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ORIGINAL ARTICLE

Is hydrogen peroxide an effective mouthwash for reducing the viral load of SARS-CoV-2 in dental clinics?

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Abstract *Background:* Previous studies have demonstrated that SARS-CoV-2 is mainly transmitted by inhalation of aerosols and can remain viable in the air for hours. Viruses can spread in dental settings and put professionals and patients at high risk of infection due to proximity and aerosol-generating procedures, and poor air ventilation.

Objectives: The aim of this study was to investigate the effects of a 1% hydrogen peroxide (H_2O_2) mouth rinse on reducing the intraoral SARS-CoV-2 load.

Methods: Portable air cleaners with HEPA filters exposed for 3 months were analysed to test for virus presence in a waiting room (where patients wore a face mask but did not undergo mouth rinsing) and three treatment rooms (where patients wore no mask but carried out mouth rinsing). As CO_2 is co-exhaled with aerosols containing SARS-CoV-2 by COVID-19 infected people, we also measured CO_2 as a proxy of infection risk indoors. Specific primer and probe RT-PCR were applied to detect viral genomes of the SARS-CoV-2 virus in the filters. Specifically, we amplified the nucleocapsid gene (Nuclv) of SARS-CoV-2.

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Results: CO_2 levels ranged from 860 to 907 ppm, thus indicating low ventilation and the risk of COVID-19 transmission. However, we only found viral load in filters from the waiting room and not from the treatment rooms. The results revealed the efficiency of 1-minute mouth rinsing with 1% H_2O_2 since patients rinsed their mouths immediately after removing their mask in the treatment rooms.

Conclusions: Our findings suggest that dental clinics would be safer and more COVID-19 free by implementing mouth rinsing 1 min with 1% H_2O_2 immediately after the patients arrive at the clinic.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has caused a large number of infections worldwide. The transmission of SARS-CoV-2, from asymptomatic, presymptomatic, and symptomatic carriers, occurs mainly via respiratory droplets, aerosols, and to a lesser extent, fomites (Guo et al., 2020; Mondelli et al., 2021). The virus can stay suspended and viable for hours in aerosols depending on factors like heat and humidity (van Doremalen et al., 2020), and it can be transmitted up to distances of 4 m (Guo et al., 2020).

Dentistry is considered a high-risk profession during a transmissible infectious disease pandemic due to proximity to the patient's respiratory tract openings during dental exams and treatments, despite the use of basic personal protective equipment (Harrel and Molinari, 2004). Since the emergence of the COVID-19, appropriate guidance for dental professionals to protect both themselves and their patients against SARS-CoV-2 has become a critical topic. However, the evidence on this issue is still controversial (Shamsoddin et al., 2021). The World Health Organization (WHO) has considered "airborne precautions" for healthcare workers, particularly in dental clinics where, due to the nature of dental procedures (Coulthard, 2020), saliva may become aerosolized and thus contribute to the spread of the virus from the oral cavity (Askarian et al., 2005; Benzian et al., 2021). Therefore, preventive measures that minimize the transmission of the virus in this setting are essential.

Mouthwashes are widely used before oral surgery due to their ability to reduce the number of microorganisms in the oral cavity (Dominiak et al., 2020; Kosutic et al., 2009) and colony forming units in dental aerosols (Marui et al., 2019). Moreover, based on the existing evidence, the use of mouthwashes in COVID-19 patients admitted to ICU (intensive care units) is currently suggested before performing daily routine procedures with a potential risk of generating aerosols, and/or droplet emission (e.g., Tovani-Palone and Shamsoddin, 2021). Recent studies have found that rinsing solutions successfully inactivate infectious SARS-CoV-2 particles (e.g., Koch-Heier et al., 2021), and reduce salivary viral load (Meister et al., 2020; Moosavi et al., 2020). In this context, the CDC (Centers for Disease Control and Prevention) have recommended the use of mouthwashes before oral procedures (CDC, 2020; Tovani-Palone et al., 2021). A variety of oral antiseptic rinses have been suggested in recent literature for preprocedural use to reduce viral transmission. Oral rinses ranging from chlorhexidine gluconate, ethanol, essential oils, povidone-iodine (PVP-I), hydrogen peroxide (H_2O_2) chlorinated water, hypertonic saline, bioflavonoids, cyclodextrins, cetylpyridinium chloride have been recommended (Vergara-Buenaventura and Castro-Ruiz, 2020).

