Original Article

Changes in reverse transcription polymerase chain reaction cycle threshold values of SARS-CoV-2 with topical povidone iodine use: A single-arm open-label interventional study

Sumita Shankar[1], Limalemla Jamir[2]*, Rakesh Kakkar[2], Korukonda Babji[3], Mangayarkarasi V[4], Mukesh Tripathi[5], Heena Tak[6]

[1]Department of Plastic Surgery, [3]Principal, Rangaraya Medical College, Kakinada, Andhra Pradesh, [2]Departments of Community and Family Medicine, [4]Microbiology and [5]Anesthesiology, All India Institute of Medical Sciences, (AIIMS), Mangalagiri, Andhra Pradesh, [6]Department of Microbiology, Government General Hospital, Kakinada, Andhra Pradesh, India

*Present Affiliation: Department of Community and Family Medicine, AIIMS, Guwahati, Assam, India

ABSTRACT

Context: Repurposed povidone iodine (PVP-I) has been suggested as an effective adjuvant against coronavirus disease-2019 (COVID-19). **Aim:** The aim of this study was to assess the changes in RT-PCR cycle threshold (Ct) values of severe acute respiratory Syndrome Coronavirus-2 (SARS-CoV-2) genes with PVP-I intranasal and oral application. **Settings and Design:** A longitudinal (repeated measures) single-arm open-label interventional study was conducted for 200 samples of ten COVID-19 patients in South India. **Methods and Material:** Demographic and clinical information were collected. Intranasal application and oral gargle with 1% PVP-I solution was done four times a day for seven days. Nasopharyngeal and oropharyngeal samples were taken for RT-PCR test at hour-0, hour-2, hour-4 on Day-0, Day-3, Day-6, and hour-0 on Day-9. **Statistical Analysis Used:** STATA analysis software version 14.2 was used. McNemar Test was applied for paired samples. Skilling Mack Test was used to assess the association between PVP-I use (intra-day and inter-day) and *E gene/N gene* Ct values. Pearson correlation coefficients and Bland-Altman plots were used for further analyses. **Results:** Mean (SD) age of the patients was 41.5 (±8.82) years. A total of 100 pairs of nasopharyngeal and oropharyngeal samples were analysed. No significant difference was observed in the Ct values of asymptomatic and symptomatic patients. *E gene* Ct values (nasopharyngeal) at Hour-0 increased from Day-0 to Day-9 ($P = 0.005$). Ct value was higher at Hour-2 for most of the samples. **Conclusions:** RT-PCR results (qualitative) differed at various testing points in the same patients. Lower Ct values were found in the nasopharyngeal samples. Successive increase in *E gene* Ct values indicates reduced viral load with natural course of COVID-19. PVP-I may have an optimal impact within 2 h of usage. **Clinical trial registration number:** CTRI/2020/05/024962.

Keywords: Anti-viral agent, COVID-19, povidone iodine, real-time RT-PCR, reverse transcriptase polymerase chain reaction, SARS-CoV-2

Access this article online

Quick Response Code:

Website:
www.jfmpc.com

DOI:
10.4103/jfmpc.jfmpc_993_21

Introduction

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) marked the year 2020 as the year of the highly infectious coronavirus disease-2019 (COVID-19) Pandemic.[1] The

How to cite this article: Shankar S, Jamir L, Kakkar R, Babji K, Mangayarkarasi V, Tripathi M, *et al*. Changes in reverse transcription polymerase chain reaction cycle threshold values of SARS-CoV-2 with topical povidone iodine use: A single-arm open-label interventional study. J Family Med Prim Care 2022;11:480-6.

main diagnostic modality of COVID-19 has been Reverse Transcription Polymerase Chain Reaction (RT-PCR) and a rough approximation of the viral load is indicated by the Cycle threshold (Ct) value, which is a semi-quantitative measure, obtained through Real-time RT-PCR.[2]

Due to relative ease and lesser invasiveness, nasopharyngeal and oropharyngeal swabs are the most common specimens collected for SARS-CoV-2 detection.[3,4] Comparatively higher viral load has been detected in the nasopharynx than the oropharynx.[5] Moreover, the nasal epithelium contains the maximum angiotensin-converting enzyme-2 (ACE-2) receptors in the body and SARS-CoV-2 makes its cellular entry through these receptors, with subsequent replication and transmission. This has been a basis for nasopharyngeal sample collection for diagnosis and use of face masks to cover the transmission portals (nose and mouth) and prevent the spread of COVID-19.[6,7]

