Cover Story

Estimating salivary carriage of severe acute respiratory syndrome coronavirus 2 in nonsymptomatic people and efficacy of mouthrinse in reducing viral load

A randomized controlled trial

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ABSTRACT

Background. Many people infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) never develop substantial symptoms. With more than 34 million people in the United States already infected and highly transmissible variants rapidly emerging, it is highly probable that post- and presymptomatic people will form an important fraction of those seeking dental care. Salivary carriage rates in these populations are not known. Moreover, although preventing transmission is critical for controlling spread, the efficacy of mouthrinses in reducing oral viral load is poorly studied.

Methods. The authors recruited 201 asymptomatic, presymptomatic, postsymptomatic, and symptomatic people and measured copy numbers of SARS-CoV-2 in unstimulated saliva using real-time reverse transcriptase quantitative polymerase chain reaction. Subsequently, the authors inducted 41 symptomatic people into a randomized, triple-blinded study and instructed them to rinse with saline, 1% hydrogen peroxide, 0.12% chlorhexidine, or 0.5% povidone-iodine for 60 seconds. The authors measured viral load 15 and 45 minutes after rinsing.

Results. Salivary SARS-CoV-2 was detected in 23% of asymptomatic, 60% of postsymptomatic, and 28% of presymptomatic participants. Neither carriage rate nor viral load correlated with COVID-19 symptomatology, age, sex, or race or ethnicity. All 4 mouthrinses decreased viral load by 61% through 89% at 15 minutes and by 70% through 97% at 45 minutes. The extent of reduction correlated significantly with initial viral load.

Conclusions. Nonsymptomatic people can pose a risk of transmitting the virus, and mouthrinses are simple and efficacious means of reducing this risk, especially when the load is less than 10^4 copies per milliliter.

Practical Implications. At a time when resources are stretched, the findings of this study contribute to evidence-based selection of personal protection equipment and simple infection-control practices to reduce contagion at source.

This clinical trial was registered at ClinicalTrials.gov. The registration number is NCT04603794.

Key Words. Aerosol; dental; saliva; SARS-CoV-2; mouthrinse; povidone-iodine; hydrogen peroxide; saline; chlorhexidine.

JADA 2021:152(11):903-908
https://doi.org/10.1016/j.adaj.2021.05.021

This article has an accompanying online continuing education activity available at: http://jada.ada.org/ce/home.

On March 11, 2020, the World Health Organization declared COVID-19 as a pandemic. On March 16, 2020, 198,000 dentists closed their doors to patients in the United States alone.[1] Ten months later, restrictions on the use of certain instruments and key procedures were still in place,[2]

fueling concern among providers and patients alike[3] and leading to major long-term changes in workflow patterns and reconfiguration of operatories.[4] Regulators and health authorities based this guidance on studies reporting that aerosols are potential vehicles for transmission of respiratory pathogens,[5] and because severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified in saliva,[6-8] the potential for disease transmission through saliva has become a concern.

Preprocedural mouthrinses have been used in dentistry for many years to reduce the microbial load in saliva.[9-11] Indeed, evidence from in vitro studies suggests that preprocedural mouthrinses containing hydrogen peroxide or povidone-iodine may help mitigate COVID-19 transmission,[12,13] although the evidence from limited studies is equivocal.[14,15] Therefore, we aimed to examine the risk posed by potential patients who report no symptoms of COVID-19 and to investigate the efficacy of a simple intervention (that is, preprocedural mouthrinsing) on reducing in salivary viral load. We achieved this aim through a 4-arm randomized controlled trial. To the best of our knowledge, this provides the first estimate of viral load in saliva of asymptomatic patients as well as an in vivo comparison of mouthrinses in patients with COVID-19.

METHODS

Ethics statement

The 2 parts of the study were approved by the institutional review board of The Ohio State University (protocols 2020H0155 and 2020H0356) and carried out in accordance with the approved guidelines and Strengthening the Reporting of Observational Studies in Epidemiology guidelines for human observational investigations.