Hydrogen peroxide oral rinse is a popular rinse anecdotally used by dentists due to its long history of use in teeth whitening procedures. Some of the advantages of H_2O_2 include easy accessibility, low cost, and long track record in dentistry. However, its disadvantages include its potential for toxicity. In this study we assessed the potential efficacy of rinsing with H_2O_2 as a virucidal mouthwash in reducing the risk of coronavirus transmission. We used a relatively low concentration of 1% (lower concentration than indicated in previous studies 1.5–3%) (e.g., Bidra et al., 2020; Tovani-Palone and Shamsoddin, 2021), for 1 min to reduce its potential toxicity. This could be considered as the main preventive option to control the viral load in the saliva and the aerosol during any dental procedure. As an additional preventive measure, the CDC have considered the use of particle-size range portable air cleaners (PACs) with high-efficiency particulate air (HEPA) filters while the patient is undergoing, and immediately following, an aerosol-generating procedure. SARS-CoV-2 has a diameter of 0.06–0.14 μm, with characteristic spikes ranging from 0.009 to 0.012 μm (Wiersinga et al., 2020). The spreading of coronavirus through aerosols, in the size range up to 5 μm diameter, is a significant transmission pathway of COVID-19 (Cai et al., 2020). These diameters can be captured by PACs with HEPA filters efficiently (Hammond et al., 2021; Rodríguez et al., 2021). These devices typically consist of a fan and a HEPA filter where the air purifier pulls air in, passes it through the filter to remove particles, and then dumps clean air back into the room, thus removing potentially contaminated air. Therefore, PACs with HEPA filters could reduce the spread of SARS-CoV-2 and improve the indoor air quality in rooms with low ventilation rates (Ren et al., 2021).

The purpose of this study was to assess the potential efficacy of 1 min of rinsing with 1% H_2O_2 as a virucidal mouthwash in reducing the risk of coronavirus transmission. We compared the viral loads captured by portable air cleaners (PAC) with HEPA filters exposed for 3 months in different rooms of a dental clinic during one of the COVID-19 pandemic peaks in Spain.

2. Methodology

2.1. Sampling

The study was carried out in the dental clinic "TD" in the city of Toledo (located 60 km south of Madrid), one of the Spanish cities with a high rate of infection, during the third wave COVID-19 pandemic peak (https://cnecovid.isciii.es/)

(Fig. 1). PACs with HEPA filters exposed for 3 months (from January to March 2021) were analysed to test for virus presence in a waiting room (where patients wore a face mask but did not undergo mouth rinsing), and three treatment rooms (where patients wore no mask but carried out 1 min mouth rinsing with 1% H_2O_2).

Patients attended the clinic with prior appointment and one by one after a temperature check. No companions were allowed due to the sanitary restrictions, except on a few occasions. A mask was mandatory, and hands were cleaned with an alcohol-based hand rub before going into the waiting room. Patients would wait for c.a. 5–10 min in the waiting room and then moved to one of the three treatment rooms in the clinic, where they rinsed their mouth for 1 min with 1% H_2O_2 mouthwash and remained without their mask for the whole dental treatment.

PACs with HEPA filters were placed in the centre of the treatment rooms, ensuring unobstructed airflow. However, the PAC in the waiting room was placed behind the seats where patients sat while waiting for their appointment. The PAC models used for this study were Blueair 403 (placed in the waiting room, measuring 22.75 m^2) and Blueair 203 (placed in the treatment rooms, each measuring 9.80 m^2). The specifications of both models were the same in terms of the pore size of the filters. They only differed in the clean air delivery rate (CADR), with five air changes per hour for up to 34 m^2 surface area (for the 403 model) and up to 22 m^2 surface area (for the 203 model). Model 403 had four speed levels (airflow 408 m^3/h) and model 203 had three speed levels (airflow 290 m^3/h). Both models capture 99.97% of airborne particles down to 0.1 μm in size.

