Toothbrushing with a dentifrice containing antimicrobial phthalocyanine derivative for the intraoral reduction of viral load of SARS-CoV-2: a pilot study

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Research Article

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Abstract

Background: The aim of this study was to evaluate the effects of the toothbrushing with a dentifrice containing antimicrobial phthalocyanine derivative (APD) for the intraoral reduction of viral load of SARS-CoV-2.

Material and methods: Twenty COVID-19 positive dentate patients were selected and toothbrushes with a dentifrice containing APD for 2 minutes. Self-collected samples of unstimulated saliva were carried out three times: T0 (baseline, before toothbrushing), T5 (5 minutes after toothbrushing), and T30 (30 minutes after toothbrushing). The analysis of RNA viral was performed by RT-PCR using TaqPath™ COVID-19 multiplex Real-Time RT-PCR test for detection of three viral genes (ORF1ab, N and S genes). Evaluation of the effects was based on difference in cycle threshold (Ct) value. Friedman's test and pairwise comparison with Bonferroni corrections were used, with a significance level of 5%.

Results: The Ct values were significantly higher (p=0.020) at T30 in comparison to T0 and T5. The greatest difference in the Ct values was between T30 and T0 (3.83).

Conclusion: This pilot study suggests that oral hygiene action associated with an antimicrobial chemical dentifrice may be an important tool for SARS-CoV2 viral load reduction in oral cavity.

Introduction

On March 11, 2020, the World Health Organization (WHO) declared Coronavírus Disease 2019 (COVID-19) as a pandemic. Since then, a worldwide catastrophe has been reported, taking more than a million lives (1–3). Direct contact, airborne contamination and respiratory droplets are the main source of the Severe Acute Respiratory Syndrome Coronavirus – 2 (SARS-CoV-2) (2, 4, 5). Measures to prevent spread of COVID-19 have been used like mask-wearing, social distancing and handwashing (6), with emphasis on vaccines.

The mouth is potentially involved in the pathophysiological process of COVID-19 (7–10) and oral health could impact the severity of the disease (8, 10). Thus, the use of adjuvant preventive measures, such as toothbrushing, gargling, rinsing and oral care products, has been reported (11–19).

Recently, an innovative antimicrobial phthalocyanine derivative (APD) compound was incorporated in oral hygiene products and has been used in Dentistry (16–19). Also, the beneficial application of APD mouthwash gargling/rinsing protocol with COVID-19 positive patients, such as rapid recovery of sore throats, cough, mouth ulcers and significant decrease in the length of hospitalization was previously reported (16, 17). Additionally, in a recent *in vitro* study demonstrated 90% and 99,99% SARS-CoV-2 inactivation with an oral rinse and a dentifrice containing APD with, respectively (20).

Therefore, the aim of the present pilot study was to investigate the effect of the toothbrushing with a dentifrice containing APD on the intraoral reduction of viral load in SARS-CoV-2-positive subjects.

Material And Methods

Ethical Aspects

This project was approved by the Ethical Committee of the Federal Institute of Paraná (CAAE 35194520.0.0000.8156) upon acquiescence of the Londrina Municipal Health Authority.

Study Design and Subjects

The present study is a cross-sectional clinical pilot study to investigate the effects of the toothbrushing with a dentifrice containing APD on the intraoral viral load of SARS-CoV-2.

The subjects consisted of 20 dentate adult patients in a convenience sample, without comorbidities, non-smokers, of both genders, who were diagnosed with COVID-19 by qPCR in nasopharyngeal swab samples, and with mild symptoms.

An online questionnaire was sent to collect the demographic characteristics of the patients and clinical data about COVID-19 symptoms using the Mentimeter system (Mentimeter AB, Stockholm, Sweden).

Samples Collection

After telephone contact and agreement to participate in the research, the researchers took to the volunteers' residence a kit containing three 15ml falcon tubes (Corning Incorporated, USA), a dentifrice containing APD, and a toothbrush (DentalClean, Rabbit Corp, Londrina, Brazil). The videos with instructions for performing the saliva self-collection and toothbrushing were sent via WhatsApp.

The self-collected samples of unstimulated saliva were performed in the morning, before breakfast. Collections were carried out three times in the same day: T0 (baseline, before toothbrushing); T5 (5 minutes after toothbrushing); and T30 (30 minutes after toothbrushing). The patients did not eat or drink and or use any oral product or medication during the entire collection period (30 minutes). For acquiring a baseline saliva specimen (T0) for the SARS-CoV-2, patients were asked to mouth rinse with 5 ml of water and then all the saliva produced was poured into the tube during 10 minutes. Immediately afterwards, they were instructed to place the same amount of dentifrice and brush the teeth and tongue for 2 minutes. After 5 and 30 minutes of toothbrushing, the saliva collection procedure was repeated.

The samples were stored in freezers (-20ºC) until specialized service arrived to transport them to the laboratory of COVID-19. Then, the samples were stored at − 80ºC until analysis.