Participants

Two-hundred one people were recruited from the dental clinics of The Ohio State University College of Dentistry and Wexner Medical Center and signed informed consent. Inclusion criteria were adults aged 21 through 80 years admitted to The Ohio State University Wexner Medical Center with a diagnosis of COVID-19 confirmed via polymerase chain reaction (PCR) for the symptomatic group and absence of any COVID-19 screening symptoms (based on the American Dental Association questionnaire and body temperature) for the asymptomatic, presymptomatic, and postsymptomatic groups.[16] Exclusion criteria were allergy to any study mouthrinse, active uncontrolled thyroid disease, pregnancy, and undergoing radioactive iodine therapy. Participants who were asymptomatic at initial presentation were followed up 48 and 72 hours later. We categorized participants who reported no COVID-19 symptoms at presentation and 48 and 72 hours after sampling as asymptomatic, those who were asymptomatic at initial presentation but reported any of the symptoms listed in the American Dental Association questionnaire at 48 or 72 hours after sampling as presymptomatic, and those who reported a history of COVID-19 (as confirmed by clinical symptoms and positive nasopharyngeal quantitative reverse transcription PCR test) as postsymptomatic (Figure 1).

Experimental design

All participants completed demographic, behavioral, and health history questionnaires. We asked participants to collect saliva in their mouths for 3 minutes and then continuously drool into a tube containing RNA stabilizer.[17] We randomly assigned 40 patients being treated for COVID-19 to receive a mouthrinse containing 15 mL of normal saline, 1% hydrogen peroxide, 0.12% chlorhexidine gluconate, or 0.5% povidone-iodine. The primary outcome measure was reduction in viral load at 15 minutes. On the basis of previous studies,[14,15] we estimated the reduction to range from 0% through 40%. Using an alpha of 0.05 to estimate effect size of 0.25 or less between groups, we estimated a group size of 10 using the design of experiments tool in JMP statistical software (SAS). The mouthrinse was dispensed in premeasured quantities in colored bottles, and the person dispensing the product was blinded to the type of mouthrinse. We used a block randomization protocol to create the randomization schedule in GraphPad. Participants were also blinded to the identity of the mouthrinse to the extent that it was possible given the variations in taste. Participants vigorously rinsed with 7.5 mL of the mouthrinse for 30 seconds, expectorated, and rinsed with the remaining 7.5 mL for a further 30 seconds. The 2 expectorants were

ABBREVIATION KEY

Ct: Cycle threshold.
PCR: Polymerase chain reaction.
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

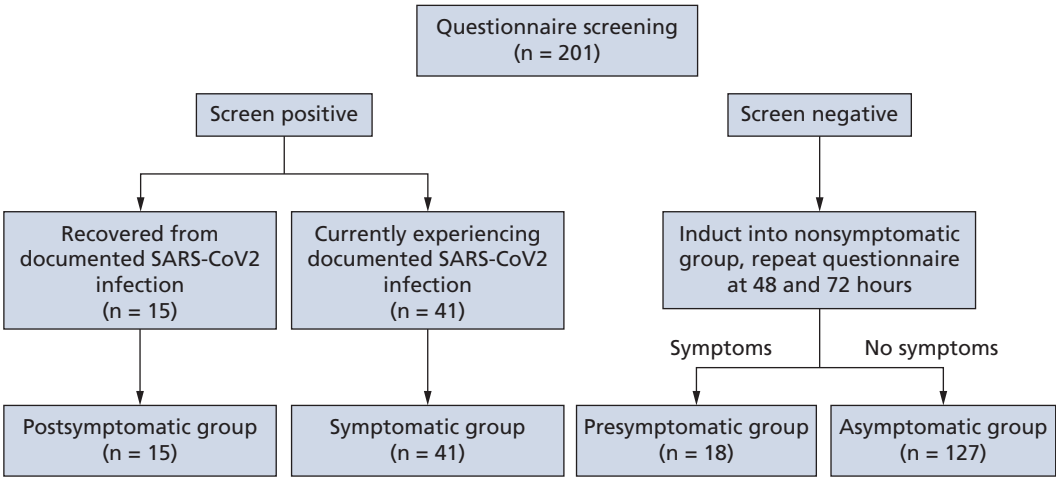

Figure 1. Patient selection workflow. SARS-CoV-2: .Severe acute respiratory syndrome coronavirus 2.

collected and pooled. Saliva samples were collected 15- and 45-minutes after rinsing in tubes containing RNA stabilizer.

Virus identification

Investigators conducting the virus identification and quantification were blinded to the type of mouthrinses. RNA-extraction free, dual-plexed reverse transcriptase quantitative PCR method for SARS-CoV-2 detection (SalivaDirect Version 5) according to the developers' instructions.[18] We mixed 50 μl of homogenized saliva with 2.5 μl of 50 mg per mL proteinase K. We used 5 μl in a 20 μl reaction containing fluorescein amidites—labeled primers and probes targeting SARS-CoV-2 N region and amplified for 44 cycles in triplicate reactions. We used RNA from trizol-inactivated virus (obtained from Dr. Qiuhong Wang of The Ohio State University) as positive control and to generate standard curves. We recorded cycle threshold (Ct) values for each run and averaged the values over the replicates. We used Ct values to compute viral copy numbers based on the positive control. Ct values greater than 40 were regarded as negative for viral presence.

