

Do mouthwashes reduce covid-19 viral load during dental procedure and oropharyngeal examination? A systematic review

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Abstract:

There is a relatively high risk of virus transmission in dental procedures and oropharyngeal examinations. We investigated the effects of mouthwashes on covid-19 viral load reduction during dental practices and oropharyngeal assessments.

We performed a systematic search in PubMed, EMBASE, Scopus, Web of Science, Cochrane library for relevant studies up to February 2021. Papers evaluating patients with covid-19 infection (patients) who rinse mouthwashes (intervention) compared to patients who don't rinse them (comparison) for reducing covid-19 viral load or reducing cross-infection of covid-19 (outcome) in the randomized and non-randomized clinical trial and quasiexperimental studies (study) were included due to PICOS question. Three independent authors conducted literature screening and data extraction. We extracted the most relevant data and we evaluated the risk of bias from the included studies.

Out of 344 potentially eligible articles, six studies were included in this systematic review. Regarding viral load and negative cycle threshold (ct) values, 1% PVP_I and Listerine mouthwash were effective. 0.12% CHX mouthwash was effective 0-2 hours post rinsing, but it was not effective after 2 hours. A mixture solution of 0.2% Chlorhexidine gluconate and 6% Hydrogen peroxide was effective on day 5 of intervention. Gargling 1% hydrogen peroxide, 0.075% Cetylpyridinium Chloride (CPC), 0.5%PVP-I and 0.2% CHX mouthwashes was not effective on SARS-COV-2. It cannot be guaranteed that rinsing a specific kind of mouthwash prevents cross-infection of covid-19; however, the viral load of SARS-COV-2 in saliva will be decreased after rinsing mouthwashes containing 1%PVP-I and Listerine.

Keywords: mouthwash, mouth rinse, oral rinse, covid-19, SARS-COV2, coronavirus

Introduction

SARS-CoV-2, the cause of coronavirus disease 2019 (covid-19), relates to Betacoronavirus of coronaviride family. It is a single-stranded, positive-sense RNA virus. The spike surface glycoprotein of this enveloped virus causes binding to receptors on the host cells [1]. This virus shows a tendency towards cells with a membrane that contains the angiotensin-converting enzyme-2 (ACE2) receptors [2]. These receptors are expressed in multiple human systems and tissues such as lung and salivary glands and epithelial cells of nasopharynx and oropharynx [3, 4].

There is evidence that the oral cavity is a reservoir of SARS-COV-2 because ACE2 highly expresses on the mouth's non-keratinizing squamous epithelium. Also, scientists successfully detected the SARS-CoV-2 RNA in saliva [5], so saliva is a source of SARS-COV-2 dissemination.

The main transmission route of SARS COV2 is through respiratory droplets. These droplets cause direct contact infection during coughing, sneezing, and speaking or indirect contact infection via touching infected objects and the environment [6].

There is a relatively remarkable risk of virus transmission in dental procedures and oropharyngeal examinations because of face-to-face proximity treatments and aerosol-generating equipment [7, 8]. The ultrasonic scalers and high-speed handpieces spray saliva, blood, and fomites result in microbial transmission between patients and clinic staff. Viral shedding was detected in the oral cavity of symptomatic and asymptomatic patients [9].

In dental clinics, breaking the virus transmission chain between patients and staff is very important. For this aim, the first step is to use personal protective equipment. After that, patient evaluation and recognizing patients with potential infection of covid-19 is very crucial. Using a contact-free thermometer is recommended for determining patients with fever. A questionnaire can screen patients; it should contain questions such as if the patient had any covid-19 infection symptoms (such as fever and respiratory problems) during the past 14 days and if they had close contact with a patient with confirmed covid-19 infection within the past two weeks [10]. With body temperature above 37.3°C or an affirmative answer to the questionnaire, the patient is suspected or at risk of covid-19 infection, so the dentist should postpone the appointment and refer the patient to local health departments [11].

It is crucial to reduce oral microbial load before starting dental processes. One way is using mouthwashes. Today, a large number of antimicrobial mouthwashes are available in the market. They have natural or synthetic antiseptic compounds. Preoperative antiseptic mouthwashes are frequently used in dental offices [12]. Different concentrations of these mouthwashes have antibacterial and antiviral effects [13, 14].