For protection of these portals of entry and transmission, Povidone Iodine (PVP-I) has been recommended as an effective anti-viral agent against SARS-CoV-2.[8] Iodine in PVP-I is a known antiviral, antibacterial and fungicidal agent.[9] PVP-I has shown efficacy against two coronaviruses, the severe acute respiratory syndrome coronavirus (SARS-CoV) causing SARS and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) causing the MERS epidemic.[10,11] Recent studies have reported high virucidal activity of PVP-I, *in-vitro* and *in-vivo*, against SARS-CoV-2 within 30 s and 60 s of contact, respectively.[8,12]

This study was conducted to assess the impact of PVP-I oral and nasal application on the SARS-CoV-2 viral load as indicated by Ct-values of rRT-PCR and to assess the correlation between nasopharyngeal and oropharyngeal sampling in terms of RT-PCR assay SARS-CoV-2 gene Ct values.

Methods

Study design and participants

This study was a longitudinal (repeated measures) single-arm open-label interventional study with reliability analysis. The study was a validation of the diagnostic method used in the main study investigating the effect of oral and intra-nasal application of PVP-I against COVID-19.

The study site was located in a State in South India which had reported one of the highest rates of COVID-19 cases in the country.[13] Study participants were laboratory-confirmed (RT-PCR) COVID-19 patients, aged 18 years and above. Patients with known allergy to any form of povidone iodine, history of thyroid disorders, pregnant or unconscious patients and patients on ventilator were excluded from the study. The study was conducted in June-August 2020.

With a threshold probability of rejecting the null hypothesis of 0.05 and a probability of failing to reject the null hypothesis under the alternative hypothesis of 0.2, a sample size of 10 was needed

to prove a correlation coefficient of 0.8 between nasopharyngeal and oral samples.

Study procedure

Enrolment was done after obtaining written informed consent of COVID-19 patients. A pre-tested questionnaire in the local language was administered to the patients. Questions included demographic profile, travel history, contact with COVID-19 case, symptoms, comorbidities, date of positive report etc., Symptoms were documented in the symptom record sheet daily.

PVP-I bottles were provided to the participants with verbal and video-recorded instructions on dilution and usage. This was a commercially available iodine-based 'mouthwash' in India, a 2% solution with mint flavour, licensed for oral mucosal use.

Participants were asked to prepare fresh PVP-I solution daily by 1:1 dilution with water to yield a 1% solution. Intranasal application of PVP-I (0.3 ml) comprised external application in the anterior nares and along the walls of nasal cavity, as far as possible with cotton buds four times a day at six hourly intervals, preferably, 5-10 minutes after meals for a period of 7 days. This was according to the trough and peak action time of PVP-I applied locally over mucous membrane.

For oral gargle, 25 ml of the 1% solution was introduced into the oral cavity and used as a mouthwash whilst ensuring the solution was distributed throughout the oral cavity for 30 s and then gently gargled or held at the back of the throat for another 30 s before spitting out. The procedure was to be done four times a day, subsequent to- and for the same duration as the nasal application. Each patient was monitored telephonically on a daily basis by trained research personnel.

Clinical sample collection was done on Day-0, Day-3, Day-6, Day-9 of enrolment at three different timings: Hour-0 (five minutes before PVP-I use); Hour-2 (2 h after using PVP-I) and Hour-4 (4 h after using PVP-I) and only Hour-0 for Day-9.

Real-time reverse transcription polymerase chain reaction assay for SARS-CoV-2

RT-PCR assay was performed at the biosafety level-2 COVID-19 Diagnostic Laboratory of the study Institute. Under strict aseptic precautions, nasopharyngeal and oropharyngeal swabs were collected by trained research personnel in Viral Transport Medium (Poly Medicure Ltd.) and transported in cold chain of 2–4°C. Nucleic acids were isolated and purified using QIAamp Viral RNA Mini extraction kit. Purified nucleic acid including those of target genes- small envelope protein (E) and nucleocapsid protein (N) was directly amplified using TRUPCR® SARS-CoV-2 Kit (Genophyll Enterprises, mail@genophyll.com) based on rRT-PCR SARS-CoV-2 detection on QuantStudio™ 5 Real-Time PCR Instrument (HID Real-Time PCR Analysis Software v1.3 -Thermo Fisher scientific company). RT-PCR machine was calibrated and set threshold above the maximum

level of no Template control curve (random noise curve). Cycle threshold (Ct) cut-off value for positive test result was 36 and Ct-value cut-off of 37 was defined as negative result.