The clinic used an air conditioner as a ventilation system, and large fixed frameless windows were the only source of natural light, so there was no natural ventilation. Carbon dioxide (CO_2) concentration is an important indicator of ventilation in indoor environments. Moreover, CO_2 is co-exhaled with aerosols containing SARS-CoV-2 by COVID-19 infected people and can be used as a proxy of SARS-CoV-2 concentrations indoors (Peng and Jimenez, 2021). For this reason, we also measured CO_2 with a portable infrared sensor Testo 315-3 in each room during the 3 months of the study. The sensor was calibrated within the year preceding the study.

2.2. Filter analysis

2.2.1. HEPA filter samples

We analysed HEPA filter samples from four rooms of the dental clinic (waiting room and three treatment rooms). The filters were randomly cut into pieces of 3 × 3 cm, using sterilized scissors, and placed in vials containing 1.5 ml of diethylpyrocarbonate (DEPC)-treated water with 0.5% bovine serum albumin (BSA) in order to inhibit the ribonucleases and protect the viral structures present on the filters, and stored at 4 °C until assayed. Six pieces of each filter were analysed.

2.2.2. RNA extraction, reverse transcription (RT), and real-time PCR

Prior to RNA extraction, the filter samples were incubated while being shaken for 45 min at high speed, after which the filter pieces were removed and 300 μl of QIAzol lysis reagent was added. Total RNA was extracted according to the QIAzol manual (Qiagen) and was quantified using the NanoDrop 2000 (Take3, BioTek). Complementary DNA (cDNA) was synthesised from 1 μg of DNase-treated RNA. Real-time PCR was performed in duplicate, in a total volume of 20 μl, containing 5 μl of the RT reaction, in an ABI Prism 7500 Fast Sequence Detection System (Applied Biosystem, Foster City, CA) as previously described (Burgos-Ramos et al., 2012). SYBRGreen gene expression assays were used to detect SARS-CoV-2 nucleocapsid (Nuclv) as a marker of viral presence, and 18S ribosomal as an endogenous control of the method. The forward and

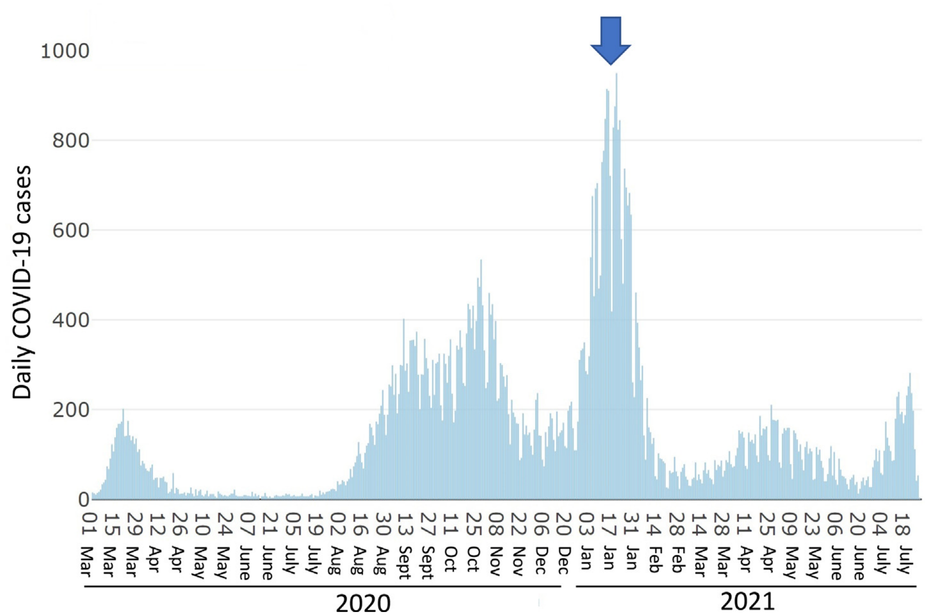

Fig. 1 Epidemic curve of the COVID-19 pandemic in Toledo. The arrow indicates the start of the third outbreak (Modified from: https://cnecovid.isciii.es/covid19/#provincias).

