant than 0.005. There was, however, some negative correlation between the viral load before use and after use of $-0.09$. It shows that the viral load somehow reduced with frequent use of the mouth wash. The relationship was, however, small. About 0.5% of the results can be explained using this data, $R^2 = 0.09$. Paired sample t-test was used to compare the mean Ct value before and immediately after mouthwash (Fig. 1). There was no statistically significant difference between C0 and C1 in participants taking normal saline ($p = 0.915$).

Discussion

With the increasing prevalence of SARS-CoV-2 infection worldwide, it is necessary to provide guidelines for reducing the risk of its transmission. Dental offices are important places for transmitting the virus because patients cannot use protective equipment during their dental procedures. In this regard, mouthwashes that reduce the viral load in patients’ mouths can reduce the risk of virus transmission to dentists.

Imran et al emphasized that there is an immense demand to raise consciousness among specialists regarding the viricidal movement of commercially accessible mouthwashes as illustrated by numerous in vitro ponders and encourage specialists to carry out more clinical trials and to get a translational step toward clinical procedure. This study, therefore, evaluated the effects of four types of mouthwash, including Betadine, hydrogen peroxide, chlorhexidine, on the viral load in a patient’s mouth admitted to the dental clinic of different universities of Iran. According to the results, after using the mouthwashes, the percentage of negative PCR results or false-negative for hydrogen peroxide, CPC, was 26.67, 7.14, 8.33, 33.33, and 20.34%.

The effectiveness of chlorhexidine in viral load SARS-CoV-2 has been previously reported in various studies. By irrigating, chlorhexidine acts as a solvent and washes the mucus crusts and other debris from the nose of patients and hence can decrease the virus load in the oral cavity, sinus spaces, nose, and nasal cavity. The mentioned study has also proposed that no dental patient should be examined before disinfection by Betadine. In another study, 99.99% reduction was reported in coronavirus titers after 20 minutes of chlorhexidine.

The results showed that CPC mouthwash was also used in COVID-19 patients to decrease viral load in their oral cavity and nasopharyngeal and nasopharyngeal regions. According to the current results, the CPC mouthwash showed the highest efficiency, higher than Betadine, hydrogen peroxide, and normal saline as mouthwashes. CPC is a well-known and commonly used mouthwash effective on a wide range of microbial agents. This mouthwash acts by penetrating the cell membrane. Previous studies have reported statistically significant reductions in the range of 16 to 28% in plaque and gingivitis in the application of CPC mouthwash. Numerous studies have studied the application of CPC on bacterial agents, but few studies have investigated its effect on viruses. In this regard, a recent study showed that CPC disrupted the integrity of the viral envelope and its morphology. Influenza viruses demonstrated no resistance to CPC despite prolonged exposure. This study may be attributed to the mechanism of CPC’s effect on microbes that mainly affects the cell membrane of bacteria and is effective on proteins of viral membranes. Moreover, the presence of chlorine in the structure of this mouthwash may cause the degradation of the viral genome and inactivation. In contrast, our findings show that CPC as a mouthwash has the highest efficiency in reducing SARS-CoV-2 in COVID-19 patients.

As observed, after chlorhexidine, hydrogen peroxide showed the highest efficiency for reducing viral load in the upper respiratory tract of the patients. The effectiveness of $H_2O_2$ on adenovirus types 3 and 6, adeno-associated type 4, rhinoviruses 1A m1b, respiratory syncytial virus strain long, and coronavirus strain 229E had been studied in vitro using different concentrations and time of exposure. $H_2O_2$ is an oxidizing agent used in dentistry alone or combination with salts for more than 70 years. One to three percent of this mouthwash has been employed in the control of plaque and the treatment of acute ulcerative gingivitis.
good efficiency for the $\text{H}_2\text{O}_2$ is attributed to the low contact time during its usage. Because peroxide hydrogen as a powerful oxidizing agent needs at least 1 minute to inactivate viruses. Overall, the present study showed that $\text{H}_2\text{O}_2$ reduced the viral load in the upper respiratory tract of the patients.

As observed in the current study, the normal saline was not an effective agent for reducing viral load in the mouth of confirmed COVID-19 patients. No obvious and direct inactivation effect of NaCl compounds has been reported for various types of viruses. Our findings reveal that normal saline has the lowest efficiency for reducing SARS-Co-2 load in the patients’ oral cavity and nasopharyngeal region.