Statistical analysis

Data analysis was done in STATA analysis software version 14.2 (StataCorp, College Station, TX). Data were summarized as mean (standard deviation) and median (inter-quartile range) for quantitative data and as frequency (percentages) for qualitative data. McNemar Test was applied for the paired nasopharyngeal and oropharyngeal samples for qualitative RT-PCR. Skilling Mack Test was used to study assess the association between PVP-I use (intra-day and inter-day) and *E gene*/*N gene* Ct values. A value of *P* < 0.05 was considered statistically significant. Pearson Correlation coefficients were calculated to assess concordance between the nasopharyngeal and oropharyngeal isolates and agreement was assessed using Bland-Altman plots in terms of two gene sets.

Ethics

Prior ethical approval for the study was obtained from the Institutional Ethics Committee of the study institute (AIIMS/MG/IEC/2019-20/16). All procedures pertaining to protection of human participants were followed as per the Declaration of Helsinki.

Results

Demographic and clinical characteristics of COVID-19 patients

Ten (male-9; female-1) COVID-19 cases were enrolled in the study. The mean (SD) age of the patients was 41.5 (8.82) years (range: 30 to 58 years). Two patients had recent travel history. There was no known close contact with COVID-19 case (s). Four patients were symptomatic (fever, cough, breathing difficulty, diarrhoea, weakness); two patients were recently treated for tonsillitis and typhoid fever. Two patients had chronic diseases (hypertension, diabetes mellitus, bronchial asthma).

Qualitative result of SARS-CoV-2 RT-PCR assay

PVP-I was used for a week by the patients and on Day-8, there was neither PVP-I use nor sample collection. A total of 200 samples were tested for SARS-Cov-2 by RT-PCR. This comprised 10 nasopharyngeal and 10 oropharyngeal samples per patient, collected thrice each on Day-0, Day-3, Day-6 and once on Day-9. Six out of ten and five out of ten nasopharyngeal and oropharyngeal isolates of Day-9 respectively were RT-PCR positive. Results did not differ across days of testing for nasopharyngeal (*P* = 0.687) and oropharyngeal (*P* = 0.219) isolates [Table 1].

Cycle threshold (Ct) values of SARS-CoV-2 *E gene* and *N gene* on RT-PCR assay

Table 2 presents the hour-wise SARS-CoV-2 Ct values of *E gene* and *N gene*. Significant difference was found in *E gene* Ct

values between hours of nasopharyngeal sample collection on Day-0 (*P* = 0.030). For *N gene* Ct values, significant difference was found between hours of oropharyngeal sample collection on Day-0 (*P* = 0.011) and Day-3 (*P* = 0.022).

Overall median (IQR) for *E gene* Ct values of nasopharyngeal and oropharyngeal samples was 28.32 (22.00-31.78); *P* = 0.076 and 28.65 (25.84-31.85); *P* = 0.218, respectively. Overall median (IQR) for *N gene* Ct values of nasopharyngeal and oropharyngeal swabs were 28.43 (22.08- 32.11); *P* = 0.077 and 29.79 (26.95- 33.13); *P* = 0.364, respectively.

Table 3 presents the *E gene* and *N gene* Ct values across sampling days. A gradual rise in the *E gene* Ct values (nasopharyngeal) were detected at Hour-0 between Day-0 and Day-9 (*P* = 0.005). Although not statistically significant (*P* = 0.308), there was increase in the *E gene* Ct values at Hour-2 from Day-0 to Day-6. *N gene* Ct values were also higher at Hour-2 and Hour-4 of Day-6 as compared to previous sampling days, without statistically significant differences. The trend lines are displayed in Figure 1.