Recent publications have recommended that using antiseptic mouthwashes may control the viral load of SARS-COV-2 in saliva. However, scientific evidence for anti-SARS-COV-2 effects is lacking and unclear. Although researchers investigated the in-vitro effects of antiseptic mouthwashes on covid-19 [15-19], limited clinical trial studies examined the effects of antiseptic mouthwashes on covid-19 viral load. In the current study, we aimed to review clinical trial and quasi-experimental studies reporting the effects of mouthwashes on reducing covid-19 viral load.

2. Materials and Methods

We systematically reviewed studies comprising patients with SARS-COV-2 positive test and underwent rinsing a mouthwash for SARS-COV-2 viral load reduction. In this study, we adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement 2009 recommendations provided by Liberati [20].

Search strategy:

We searched PubMed, EMBASE, Scopus, Web of Science and Cochrane CENTRAL databases with the MeSH and nonMeSH terms and the keywords of 'coronavirus' OR 'covid-19' OR 'SARS-COV-2' OR '2019-ncov' AND 'mouthwash', "mouth rinse", "oral rinse".

Google scholar and MedRxiv and clinicaltrials.gov were also searched with these keywords manually to retrieve the gray literature. The publication date was limited to February 2021. Except for the time, no other restriction was considered for our search. We also looked into the references of the included papers for more relevant studies. The screening was done independently by T.E and S.Z.M and A.S.H.

Firstly, duplicated retrieved search results were identified and excluded. We screened the titles and the abstracts of the papers to exclude the irrelevant studies. Accordingly, search results were categorized into three categories of included, excluded and unclear. Then, the full texts of the retrieved studies were reviewed for final inclusion. Any disagreement was discussed between the three reviewers.

Eligibility criteria and study selection:

We included randomized clinical trials, non-randomized clinical trials and quasi-experimental studies. We excluded reviews, letters to the editor, technical notes, in vitro studies and studies carried out on animals.

Studies had to be available in English. We included studies that fulfilled the following inclusion criteria according to the PICOS acronym:

Patients were subjects diagnosed with covid-19 infection with no age or gender restrictions. The rinsing of mouthwashes was an intervention for patients infected with covid-19. No mouthwash use is the comparison. Reduction of viral load in the saliva is the outcome of the study. Randomized and non-randomized clinical trial studies were included.

Data extraction:

We extracted the following data from eligible articles: study characteristics (study title, authors, date of publication, study design, number of patients); baseline data (kind of mouthwash, type of examination for determining viral load, type of analyses of viral load) and clinical outcomes.

Assessing of risk of bias:

Two reviewers (T.E, A.S.H) independently assessed the risk of bias for the included studies as a part of data extraction. A modified Down and Black (D&B) Risk of Bias checklist [21] was used for measuring the quality of the included studies. A quantitative method regarding the quality level of each study was used.

Each satisfactory response of the modified D&B items received a score of 1, otherwise the score was 0. Studies with a modified D&B level ≥ 5 were considered as a study with a low risk of bias. Those with a modified D&B level < 5 points were considered as a study with a high risk of bias. and listed in table 1. We used Endnote software for organizing the references.