Statistical analysis

We made nonparametric comparisons between all pairs using Dunn test for joint ranking, a method that incorporates Bonferroni adjustment for multiple tests. We used χ^2 tests to evaluate differences in frequency of detection of COVID-19 among groups of participants. We calculated confidence intervals to confirm significant findings.

RESULTS

Of the 201 participants, 127 were asymptomatic, 18 were presymptomatic, 41 were symptomatic, and 15 were postsymptomatic. Data were not provided for 1 patient. Postsymptomatic participants reported 14 or more days of having a normal temperature.

SARS-CoV-2 was detected in 23% of asymptomatic, 28% of presymptomatic, 99% of symptomatic, and 60% of postsymptomatic participants (Table); however, the frequency of detection was significantly lower in asymptomatic and presymptomatic participants than the frequency in symptomatic or postsymptomatic participants ($P = .001$, χ^2 test). The viral load in saliva was also significantly lower in asymptomatic and presymptomatic participants than the load in symptomatic or postsymptomatic participants ($P = .0007$, Dunn test with joint ranking). In the symptomatic patients, those treated with remdesivir or convalescent plasma or those with demonstrable COVID-19 immunoglobulin titers tended to have lower salivary loads. SARS-CoV-2 salivary carriage was greater in patients who were in the early stages of the disease process. These tendencies were not statistically significant. Salivary carriage of SARS-CoV-2 did not correlate with fever or loss of taste or smell in this group ($P > .05$, χ^2 test).

Table. Salivary carriage of SARS-CoV-2* in asymptomatic, presymptomatic, symptomatic, and postsymptomatic participants.

PATIENT METADATA	ASYMPTOMATIC (n = 127)	PRESYMPTOMATIC (n = 18)	SYMPTOMATIC (n = 40)	POSTSYMPTOMATIC (n = 15)
Race or Ethnicity				
White	112	15	23	12
African American	7	0	16	2
Asian	3	1	1	0
Hispanic	5	2	0	1
Age, Y, Mean, Median (Range)	49.3, 43 (24-71)	43, 29 (25-69)	61.1, 64 (25-82)	42.9, 39.5 (25-65)
Sex, No. (Males)	69	7	33	10
SARS CoV-2 Detection Frequency, No.	29†	5‡	39§	9¶
SARS CoV-2 Copy No. in 1 Milliliter of Saliva, Mean, Median (Range)	234, 427 (127-912)†	198, 658 (165-1,210)‡	24×10^7, 13×10^5 (10×10^4-96×10^8)§	6,491, 5,219 (897-11,256)¶

* SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2. † The groups are statistically different ($P < .05$, χ^2 test for detection frequency and Dunn test for viral copy numbers). ‡ The groups are statistically different ($P < .05$, χ^2 test for detection frequency and Dunn test for viral copy numbers). § The groups are statistically different ($P < .05$, χ^2 test for detection frequency and Dunn test for viral copy numbers). ¶ The groups are statistically different ($P < .05$, χ^2 test for detection frequency and Dunn test for viral copy numbers).