Correlation between nasopharyngeal and oral samples of SARS-CoV-2

In the correlation analysis between nasopharyngeal and oropharyngeal samples, concordance correlation coefficient for *E gene* was 0.6178 (95% CI: 0.449-0.743) and [0.428 (95% CI: 0.212-0.603)] for *N gene*. As depicted by Bland-Altman plot [Figure 2], mean difference between nasopharyngeal and oropharyngeal for *E gene* and *N gene* Ct values were -2.3 (-9.5%) and -2.0 (-8.2%), respectively. The differences established superiority of nasopharyngeal measurements in the range of Ct values for COVID-19 diagnosis. Ct values were lower in nasopharyngeal samples than oropharyngeal samples by a factor of 2.3 and 2.0 points. The regression line of differences showed that values were more prominently diagnostic at lower levels of Ct than higher values.

Discussion

This is one of the few in-vivo studies which assessed the impact of povidone iodine on the RT-PCR cycle threshold values of SARS-CoV-2 genes in COVID-19 patients. Of the ten COVID-19 patients enrolled in the study, six patients were asymptomatic. Asymptomatic viral shedding by SARS-CoV-2 infected cases has been reported earlier.[6,14] The four symptomatic patients in our study had clinical manifestations and co-morbidities similar to those reported earlier among COVID-19 patients.[15]

In this study, there was variability in the qualitative RT-PCR results across the sampling points. This is congruent with similar reports regarding qualitative RT-PCR which can vary greatly.[16] Xiao *et al*.[17] reported a positive third-time RT-PCR test in 30% of patients, despite previous two negative tests. Since SARS-CoV-2 virus-laboratory-patient dynamics influence RT-PCR results, cautious interpretation of results have been advocated.[18] Moreover, prolonged viral shedding

Table 1: Distribution of RT-PCR qualitative result by site of sample collection											
Site	Result	Day-0 (*n*=10)			Day-3 (*n*=9)			Day-6 (*n*=10)			Day-9 (*n*=10)	P*
		Hr0	Hr2	Hr4	Hr0	Hr2	Hr4	Hr0	Hr2	Hr4	Hr0	
Nasal	Positive	8	8	9	6	6	5	5	6	6	6	0.687
	Negative	2	2	1	3	3	4	5	4	4	4	
OralOral	Positive	9	9	7	5	5	5	5	3	5	5	0.219
	Negative	1	1	3	4	4	4	5	7	5	5	

*McNemar Test

Table 2: Intra-hour distribution of *E gene* and *N gene* Ct values								
Gene	Site	Day-0 Median (IQR)			P*	Day-3 Median (IQR)		
		Hour0	Hour2	Hour4		Hour0	Hour2	Hour4
E gene	Nasopharyngeal	22.01 (17.9-27.77)	25.13 (21.99-30.01)	26.78 (22.27-33.18)	0.030#	25.59 (24.69-29.64)	25.68 (21.56-29.33)	27.39 (19.76-27.50)
	Oropharyngeal	28.67 (26.8-36.79)	28.78 (25.84-32.94)	28.79 (26.19-30.83)	0.052	27.67 (27.55-29.39)	25.16 (21.28-25.88)	27.57 (23.70-27.74)
N gene	Nasopharyngeal	24.94 (15.4-31.63)	28.98 (20.80-31.61)	28.43 (21.51-34.34)	0.196	25.74 (21.67-31.11)	28.92 (23.51-30.66)	30.20 (26.86-30.68)
	Oropharyngeal	30.78 (28.17-34.62)	27.69 (26.99-34.10)	29.80 (27.72-33.50)	0.011#	29.78 (27.47-30.43)	19.67 (18.38-25.49)	30.02 (29.42-31.39)

Gene	P*	Day-6 Median (IQR)			P*
		Hour0	Hour2	Hour4	
E gene	0.818	31.48 (27.98-32.50)	34.18 (23.93-34.99)	24.90 (21.85-31.16)	0.496
	0.246	26.40 (25.90-29.09)	29.61 (23.39-34.11)	28.74 (26.98-31.15)	0.690
N gene	0.548	31.20 (29.83-33.41)	26.94 (20.89-30.25)	26.97 (24.14-31.01)	0.055
	0.022#	31.90 (30.96-35.14)	31.60 (25.58-31.83)	31.40 (28.90-34.56)	0.762