Table 1: Modified Downs and Black checklist

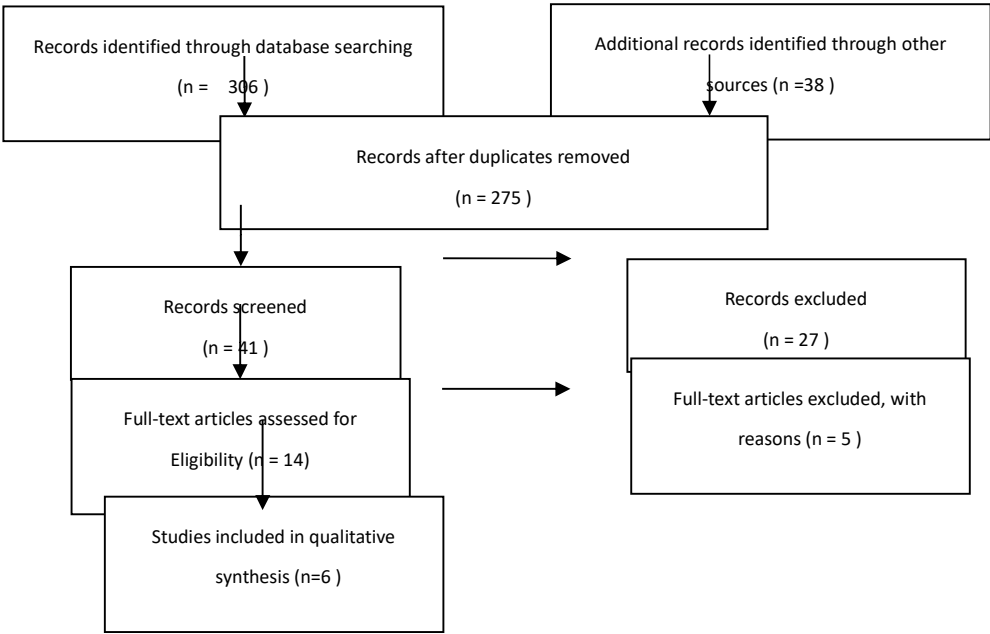
		Gottsaunerr et al. [22]	Mohamed et al. [23]	Mukhtar et al. [24]	Lamas et al. [25]	Yoon et al. [26]	Seneviratne et al. [27]
1	Objective Clearly Stated ¹	1	1	1	1	1	1
2	Main outcomes clearly described ²	1	1	1	1	1	1
3	Patients characteristics clearly defined ³	1	1	1	1	1	1
4	Main findings clearly defined ⁴	1	1	1	1	1	1
5	Random variability in estimates provided ⁵	1	1	1	1	1	1
6	Sample targeted representative of population ⁶	0	0	1	0	0	0
7	Sample recruited representative of population ⁷	0	0	1	0	0	0
8	Primary outcomes valid/reliable ⁸	1	1	1	1	1	1
	Total	6	6	8	6	6	6

Selected items from Downs and Black checklist 1: q1 2: question 2; 3: question 3; 4: q6; 5: q7; 6: q11; 7: q12 and 8: q20
Total possible score for the modified D&B checklist=8

3. Results

In the initial search, 344 references were obtained on PubMed, EMBASE, Scopus, Web of Science and Cochrane Central, Google scholar and MedRxiv and clinicaltrials.gov (figure 1). After screening titles, abstracts and full texts of 14 articles, six articles were included in our study. Details are given in the PRISMA flow chart.

Figure 1 :PRISMA diagram



Study characteristics:

The reviewed articles had sample sizes between 2 and 92 patients infected with covid-19. In total, 144 patients were included in the studies. More details are given in Tabel 2.

As shown in Table 1, five studies obtained a score of 6 points [22, 23, 25-27]. One study obtained a score of 8 points [24]. All studies were considered to have a low risk of bias.

Due to inconsistency in the kind of mouthwashes (intervention), diagnostic kits, basement specimens (saliva or nasopharynx or oropharynx swab) and the time of experiments (the time between first rt-PCR test and rinsing the mouthwash) between studies, we decided to forbear from performing Meta-analysis. Hence five studies used semi-quantitaive experimental kits; we considered negative ct values as main outcome, and decreasing in viral load as second for reporting the results.

The mouth-rinses used in these six articles were 0.01 Hydrogen peroxide [22], 0.01PVP-I, Listerine and tap water [23], hydrogen peroxide in combination with CHX[24], 1%PVP-I [25], 12%CHX [26], 0.5% PVP-I , 0.2 CHX, 0.075CPC [27].One study used tap water as an intervention [23] and in another study, tap water was used in the control group [27]. Three studies had control group [23];[24];[27],other three studies had no control groups and baseline sapmles were compared with experimental samples [22];[25];[26].

In five studies [22-24, 26, 27], the populations under evaluation were patients with positive PCR test for SARS-COV-2 in hospitals and in one study, patients were isolated at home or they were hospitalized [25].

In one study, if patients started one treatment for covid-19, they were excluded from the study [23]. In another study, all patients received antiviral agents [26].

In a study, patients received different treatment for covid-19 during the experiment such as antibiotics or hydroxyl chloroquine or antiviral agents or a combination of them[24]. In three studies, using antiviral or other medications during the study was not mentioned [22];[25];[27].

In one study, nine patients out of ten had different underlying diseases such as chronic renal failure, Multiple Myelo-ma and arterial hypertension [22]. In another study, only two subjects out of twenty had asthma and obesity as comorbidities[23]. Another study mentioned that 21% of respondents had different underlying diseases (diabetes mil-lets, hypertension, and chronic kidney disease)[24]. History of non-Hodgkin's lymphoma and diabetes and ischemic stroke was reported for two subjects out of four [25]. Two studies did not mention any underlying diseases [26, 27].