reverse primers were as follows: 5′-CAATGCTGCAATCGTGCTAC-3′ and 5′-GTTGCGACTACGTGATGAGG-3′ for Nuclv, and 5′- TCTGCCCTATCAACTTTCGATGG-3′ and 5′-TAATTTGCGCGCCTGCTG-3′ for 18S. According to the manufacturer's guidelines, the Cycle threshold (Ct) method was used for relative quantification, considering a sample positive for SARS-CoV-2 when the Ct value was less than or equal to 41 (Rodríguez et al., 2021).

2.2.3. Statistical analysis

Data are expressed as means ± SEM (standard error of the mean) of six pieces of each filter analysed in duplicate. Statistical analysis was performed using one-way ANOVA (GraphPad Prism 5.03 software, GraphPad Software, Inc., San Diego, CA). Statistical significance was set at $p < 0.05$.

3. Results

SARS-CoV-2 was detected in the filter of the waiting room (mean Ct value = 36.41 ± 1.51), although the use of a mask was mandatory (Fig. 2 Panel A). This could be due to the type of mask used or its incorrect use, i.e., because masks were not well-fitted (leaving gaps), or because they were used longer than recommended. However, we did not detect virus in the filters from the treatment rooms. We ruled out the possibility that this absence was due to a technical failure, as 18S expression was homogeneous in all analysed filters, as demonstrated in the ANOVA (p value = 0.325) (Fig. 2 Panel B). The mean Ct value for 18S expression was 16.18 ± 0.73 in the waiting room; 15.84 ± 1.09 in treatment room 1; 17.71 ± 1.01 in treatment room 2 and 17.87 ± 0.84 in treatment room 3. Moreover, some patients notified the clinic of a positive PCR test after dental care. In addition, asymptomatic patients may have attended the clinic.

4. Discussion

Our results demonstrated that the dental treatment room could be considered a COVID-free room, because patients rinsed their mouths with 1% H_2O_2 solution for 1 min immediately after removing their mask, deleting viral particles from their mouth. This could explain the absence of viral load in filters from these rooms. The use of mouthwashes has been found to be effective at reducing the microbial count in the oral cavity (Marui et al., 2019). Recent evidence has confirmed that rinsing the mouth with 0.5% povidone-iodine (PVP-I) (iodine with water-soluble polymer polyvinylpyrrolidone) for 30 s can reduce SARS-CoV-2 virus infectivity (Chopra et al., 2021), as can chlorhexidine 0.2% and 1% PVP-I oral solutions (Elzein et al., 2021). However, there are conflicting reports on the *in vivo* efficacy of H_2O_2 against SARS-CoV-2 (Ather et al., 2021; Ortega et al., 2020). H_2O_2 contains one more atom of oxygen than water (H_2O) that is eventually used in oxidation reactions. No adverse effects were reported from the use of a mouth rinse containing 1% H_2O_2 when it was not used often (Gusberti et al., 1988). H_2O_2 degrades into oxygen and H_2O when it is in contact with an enzyme (catalase) present in almost all living beings, including microorganisms within the oral microbiota, and this oxidative process would be effective against SARS-CoV-2 because the virus is sensitive to oxidation (Peng et al., 2020; Rowen and Robins, 2020).