*Skilling Mack Test #*P*<0.05

Table 3: Ct values (*E gene* and *N gene*) across days by hour of sample collection						
Gene	Sample Timing	Nasopharyngeal Median (IQR)				P*
		Day 0	Day 3	Day 6	Day 9	
E gene	Hour 0	22.01 (17.96-27.77)	25.59 (24.69-29.64)	31.48 (27.98-32.50)	30.94 (28.66-32.14)	0.005#
	Hour 2	25.13 (21.99-30.01)	25.68 (21.56-29.33)	34.18 (23.93-34.99)	NA	0.308
	Hour 4	26.78 (22.27-33.18)	27.39 (19.76-27.50)	24.90 (21.85-31.16)	NA	0.897
N gene	Hour 0	24.94 (15.49-31.63)	25.74 (21.67-31.11)	31.20 (29.83-33.41)	29.89 (26.67-36.16)	0.164
	Hour 2	28.98 (20.80-31.61)	28.92 (23.51-30.66)	26.94 (20.89-30.25)	NA	0.289
	Hour 4	28.43 (21.51-34.34)	30.20 (26.86-30.68)	26.97 (24.14-31.01)	NA	0.636

Gene	Oropharyngeal Median (IQR)				P*
	Day0	Day3	Day6	Day 9	
E gene	28.67 (26.87-36.79)	27.67 (27.55-29.39)	26.40 (25.90-29.09)	33.74 (30.77-34.84)	0.779
	28.78 (25.84-32.94)	25.16 (21.28-25.88)	29.61 (23.39-34.11)	NA	0.096
	28.79 (26.19-30.83)	27.57 (23.70-27.74)	28.74 (26.98-31.15)	NA	0.074
N gene	30.78 (28.17-34.62)	29.78 (27.47-30.43)	31.90 (30.96-35.14)	29.54 (26.35-29.66)	0.686
	27.69 (26.99-34.10)	19.67 (18.38-25.49)	31.60 (25.58-31.83)	NA	0.180
	29.80 (27.72-33.50)	30.02 (29.42-31.39)	31.40 (28.90-34.56)	NA	0.969

*Skilling Mack Test #*P*<0.05

and positive reports after recovery from COVID-19 have been reported.[19,20] This is also one of the reasons for policy change in discharge of COVID-19 patients by the Health Ministry, Government of India, from "two negative results on RT-PCR within a span of 24 h prior to discharge" to "no testing prior to discharge".[13]

As reported earlier, nasopharyngeal samples were found to be relatively superior to oropharyngeal samples along with lower Ct values in most testing points, especially for *N gene*.[21] Several other studies have also reported better viral detection from nasopharyngeal or nasal specimens.[17,22,23] Liu *et al.*[24] stated that inhibitory components in the oropharynx (close vicinity to the

oral cavity) might attribute to lower SARS-CoV-2 detection in oropharyngeal samples.