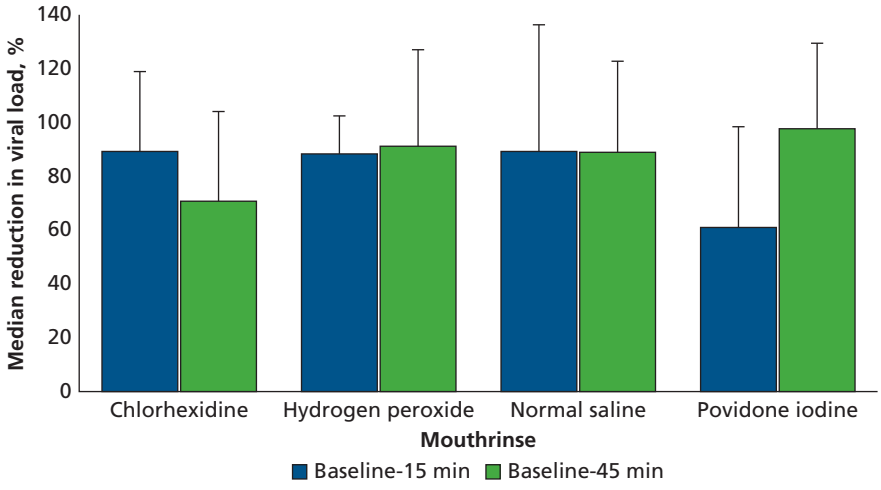

Figure 2. Reduction of severe acute respiratory syndrome coronavirus 2 load 15 and 45 minutes after rinsing for 1 minute with 15 milliliters of normal saline, 1% hydrogen peroxide, 0.12% chlorhexidine gluconate, or 0.5% povidone-iodine. Bars represent median reduction from baseline at each time point, and error bars indicate standard error of the mean.

No adverse events related to the mouthrinse (pain or burning in mouth, oral dryness, or difficulty in swallowing) were recorded during the trial. All 4 mouthrinses reduced salivary carriage of SARS-CoV-2 (Figure 2). We observed a median reduction of 61% through 89% (mean, 25%-74%) at 15 minutes, whereas the median reduction ranged from 70% through 97% at 45 minutes (mean, 30%-43%). Neither the 15-minute reduction in viral load nor the persistence of reduction at 45 minutes differed among the mouthrinses ($P > .05$, Dunn test). There was a significant correlation between baseline viral load and reduction at 15 minutes ($P = .0073$, Spearman rank correlation) and persistence at 45 minutes ($P = .0087$, Spearman rank correlation). In all participants with a baseline viral load less than 10^4 copies per mL of saliva (n = 6), there was 100% reduction at 15 and 45 minutes.

DISCUSSION

The oral cavity forms a continuum with the nasopharynx and lower respiratory tract, and hence, aerosol-generating dental procedures on patients with any type of infectious respiratory disease have the potential to create pathogen-rich aerosols, a concern that has gained immediacy in the

SARS-CoV-2 pandemic. Moreover, oral epithelial cells, especially those in the tongue and salivary glands, express transmembrane protein angiotensin-converting enzyme and transmembrane serine protease 2,[19,20] the primary receptors and portals of entrance of SARS-CoV-2 into cells.[21] Because the oral cavity is a primary external interface, it is likely that these surfaces provide an avenue for viral colonization. This is borne out by our findings for asymptomatic, presymptomatic, and post-symptomatic participants (collectively referred to as nonsymptomatic), and it suggests that questionnaires based on disease symptomatology or temperature records might not be diagnostic of infective potential. To the best of our knowledge, our study is the first to provide this information and sets the stage for future work on risk of transmitting the disease among dental health care workers and patients.

Although we discovered that more than 40% of nonsymptomatic participants (including pre-, post- and asymptomatic participants) carried the virus in their saliva, 2 lines of evidence point to a low risk of transmitting the disease from nonsymptomatic patients. The first is based on mathematical modeling that contagion requires salivary viral loads of 10^8 copies per mL or greater[22] and the clinical evidence that supports the model.[23] The second is from our earlier discovery that the primary source of aerosol microbiota is the irrigant that cools dental handpieces and ultrasonic scalers and that SARS-CoV-2 is not present in measurable levels in these aerosols when procedures are performed in conjunction with preoperative mouthrinses and intraoral high-volume evacuation.[24]

A 2020 survey reported that only 12% of US dentists administer preoperative mouthrinses.[25] In our investigation, we report on the efficacy of using any mouthrinse, irrespective of its mechanism of action, on reducing salivary SARS-CoV-2 levels for up to 45 minutes. This efficacy is correlated directly with salivary loads, further attesting to the benefits of preprocedural mouthrinsing in nonsymptomatic patients. We found a higher viral load at 45 minutes after rinsing in 6 participants compared with baseline; in all of these participants, episodes of coughing were recorded during this period. COVID-19 viral loads in saliva, including deep cough sputum, are higher than nasopharyngeal loads at disease onset and decrease slower.[26] It is possible that episodes of coughing might increase salivary viral load in nonsymptomatic patients, and further studies are required to evaluate this.

CONCLUSIONS

Within the limitations of a small sample size, we discovered a high rate of SARS-CoV-2 carriage in the saliva of nonsymptomatic participants, although these levels are well below those required for disease transmission. We also discovered that mouthrinses are a simple and highly efficacious means of reducing the virus from the oral environment for up to 45 minutes and may be a valuable tool in disease mitigation. ■

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Disclosures. None of the authors reported any disclosures.

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