In the study of Gottsauner et al. [22] envelope (E) gene of SARS-COV-2 was amplified. Four patients showed an increase in viral load after intervention and four patients showed a decrease in viral load. There was no difference

between the viral load of basement and intervention swab tests of two patients. So they reported no significant reduction of intraoral viral load after rinsing 1% hydrogen peroxide mouthwash.

Mohamed et al. [23] reported the result of swab tests as either "negative=ct value >45" or "positive ≤45 or intermediate" for E gene and RNA-dependent RNA polymerase (RdRp) gene. Intermediate results are not positive or negative; rather the investigators should repeat that test for intermediate results but they did not. So only negative results are reliable and we considered them for reporting the results. SARS-CoV-2 test was negative in all specimens of PVP-I group on days four, six and twelve. In Listerine group, four out of five swab tests were negative on subsequent days. Two samples were negative within the tap water group on days four, six and twelve. Within the control group, one swab sample was negative on day four, twelve and on day 6 there was no negative sample. In this study, rinsing 1% PVP-I and Listerine mouthwashes three times a day effectively reduced SARS-COV-2 viral load.

Mukhtar et al. [24] reported the result of swab tests as either "negative=ct value >40" or "positive ≤40 or inconclusive" for ORF-1a/b and E-genes. In baseline, ct values of none of the swab tests were negative in both the intervention and control groups (0 out of 46 swab test was negative). After 5 days, 6 out of 45 swab tests were negative in the intervention group and no swab test of the control group was negative. After 15 days, 15 out of 43 swab tests were negative in the intervention group and 9 out of 44 were negative in the control group.

They showed a significant difference between the two groups which rinsed the mouthwash (mixture of 0.2% Chlorhexidine gluconate and 6% Hydrogen peroxide) in terms of PCR results on day 5, but it was not significant on day 15.

Lamas et al [25] reported ct values of three SARS-COV-2 genes: E, RdRp and nucleocapsid (N) in baseline samples and after 5 min, 1 h, 2h, and 3h in four patients. ct value of baseline saliva sample was not reported for E and RdRp genes in patient 1. Hence we compared ct values of experimental samples with baseline sample; we only reported ct value results of N gene in this study. In patient 1, ct value of baseline sample was negative and experimental sample results remained negative. In patient 2, baseline was positive, and only after 1 and 2 hour it became negative. In patient 3, ct value of baseline sample was negative and experimental sample results remained negative. In patient 4, baseline sample was positive and it changed to negative only after 2h and 3h. So gargling PVP-I mouthwash was effective in 2 patients out of four not after a short time (5 min) but after a longer time (1, 2 h for patient 2 and 2, 3h for patient 4). Due to viral load reduction, in two of the four participant, PVP-I mouthwash resulted in a drop in viral load after 1, 2 and 3 hours.

In the study of Yoon et al. [26] after baseline specimen sampling of E and RdRP genes of SARS-COV-2, results were reported on day 3 and 6 after the intervention. On day 3, SARS-COV-2 was not detected in the specimens, 1 and 2 hours after rinsing 0.12% chlorhexidine and data was not available for these two points of time. Four hours after intervention, ct value was not negative for both two patients. On day 6, ct value of one out of two patients was negative 1, 2 and 4 hours after the intervention. In another patient, 1 hour after gargling with mouthwash, ct value was positive, but after 2 hours ct value was negative and after 4 hours it was positive again. Due to viral load, after rinsing CHX mouthwash, SARS-COV-2 viral load decreased transiently for 2 hours, but it increased again after two hours.

In the study of Seneviratne et al., E gene of SARS-COV-2 was targeted. Ct values were calculated for each mouth rinse group at 5 minutes, 3 and 6 hours after rinsing mouthwashes compared with the water group. In none of the specimens, ct value was negative after the intervention. With quantitative and semi-quantitative analysis, there were no difference between groups, so it can not be reported that gargling 0.5% PVP-I, 0.075% CPC and 0.2% CHX mouthwash is effective [27].

Other characteristics of the six included studies are summarized in Table 2 and Table 3.