Studies have reported the utility of Ct values in clinical progression of COVID-19.[25-27] However, lack of association between Ct values and clinical course of COVID-19 has also been reported.[28,29] In this study, a significant rise in the Ct values was observed for Hour-0 (prior to PVP-I usage for the day) across days of testing, which may be indicative of the natural course of SARS-CoV-2 infection and decline in viral load, irrespective of the positive qualitative results, as reported earlier.[2,30] Another aspect in SARS-CoV-2 sample collection which is reinforced by our study, is the possibility of higher
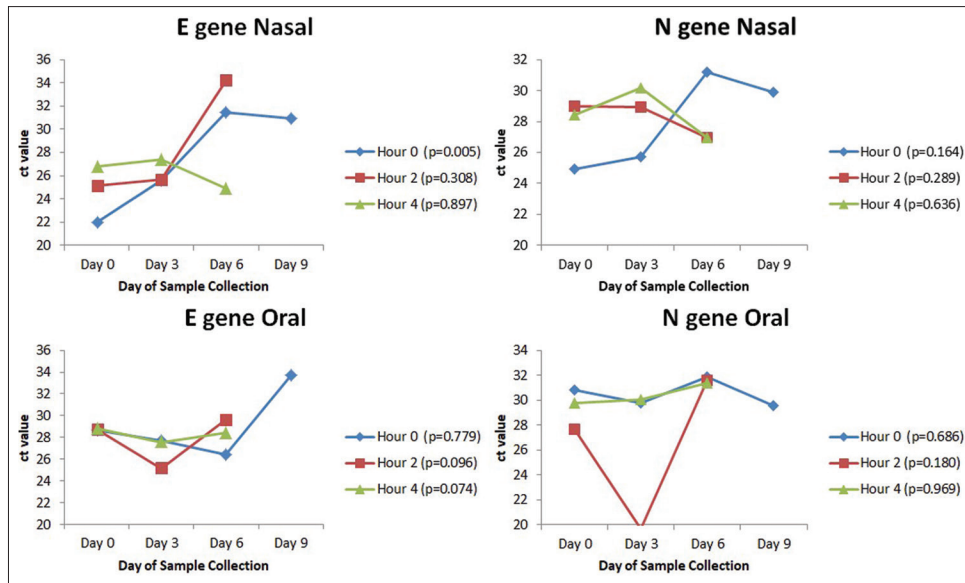
Figure 1: Ct values of *E gene* and *N gene* across days by hour of sample collection
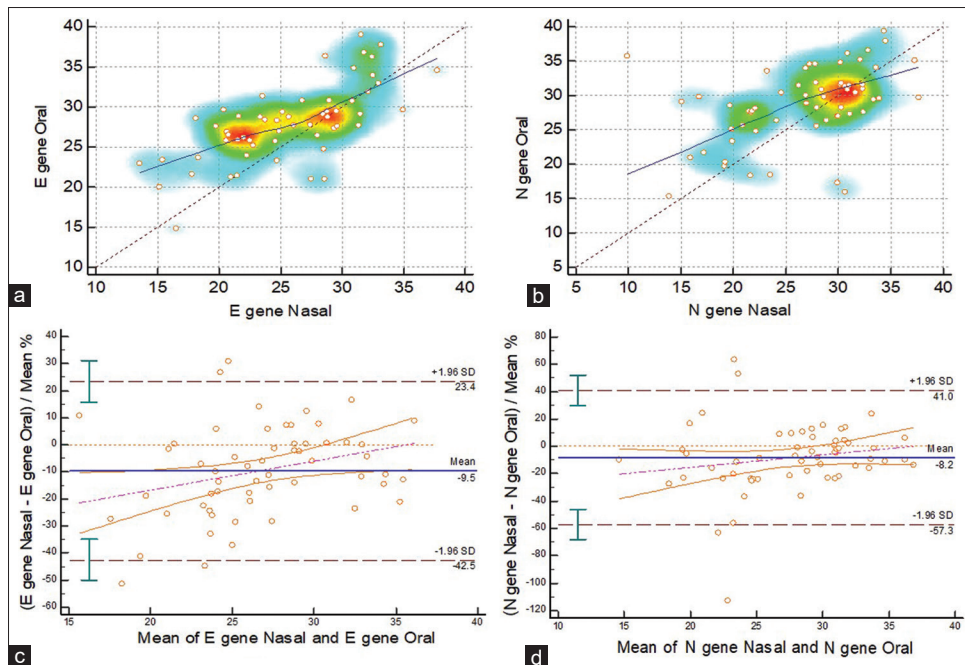

Figure 2: Bland-Altman diagram using mean of nasal and oral Ct values depicting a) Nasal Vs Oral *E gene* differences, b) Nasal Vs Oral *N gene* differences, c) Nasal Vs Oral *E gene* differences in %, d) Nasal Vs Oral *N gene* differences in %. The neutral line (0) with limits (+1.96 × SD) are shown as horizontal lines. Regression lines and the 95% CI are also included in each graph

yield of virus in the early part of the day or before washing, as reported earlier.[24]

Moreover, viral carriage and transmission by asymptomatic or convalescent COVID-19 cases through the nasal cavity and oropharynx have been reported.[31,32] In this study, 1% PVP-I was used. Similarly, studies have reported rapid inactivation of SARS-CoV-2 at concentrations ranging from 0.5% to 2.25% with intranasal safety concentrations reported to be 1.25% and recommendations for six hourly prophylactic usage of PVP-I.[8,33] Allaying apprehensions regarding PVP-I topical applications,

several studies have reported low allergenic properties, low cytotoxicity, and no or minimal thyroid dysfunction with PVP-I usage.[34-36] Hence our study provides a direction in the usage of PVP-I 1% solution as an adjuvant therapy against COVID-19.