RNA Extraction of SARS-CoV-2 Using Magnetic Beads

Viral RNA was extracted from 100 µL of saliva of each time, using the automatized extractor EXTRACTA 32 (LOCCUS, Cotia, Brazil) and magnetic beads extraction kits (MVXA-P016 FAST), following the

manufacturer's instructions (LOCCUS, Cotia, Brazil). A negative extraction control (UltraPure™ DNase/RNase-Free Distilled Water, Thermo Fisher Scientific, Waltham, MA, USA) was added to each extraction run.

SARS-CoV-2 RNA Detection by RT-qPCR

The qualitative analysis of RNA viral was performed by real-time reverse transcription-polymerase chain reaction (RT-PCR) using TaqPath™ COVID-19 multiplex Real-Time RT-PCR (qPCR) test for detection of three viral genes (ORF1ab, N and S genes) (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Positive and negative controls were analyzed simultaneously with the samples. All stages of RT-PCR, including cDNA synthesis and amplification of the target sequences, were performed on the QuantStudio™ 6 FLEX Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). Results were considered positive when the cycle threshold (Ct) values were ≤ 37 for two or more genes and negative when the Ct values were > 37 for three SARS-CoV-2 targets (ORF1ab, N, and S genes). This was the same methodology for the diagnosis of COVID-19 in nasopharyngeal swab samples.

Statistical analysis

The evaluation of the effects was based on differences in Ct values. The medians of the Ct values of the three genes (ORF1ab, N, and S) were calculated to avoid the influence of the extremes of the data set and were used for statistical analysis. The Kolmogorov-Smirnov test was used to assess the normality of the variables. Continuous variables were described with median values with interquartile range (IQR) for non-normally distributed variables and with mean ± standard deviation (SD) values for mean age (normally distributed variable). Friedman's test and pairwise comparison with Bonferroni corrections were used to compare the differences in the Ct values between the groups. The infectivity was divided according to the Ct value obtained: high viral load (Ct < 25), intermediate viral load (Ct: 25–30) and low viral load (Ct > 30) (21). Statistical tests for $p < 0.05$ were reported as statistically significant. All statistical analyses were performed using IBM SPSS Statistics, version 27 (IBM Corp., Armonk, NY, USA).

Results

Twenty patients were initially included in this study, but two patients (10%) were excluded because no SARS-CoV-2 could not be detected in the saliva specimen. The patient characteristics as shown in Table 1. Eighteen patients (8 female and 10 male) had a mean age of 30.6 years (SD: 8.50). No adverse events were reported with any of the patients. The median period between symptoms onset and swab collection was 4 days (IQR: 3-6). According to the viral load on swab, the median Ct value was 19.77 (IQR: 18.88-21.09), seventeen patients (94.4%) had a high viral load and one patient (5.6%) intermediate viral load. The median period between symptoms onset and toothbrushing/saliva collections was 8 days (IQR: 7-10), and the baseline saliva Ct value was 29.59 (IQR: 22.08-32.34).

Analysis of Ct value prior and after toothbrushing

Figure 1 shows the Ct values of detection of SARS-CoV-2 genes in saliva at T0, T5 and T30. There was an increase in the Ct value from T0 to T5 in 13 patients (72.22%), and from T0 to T30 in 14 patients (77.77%). In two patients (11.11%) no SARS-CoV-2 was detected at T5, increasing to five patients (27.77%) at T30.

The Ct values were significantly higher (p=0.020) at T30 in comparison to T0 and T5 (Table 2). The greatest reduction in the Ct values was between T30 and T0 (3.83).

Discussion

The present pilot study demonstrated intraoral reduction of SARS-CoV-2 viral load after 30 minutes of the toothbrushing using a dentifrice containing APD, and comprised 18 subjects, who served as their own controls (22). The intraoral viral load of each patient was examined in the saliva at baseline (T0) and 5 and 30 minutes after toothbrushing, and the patients themselves carried out the saliva collections at home (23, 24). This methodology is in accordance with Valentine-Graves *et al.* (2020) who concluded in a study that at-home self-collection offers possibilities to reduce the exposure of people, reduce the need for personal protective equipment/cost, and offer options for screening populations without symptoms (23).

The pandemic situation decreed just over a year ago and experienced to date, has led scientists from all areas of knowledge in the world to fight together in this “war for life” against COVID-19. The understanding of the pathophysiology of COVID-19, with SARS-CoV2 having its entrance through the upper airways and affinity for the mucous membranes of the nose, mouth and oropharynx. In addition, the salivary glands being reservoirs of the coronavirus, makes strategic for research such as this study (7, 25).

In a recent study by Huang *et al.* (2021) confirmed SARS-CoV-2 infection in the glands and oral mucosa from identification of the host entry factors (ACE2 and TMPRSS) (7). The authors reported that glands and oral mucosa could play an important role in transmitting the SARS-CoV-2 to the lungs or the gastrointestinal tract via saliva. Concluded that the oral cavity is an important site for SARS-CoV-2 and saliva as a potential route of COVID-19 transmission from oral droplets containing infectious virus and infected cells. In another study by Matuck *et al.* (2020) demonstrated the presence of SARS-CoV-2 in periodontal tissue in severely ill patients (26). The authors highlight that periodontal tissue can be a target for SARS-CoV-2, and contribute to the presence of the virus in saliva. Therefore, the hole of oral hygiene in this circumstance is crucial for preventing the spread of the virus and the use of antiviral oral care products are of paramount importance (27). In this sense, the WHO recommends for dental practices routine asking patients to decontaminate their oral cavity with antiviral mouthwashes prior to examination (13).