Table 2: Study details

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis	
Gottsauner [22]	Clinical Pilot Study	10	positive covid19 infected patients within the last 72 h in a hospital	Patients who need intubation or mechanical ventilation or severe stomatitis.	1 to 5 days (median 3 days) 12 patients (6 female and 6 male) had a median age of 55 years (range: 22–81 years). Two with neg. RT-PCR test.	RT-PCR test of oropharyngeal specimens	Hydrogen peroxide 1%	Gargle for 30s	oropharyngeal	30 min	5	1/5(culture)	0/5(culture)	
Mohamed [23]	4-arms preliminary interventional study	20	Adults older than 18 years, covid-19 positive patients with no symptom ,less than five days from diagnosis.	Objects who cannot understand instructions, express symptoms of covid-19 infection such as fever or respiratory problems or, abnormal chest computed tomography , patients started treatments for covid-19 , objects infected with	age range from 22-56 years old(16male,4female)	RT-PCR test was performed on nasopharyngeal and oropharyngeal swabs targeting the E gene and RNA-dependent RNA polymerase gene (RdRP) and provided with a cycle threshold(ct) value.	1% PVP-I	gargle for 30 seconds, three times per day for 7 days.	nasopharyngeal and oropharyngeal swab	4d	5	—	0/5	
										6d	5	—		
										12d	5	—		
							Listerine (essential oil)	gargle for 30 seconds, three times per day for 7 days.		4d	5	—	1/5	
										6d	5	—	1/5	
										12d	5	—	0/5 (1 intermediate)	
							Tap water	gargle for 30 seconds, three		4d	5	—	3/5	
										6d	5	—	1/5 (2 intermediates)	

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis
				SARS-CoV-2 again, thyroid dysfunction, allergy to povidone-iodine				times per day for 7 days.		12d	5	—	2/5 (1 intermediate)
							No intervention	—		4d	5	—	2/5 (2 intermediates)
										6d	5	—	3/5 (2 intermediates)
										12d	5	—	3/5 (1 intermediate)

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis
Mukhtar [24]	an investigator-initiated, randomized, unblinded, phase IV clinical trial	92	patients with positive PCR test for covid-19 through combined Nasopharyngeal Oropharyngeal swab who were hospitalized within 24 hours	Objects under 18 years of age, mental or cognitive problems, pregnant women, head and neck injuries, patients who need intubation	The mean age was 49 ; the age range had no significant difference between the objects (P=0.89). number of males gender were higher (72 Vs. 10). Intervention group (n=46): 1 Non-covid Pneumonia (NCP) Asymptomatic; 11 NCP mild symptoms; 24 MILD covid Pneumonia (CP); 1 MODERATE CP; 9 SEVERE CP Control group (n=46): 4 NCP Asymptomatic; 10 NCP	RT-PCR test of nasopharyngeal and oropharyngeal swabs targeting the S, N and E-genes Obtaining CT value >30 in subsequent covid-19 RT-PCR test	10 ml of 0.2% Chlorhexidine gluconate and 5 ml of 6% Hydrogen peroxide (a final concentration of 2%).	gargling 15 ml three times daily. 30 seconds for 2w Initially, they were advised to use the mouthwash for one minute (not exceeding 2 minutes contact time with the oral cavity); however, due to the difficulty of prolonged use given the high oxygen requirements ...		5d	Baseline: 46 5d: 45	CT values: Negative: 0 Inconclusive: 0 35-40: 1 30-34.9: 6 25-29.9: 13 20-24.9: 13 15-19.9: 9 <15: 4 Mean: 22.6 [95% CI: 20.8-24.3]	CT values: Negative: 6 Inconclusive: 5 35-40: 0 30-34.9: 2 25-29.9: 19 20-24.9: 8 15-19.9: 4 <15: 1

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis
					mild symptoms; 18 MILD CP; 3 MODERATE CP; 11 SEVERE CP		Control	---		5d	Baseline: 46 5d: 44	CT values: Negative: 0 Inconclusive: 0 35-40: 0 30-34.9: 5 25-29.9: 12 20-24.9: 12 15-19.9: 11 <15: 6 Mean: 23.7 [95% CI: 21.9-25.5]	CT values: Negative: 0 Inconclusive: 6 35-40: 0 30-34.9: 3 25-29.9: 19 20-24.9: 11 15-19.9: 4 <15: 1