In this study, there was a significant difference in the Ct values between specific hours of testing after PVP-I usage. However, no consistent significant differences were found between successive days of testing. Although not statistically significant, higher Ct values were observed at Hour-2 for most of the samples. This points to the possibility of PVP-I action being optimal within

2 h of usage, at a contact time of 30 s as done in our study. This finding supports recommendations for usage of PVP-I by health care workers and general public immediately pre- and post-close contact with COVID-19 cases.[37] PVP-I formulations as nasal sprays or oral rinses have been particularly recommended for those involved in aerosol-generating procedures such as surgical, otolaryngological and dental practice.[38-40]

Additionally, a large proportion of general public seek primary healthcare due to easier access, especially during the pandemic as healthcare facilities have either been converted to dedicated COVID-19 facilities or have placed restrictions on out-patient services. This study provides information to primary care physicians on the potential role of povidone iodine in COVID-19 as a protective measure, particularly among high-risk contacts in the community and as a treatment adjuvant for COVID-19 patients. The study also provides scientific research update on RT-PCR (variable results of the same patients at different testing points) and in-vivo PVP-I effect on SARS-CoV-2 cycle threshold values.

Study limitations include the small sample size of COVID-19 patients and testing at various clinical stages of the disease, although the participants were enrolled in the early stages of infection. Exposure could not be ascertained as participants could not recall or did not know if they were in close contact with a COVID-19 case. PVP-I usage was self-reported and it was not done under direct observation due to infectious nature of the disease. Lack of a control group is another limitation of our study. However, Hour-0 implied pre-PVP-I usage on all testing days so this may have validated the comparisons to some extent. Despite these limitations, our study provides unique insights into the paradigm of changes in Ct values of RT-PCR with PVP-I usage.

In conclusion, lower RT-PCR Ct values were found in Hour-0 samples across successive days, indicating higher viral load before PVP-I usage and decline in viral load as part of natural course of disease. Higher Ct values at Hour-2 for most of the samples indicate optimal action of PVP-I on the surface mucosa within 2 h of usage. Nasopharyngeal swab samples were found to be relatively superior to oropharyngeal samples in SARS-CoV-2 viral detection.

Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. Report of the WHO China joint mission on Coronavirus disease 2019 (COVID 19). WHO, 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf [Last accessed on 2021 Feb 01].

2. Rao SN, Manissero D, Steele VR, Pareja J. A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Ther 2020;9:573-86.

3. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, *et al*. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. Emerg Microbes Infect 2020;9:761-70.

4. Bwire GM, Majigo MV, Njiro BJ, Mawazo A. Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: A systematic review and meta-analysis. J Med Virol 2021;93:719-25.

5. Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, *et al*. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177-9.

6. Meyerowitz EA, Richterman A, Gandhi RT, Sax PE. Transmission of SARS-CoV-2: A review of viral, host, and environmental factors. Ann Intern Med 2021;174:69-79.

7. Sungnak W, Huang N, Bécavin C, Berg M, Queen R, Litvinukova M, *et al*. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune *gene*s. Nat Med 2020;26:681-7.

8. Frank S, Brown SM, Capriotti JA, Westover JB, Pelletier JS, Tessema B. *In vitro* efficacy of a povidone-iodine nasal antiseptic for rapid inactivation of SARS-CoV-2. JAMA Otolaryngol-Head Neck Surg 2021;146:1054-8.

9. McDonnell GE. Antisepsis, Disinfection, and Sterilization. Washington DC: ASM Press; 2017.

10. Eggers M, Eickmann M, Zorn J. Rapid and effective virucidal activity of povidone-iodine products against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Modified Vaccinia Virus Ankara (MVA). Infect Dis Ther 2015;4:491-501.

11. Kariwa H, Fujii N, Takashima I. Inactivation of SARS coronavirus by means of povidone-iodine, physical conditions and chemical reagents. Dermatology 2006;212(Suppl 1):119-23.