To investigate the effects of the studied mouthwashes on viral load in the present study, real-time PCR Ct values for each used mouthwash were evaluated. The results implied no significant relationship between the mean difference of Ct value changes before and after the intervention and the type of mouthwash used ($p = 0.71$). Moreover, the Ct values of the studied mouthwashes indicated that the normal saline (Ct = 28) resulted in the highest viral load after use. In the application of mouthwashes, the Ct values were in the range of 30 to 37, which represented a moderate amount of target nucleic acid in the samples.

Our findings imply that the effects of the studied mouthwashes for the reduction in SARS-CoV-2 inpatients are in the order of CPC > $\text{H}_2\text{O}_2$ > PVP-I > normal saline. Therefore, the present study results demonstrate that chlorhexidine could efficiently reduce the load of SARS-CoV-2 in the mouth of patients, and hence it can be used as an effective strategy in dental offices to reduce the risk of SARS-CoV-2 infection among dentists.

Dental health professionals should educate dental patients on the mechanism of spread of COVID-19 through the droplets and aerosols that are generated during dental procedures, for a dental professional, and the best way to protect himself/herself and the staff from SARS-CoV-2 is with using prevention, or using mouthwashes for patients.

The guidelines from previous publications, as well as the suggestions proposed in this paper, are as they were proposed to specialists to assist them in their everyday operations and arranging until the widespread pandemic is over or under control. The ultimate decision on patient management and treatment should be made by the practitioner to provide what is in the patients’ best interest.

**Conclusion**

This study aimed at investigating the effects of four types of commonly used mouthwashes, including Betadine, hydrogen peroxide, mouthwash, and normal saline, for reducing intraoral viral load in COVID-19 patients. Our results show that the percentage of negative PCR results after using hydrogen peroxide, normal saline, Betadine, and chlorhexidine saline is 26.67%, 7.14%, 8.33%, and 33.33%, respectively. The normal saline is not effective in reducing the viral load in the mouth of confirmed COVID-19 patients. Chlorhexidine shows the highest efficiency for reducing viral load in the upper respiratory tract of the patients. $\text{H}_2\text{O}_2$ also has good efficiency for reducing SARS-CoV-2 load in patients’ oral cavity and nasopharyngeal region. Therefore, chlorhexidine and peroxide hydrogen can be used in dental clinics to reduce the risk of transmitting the SARS-CoV-2 virus from potential patients to dentists before dental procedures.

**Note**

The manuscript has not been submitted to more than one journal for consideration. The manuscript has not been published previously (partly or in full) unless the new work concerns an expansion of previous work; there is no transparency on the reuse of material to avoid the hint of text-recycling (“self-plagiarism”).

**Ethical Approval**

All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee’s ethical standards and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Authors’ Contributions**

A.H., S.Kh., A.P., S.B., and R.B. designed this research. S.Kh. and A.R.S. conceived the statistical analysis plan. M.M., F.M., F.A.J., S.S.H., A.M., F.K., and N.A. collected the data. A.P., F.M., S.K.H. drafted the manuscript. M.D., S.G., and A.N. designed this research and expanded the study design with J.J. from Augusta University in Georgia state of United States and negotiated to collaborate with Shahid Beheshti Medical Sciences University and Hamedan Medical Sciences University. M.K., P.R. and A.E. collected the data and conceived the data analysis of all the patients in Masih Daneshvari Hospital; F.Z. collected the ten patients in Mashhad Medical Sciences University. All authors read, approved, and contributed to the manuscript preparation.

**Funding**

None.

**Conflict of Interest**

None declared.

**Acknowledgments**

This research was supported by the Masih Daneshvari Hospital and Shahid Beheshti Medical Sciences, and the Hamedan Medical Sciences approved the protocol of this study with ethical code IR.SMBU.NRITTLD.1400.008 and the collaboration of the vice-chancellor for research & technology of the Hamadan University of Medical Sciences. The protocol of this study was approved by the Hamadan University of Medical Sciences with an ethical code: IR.UMSHA.REC.1398.235 and IRCT code: 20170117032025N7 and the collaboration of Mashhad University of Medical Sciences and the study were supervised by the chief of the OMFS Department of Augusta University in Georgia, USA, by ClinicalTrials.gov identifier (NCT number): NCT04719208 in USA.
References

European Journal of General Dentistry © 2022. The Author(s).