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis
							mixed solution of 10 ml of 0.2% Chlorhexidine gluconate (oral rinse) plus 5 ml of 6% Hydrogen peroxide (to make up a final concentration of 2%).	gargling 15 ml three times daily. 30 seconds for 2w Initially, they were advised to use the mouthwash for one minute (not exceeding 2 minutes contact time with the oral cavity); however, due to the difficulty of prolonged use given the high oxygen requirements ...		15d	Baseline: 46 15d: 43	CT values: Negative: 0 Inconclusive: 0 35-40: 1 30-34.9: 6 25-29.9: 13 20-24.9: 13 15-19.9: 9 <15: 4 Mean: 22.6 [95% CI: 20.8-24.3]	CT values: Negative: 15 Inconclusive: 14 35-40: 4 30-34.9: 7 25-29.9: 3 20-24.9: 0 15-19.9: 0 <15: 0

First author	Type of study	Number of participants	Inclusion criteria	Exclusion	Patients' characteristics	Lab. Test	Kind of mouthwash	Treatment schedule	Specimen	Testing time after intervention	Sample size	Baseline specimens analysis	Interventional specimens analysis
							Control	---		15d	Baseline: 46 15d: 44	CT values: Negative: 0 Inconclusive: 0 35-40: 0 30-34.9: 5 25-29.9: 12 20-24.9: 12 15-19.9: 11 <15: 6 Mean: 23.7 [95% CI: 21.9-25.5]	CT values: Negative: 9 Inconclusive: 17 35-40: 5 30-34.9: 11 25-29.9: 1 20-24.9: 1 15-19.9: 0 <15: 0

Table 3: study details

Lamas[25]	Quasi-experimental	4	Not mentioned	Not mentioned	74,73,43,54 years old patients (28-41 days after positive nasopharyngeal positive test); 2Males and 2females	rRT-PCR assay which targeted E-gene , RdRP and N genes	1% povidone iodine	15 ml for 1 min	Nasopharyngeal	---	4	2/4 Ct E 27.83±11.33 Ct RdRp 29.94±11.27 Ct N 30.12±9.82	
									Saliva	5min	4	Ct E 28.98±7.59 Ct RdRp 32.39±5.25 Ct N 32.70±5.20	.35±4.35 Ct RdRp 32.07±2.64 Ct N 32.41±3.85
									Saliva	1h	4	Ct E 28.98±7.59 Ct RdRp 32.39±5.25 Ct N 32.70±5.20	Ct E 33.62±2.30 Ct RdRp 37.08±0.59 Ct N 36.06±1.32
									Saliva	2h	4	Ct E 28.98±7.59 Ct RdRp 32.39±5.25 Ct N 32.70±5.20	Ct E 35.88±1.95 Ct RdRp 38.45±0.60 Ct N 37.46±2.43
									Saliva	3h	4	Ct E 28.98±7.59 Ct RdRp 32.39±5.25 Ct N 32.70±5.20	Ct E 35.38±3.59 Ct RdRp 35.32±2.91 Ct N 36.62±1.78
Yoon[26]	Quasi-experimental	2	Not mentioned	Not mentioned	Two hospitalized patients diagnosed with	rRT-PCR which targeted the E and RdRP genes of SARS-CoV-2. Cts were derived from supplementary tables.	CHX 0.12%	15 ml, 30 sex, Gargling	Nasopharynx	Day1	2	19.38±2.56	
										Day3	2	24.21±0.53	
										Day5	2	25.07±4.33	
										Day7	2	23.17±6.93	

					covid-19.					Day9	2	36.12±2.41	
									Oropharynx	Day1	2	25.75±1.82	
										Day3	2	35.29±3.04	
										Day5	2	30.51±1.25	
										Day7	2	0	
										Day9	2	0	
									Saliva	Day1	2	23.61±1.27	
										Day3	2	27.52±5.49	
										Day5	2	30.69±0.59	
										Day6	2	32.13±1.77	
										Day7	2	0	
										Day9	2	39.67±0.21	
									Saliva-Day 3 of hospi- talization (day 6 of disease)	1h	2	27.52±5.49	0(not detected)
									Saliva-Day 3 of hospi- talization (day 6 of disease)	2h	2	27.52±5.49	0(not detected)
									Saliva-Day 3 of hospi- talization (day 6 of disease)	4h	2	27.52±5.49	30.16±6.57
									Saliva-Day 6 of hospi- talization (day 9 of disease)	1h	2	32.13±1.77	33.55±2.13
									Saliva-Day 6 of hospi- talization (day 9 of disease)	2h	2	32.13±1.77	37.17±2.52