In a recent study that modeled factors associated with the spread of respiratory infectious disease in dental offices, CO_2 levels were found to play the most important role in the risk of disease transmission (Huang et al., 2021). CO_2 levels at 774 ppm were considered low risk, but those at or above 1,135 ppm may increase the risk of disease transmission in dental offices (Zemouri et al., 2020). In our case, the average CO_2 concentration in the waiting room was 890 ppm, and it was between 907 and 860 ppm in the treatment rooms. These

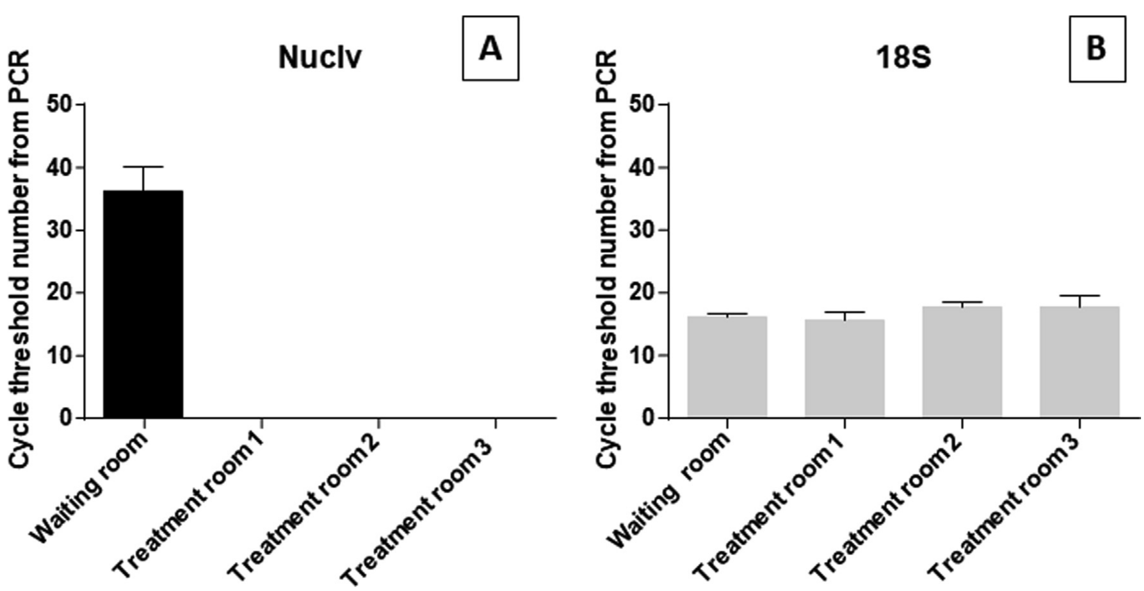

Fig. 2 Cycle threshold values obtained by real-time RT-PCR of HEPA filters from three rooms in a dental clinic. Panel A: results of SARS-CoV-2 nucleocapsid gene expression, as a marker of viral presence. Panel B: results of 18S gene expression, as endogenous control of method. Cycle threshold values ≤ 41 were considered to indicate a positive sample for SARS-CoV-2. Data are expressed as means ± SEM (standard error of the mean) of six pieces of each filter analysed in duplicate. Solid bars correspond to SARS-CoV-2 nucleocapsid gene expression and grey bars to 18S gene expression.

values indicate that transmission of the virus inside the clinic is plausible due to the poor ventilation.

5. Conclusions

Until mass vaccination is successfully implemented globally, dental practices are not totally secure for either staff or patients. Dentists should install preventive strategies to avoid the spread of COVID-19 infection. This study highlights the importance of H_2O_2 mouth wash in controlling airborne spread of SARS-CoV-2 in an indoor environment with poor ventilation and without safe social distancing.

The use of H_2O_2 solution (1%) for 1 min for mouth rinsing drastically reduced the possibility of coronavirus spread during aerosol-generating dental procedures. Our results suggest this procedure could be one of the main strategies to limit SARS-CoV-2 transmission in dental clinics. The use of a mouthwash prior to a dental procedure is recommended, as soon as a patient arrives at the clinic, to prevent the spread of SARS-CoV-2 to staff and other patients.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Statement

D.R (diana.rodriguez@uclm.es) and I.R.U (itziar.rodriguez@uclm.es) created the project, measured the CO_2 concentration, and collected the HEPA filters. E.B-R. (emma.burgos@uclm.es) did the analyses. All authors drafted the manuscript and contributed to the discussion of the results.

Ethical Statement

This article requires no human or animal in research.

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