The presence of the coronavirus colonizing the mucous membranes of the mouth and in the gingival crevicular fluid before its penetration into the cells of the epithelium was found to have a reduced amount

of virus after the use of the dentifrice containing APD (5). A APD demonstrated in vitro studies a promising antiviral action and absence of cytotoxic effects (16, 20).

The results of the present study found that after 30 minutes of toothbrushing with dentifrice containing APD increased Ct values in the saliva for 77.77% of the patients. Of this, 27.77% no SARS-CoV-2 was detected at T30, which may have substantially reduced the infectivity/non-contagious of disease. The estimate of viral load reduction was based on the mean increase in the Ct values of 3.83 units, which may correspond at least to 10-fold less target RNA as reported by Tom and Mina (2020) (28). Previous studies have shown that Ct values offer a semi-quantitative analysis of viral RNA concentration, i.e., lower Ct values correspond to higher viral RNA concentrations. Therefore, Ct value can serve as an indirect indicator of the relative viral load of SARS-CoV-2 (28, 29).

The limitations of this study were that no virus cultures were performed for SARS-CoV-2, the lack of substantivity analysis of the dentifrice after 30 minutes, and the presence of a toothbrushing group without APD and a control group without toothbrushing.

We believe that the sum of the mechanical action associated with antiviral action is one more strategy against the world pandemic. The results of this pilot study suggest the need for further studies evaluating the effectiveness of products with antiviral characteristics and the mechanical actions of reducing oral microbiota with oral hygiene in a prospective way, with more time of use and also the analysis of substantivity for a longer period of time and the antiviral effect.

Conclusion

This pilot study suggests that oral hygiene action associated with an antimicrobial chemical dentifrice may be an important tool for SARS-CoV2 viral load reduction in oral cavity.

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Tables

Table 1. Characteristics of patients with SARS-CoV-2 detected in the nasopharyngeal swab and baseline saliva by RT-PCR

Patient no.	Gender	Age	Period between symptoms onset and swab collection (days)	Nasopharyngeal swab Ct values Median	Viral load	Period between symptoms onset and baseline saliva collection (days)	Baseline saliva Ct values Median
1	M	27	2	18.65	High	5	21.28
2	W	20	6	23.67	High	9	22.35
3	W	22	4	24.59	High	8	31.08
4	W	40	2	16.08	High	5	33.81
5	W	23	7	20.57	High	10	32.34
6	M	38	6	20.22	High	9	29.74
7	M	41	5	19.82	High	8	29.12
8	M	29	3	17.61	High	7	29.44
9	M	20	4	19.73	High	7	32.36
10	M	23	4	18.81	High	8	18.96
11	M	45	4	22.66	High	7	29.96
12	W	31	2	18.73	High	4	17.91
13	M	28	6	16.30	High	10	24.59
14	M	33	3	18.26	High	8	17.42
15	M	20	4	19.73	High	9	23.56
16	W	45	7	26.84	High	10	30.79
17	W	27	7	19.90	Intermediate	10	33.34
18	W	29	6	19.82	High	11	34.38

Ct – cycle threshold . M- Male; W - Women

Table 2. Comparison of the Ct value in saliva between baseline, T5 and T30 groups.

Ct – value Median (IQR)			P value	Δ of Ct-value T5 - T0 Median (IQR)	Δ of Ct-value T30 - T0 Median (IQR)
Baseline (T0)	T5	T30			
29.59[a] (22.08-32.34)	28.97[a] (25.93-33.82)	31.39[b] (24.60-38.00)	0.020*	1.72 (-0.28-3.77)	3.83 (0.42-6.33)

* Statistically significant
Different letters represent statistical differences

Declarations

1. Conflicts of interest

Dr. Vilhena has a patent classified pending. The other authors have no conflict of interest.

2. Ethics

This study was approved by the Ethical Committee of the Federal Institute of Paraná (CAAE 35194520.0.0000.8156).

3. Source of Funding

The present study is a non funding research.

4. Authors' contributions

Danielle Gregorio and Alisson Gabriel Idelfonso Bistaffa: Acquisition, analysis and interpretation of data, drafting, revision and final approval of the manuscript. Marcelo Lupion Poleti and Thais Maria Freire Fernandes: Conception and design of the study, analysis and interpretation of data, revision and final approval of the manuscript. Fabiano Vieira Vilhena, Mayara Tiemi Enokida Mori, Nicole Perugini Stadtlober, Marcell Alysson Batisti Lozovoy, Andréa Name Colado Simão, Paulo Sérgio da Silva Santos, and Berenice Tomoko Tatibana: Analysis and interpretation of data, revision and final approval of the manuscript.