									Saliva-Day 6 of hospi- talization (day 9 of disease)	4h	2	32.13±1.77	32.85±9.75
Senevirat- ne [27]	Ran- domiz- ed clini- cal trial	16	Patients whose their nasal swabs were positive for rRt-PCR assay of SARS-COV-2 from a hospital in Singapore .	thyroid prob- lems ,patients received radioactive iodine lately, under treatment with lithium, pregnant wom- en, and renal failure 19 pts had negative PCR for saliva And one pt excluded due to non-compliance	All were males except 1 in control group	The in-house RT-PCR test of saliva sam- ples targeting the E gene of SARS-CoV-2. [Fold changes in comparison to control group also are reported in the article but we omit them as not reported in other studies.]	---		Saliva	Baseline	16	17/36 n=16: 27.7±4.8	
							PI	5ml, 0.5% w/v	Saliva	5 min	4	22.53±5.42	24.20±8.08
										3h	4	22.53±5.42	24.21±5.63
										6h	4	22.53±5.42	23.03±5.17
							CHX	15ml, 0.2% w/v	Saliva	5 min	6	29.90±2.41	27.89±2.57
										3h	6	29.90±2.41	30.01±1.82
										6h	6	29.90±2.41	27.90±2.34
							CPC	20ml, 0.075%	Saliva	5 min	4	32.08±2.27	32.91±2.48
										3h	4	32.08±2.27	30.65±3.20
										6h	4	32.08±2.27	31.86±2.76
							Wa- ter(control)	15ml	Saliva	5 min	2	26.33±1.83	25.30±2.17
										3h	2	26.33±1.83	23.16±1.13
										6h	2	26.33±1.83	22.00±2.80

Discussion:

Covid-19 is known to transfer from person-to-person through infected droplets and aerosols. Close contact of dentist with patient and aerosol-generating procedures can significantly increase airborne contamination and cross-infection of SARS-COV-2 in dental clinics.

Antiseptic mouth rinses have been suggested for various prophylactic and therapeutic purposes in dentistry. But their anti-SARS-COV-2 effect to control the viral load has not been evaluated systematically.

Oral rinses should have a high substantivity. It means that they release slowly, so they show their antimicrobial effects for an extended time; therefore, only mouth-rinses with high substantivity may be effective against covid-19.

in-vitro studies demonstrated that povidone-iodine in different concentrations has antiviral effects against SARS-COV-2 [15-18]. Other studies investigated the effects of hydrogen peroxide, Cetylpyridinium Chloride, ethanol, and essential oil mouthwashes on covid-19 [15]; [19]. An in-vitro study examined the virucidal effects of eight different oral rinses: in this study, researchers added mouth rinses to viral suspension and a particular substance that simulates the oral environment. The results showed that Dequalinium chloride, benzalkonium chloride, ethanol and povidone-iodine have more significant antiviral effects than other compounds. They concluded that commercially available oral rinses inactivate SARS-CoV-2 within the short exposure time [15].

Clinical trial studies examined the effects of hydrogen peroxide, iodine povidone (PVP-I), Cetylpyridinium chloride (CPC), chlorhexidine and essential oil mouthwashes on SARS-COV-2 viral load.

Hydrogen peroxide eliminates microorganisms of the oral cavity by degradation into oxygen and water. Hossainian et al. explained that hydrogen peroxide mouthwashes do not consistently control the microbiota of the oral cavity [28].

Despite the safety of hydrogen peroxide in a short period, long-term use might have carcinogenic effects.

Filho J et al. suggested that H₂O₂ mouthwashes should not continuously be recommended for patients with covid-19 because there is no approved evidence that H₂O₂ prevents covid-19 syndromes or prevents the virus from spreading [29]. But Peng et al. demonstrated that 1% hydrogen peroxide or 0.2% povidone-iodine reduce microbial and viral load when using a rubber dam is not possible [8]. Within the oral cavity, hydrogen peroxide will be inactivated due to the host-catalase activity [30]. In the clinical study of Gottsauner et al., they concluded that 1% hydrogen peroxide mouth rinse is not effective against SARS-COV-2 [22].

PVP-I is a water-soluble iodophor composed of iodine and polyvinylpyrrolidone as a water-soluble polymer [31]. Free Iodine molecule penetrates microorganisms, oxidizes surface proteins, and disrupts nucleotides and fatty acids, so it causes cell death [31]. Povidone-iodine has a large, broad spectrum of antimicrobial effects against bacteria, fungi and different viruses. 0.23% Povidone-iodine oral rinse showed a significant reduction in bactericidal activity and inactivated influenza virus and MERS-COV [16].

PVP-I is more effective than other common antimicrobial agents such as chlorhexidine, Ostenidine and polyhexinide [32]. It was demonstrated that PVP-I had sustained effects for more than 4 hours [33]. Oxidation mouthwashes, such as povidone-iodine, may reduce the salivary viral load of SARS-COV-2 in saliva [34]. In this systematic review, three studies used PVP-I mouthwash as an intervention, sample size was small in these three studies: 4 patients [25, 27] and 5 patients [23]. Higher concentration of PVP-I (1%) reduced SARS-COV-2 viral load [23], but lower concentration (0.5%) was not effective [27].

The study of Muhamed Khan et al. confirmed that gargling a mouthwash of 0.5% povidone-iodine is safe for health care workers and their patients before oral surgery and ENT examination. No allergy was reported [35]. Parhar et al. suggested that PVP-I reduces viral transmission of covid-19 during upper airway mucosal surgery [36].

There are some contraindications for use of PVP-I: 1) patients with an allergy to iodine, 2) thyroid disease, 3) pregnancy, 4) treatment with radioactive iodine [37].

CHX is a broad-spectrum antiseptic mouthwash and it has antibacterial and antiplaque properties [38, 39]. Bernstein et al. explained that CHX has antiviral effects on lipid-enveloped viruses and has no impact on non-enveloped viruses [40]. Peng et al. explained that CHX is not effective for covid-19 transmission reduction during dental practices [8].

In the clinical study by Yoon et al., two patients with covid-19 gargled chlorhexidine 0.12% for 30 seconds, and their saliva samples were collected after 1, 2 and 4 hours. Due to our qualitative results, this study showed that CHX was not effective on day 3, and it has controversial outcomes on day 6 for two patients, so we can not say that CHX has antiviral effects against SARS-COV-2 [26]. As the covid-19 disease is highly infectious, early viral clearance is so critical at the early stage. A combination of CHX and hydrogen peroxide mouthwash showed effectiveness against covid-19 viral load reduction on day 5, but it was ineffective on day 15 of experiment [24]. It can be related to the results of Uhm et al. that showed median time from diagnosis to negative RT-PCR assay was approximately 14 days for asymptomatic patients, so without intervention, 50% of asymptomatic patients will show clearance and intervention is less effective after 15 days.

CPC is a quaternary ammonium compound soluble in water. CPC can penetrate the cell membrane, raises the endocytic and lysosomal pH and disrupt the cell activity. In past decades, some clinical trials showed that CPC mouthwashes are effective in gingivitis and plaque control [41]. Comis et al. mentioned that CPC might have virucidal effects, especially against enveloped viruses [42]. In-vitro studies suggested that CPC disrupts different strains of the influenza virus [43]. By qualitative analysis, using CPC was not effective in reducing COVID-19 viral load [27].

Listerine mouth-rinses contain four active ingredients (eucalyptol, menthol, methyl salicylate, thymol) and inactive constituents such as water, alcohol and benzoic acid. Previous studies demonstrated Listerine's effectiveness in reducing dental plaque and gingivitis [44]. Moreover, Listerine has a significant efficacy against fungal species. Listerine disrupts the cell walls of microorganisms or inhibits the enzymatic activity of pathogens [45]. In vitro studies determined that Listerine has virocidal effects. Meiller et al. found that oral rinsing with Listerine mouthwash for thirty seconds reduce the viral load of HSV-1. They explained that this conclusion may extend to other enveloped viruses [46]. Mohamed et al. showed that Listerine mouthwash is effective against SARS-CoV-2 [23].

Conclusion:

The number of clinical trial studies that examined the effect of mouthwashes on the viral reduction of COVID-19 in saliva is very limited. The sample size of these experimental studies is small. So more clinical trial studies with standard sample size are required to assess the effect of mouth-rinses on SARS-CoV-2 virus. It cannot be guaranteed that rinsing a specific kind of mouthwash prevents cross-infection of COVID-19, however, the viral load of SARS-CoV-2 in saliva will be decreased after rinsing mouthwashes containing 1% PVP-I or Listerine. So they can be considered as a simple and inexpensive intervention during COVID-19 pandemic.

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