12. Anderson DE, Sivalingam V, Kang AE, Ananthanarayanan A, Arumugam H, Jenkins TM, *et al*. Povidone-iodine demonstrates rapid *in vitro* virucidal activity against SARS-CoV-2, The virus causing COVID-19 disease. Infect Dis Ther 2020;9:669-75.

13. Government of India, Ministry of Health & Family Welfare, Directorate General of Health Services, EMR Division. Guidance document on appropriate management of suspect/confirmed cases of COVID-19. GoI, 2020. Available from: https://www.mohfw.gov.in/pdf/FinalGuidanceon Managementof Covidcases version2.pdf. [Last accessed on 2020Dec 20].

14. Lee S, Kim T, Lee E, Lee C, Kim H, Rhee H, *et al*. Clinical course and molecular viral shedding among asymptomatic and symptomatic patients with SARS-CoV-2 infection in a community treatment center in the Republic of Korea. JAMA Intern Med 2020;180:1447.

15. Hu R, Jiang Z, Gao H, Huang D, Jiang D, Chen F, *et al*.

Recurrent positive reverse transcriptase–polymerase chain reaction results for coronavirus disease 2019 in patients discharged from a hospital in China. JAMA Netw Open 2020;3:e2010475.

16. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction–based SARS-CoV-2 tests by time since exposure. Ann Intern Med 2020;173:262-7.

17. Xiao AT, Tong YX, Gao C, Zhu L, Zhang YJ, Zhang S. Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: A descriptive study. J Clin Virol 2020;127:104346.

18. Tahamtan A, Ardebili A. Real-time RT-PCR in COVID-19 detection: Issues affecting the results. Expert Rev Mol Diagn 2020;20:453-4.

19. Kim S-M, Hwang YJ, Kwak Y. Prolonged SARS-CoV-2 detection and reversed RT-PCR results in mild or asymptomatic patients. Infect Dis (Lond) 2021;53:31-7.

20. Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, *et al.* Positive RT-PCR test results in patients recovered from COVID-19. JAMA 2020;323:1502-3.

21. Patel MR, Carroll D, Ussery E, Whitham H, Elkins CA, Noble-Wang J, *et al.* Performance of oropharyngeal swab testing compared with nasopharyngeal swab testing for diagnosis of coronavirus disease 2019—United States, January 2020–February 2020. Clin Infect Dis 2021;72:403-10.

22. Lin C, Xiang J, Yan M, Li H, Huang S, Shen C. Comparison of throat swabs and sputum specimens for viral nucleic acid detection in 52 cases of novel coronavirus (SARS-Cov-2)-infected pneumonia (COVID-19). Clin Chem Lab Med 2020;58:1089-94.

23. Yang Y, Yang M, Yuan J, Wang F, Wang Z, Li J, *et al.* Laboratory diagnosis and monitoring the viral shedding of SARS-CoV-2 infection. The innovation. 2020;1(3):100061.

24. Liu M, Li Q, Zhou J, Ai W, Zheng X, Zeng J, *et al.* Value of swab types and collection time on SARS-COV-2 detection using RT-PCR assay. J Virol Methods 2020;286:113974.

25. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020;20:411-2.

26. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, *et al.* Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis 2020;71:793-8.

27. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, *et al.* Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: Retrospective cohort study. BMJ 2020;369:m1443.

28. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, *et al.* Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. N Engl J Med 2020;382:2081-90.

29. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, *et al.* Saliva is a reliable tool to detect SARS-CoV-2. J Infect 2020;81:e45-50.

30. Salvatore PP, Dawson P, Wadhwa A, Rabold EM, Buono S, Dietrich EA, *et al.* Epidemiological correlates of PCR cycle threshold values in the detection of SARS-CoV-2. Clin Infect Dis 2021;72:e761-7.

31. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet Lond Engl 2020;395:497-506.

32. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, *et al.* Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020;382:1564-7.

33. Khan MM, Parab SR, Paranjape M. Repurposing 0.5% povidone iodine solution in otorhinolaryngology practice in Covid-19 pandemic. Am J Otolaryngol 2020;41:102618.

34. Barreto R, Barrois B, Lambert J, Malhotra-Kumar S, Santos-Fernandes V, Monstrey S. Addressing the challenges in antisepsis: Focus on povidone iodine. Int J Antimicrob Agents 2020;56:106064.

35. Kovacikova L, Kunovsky P, Skrak P, Hraska V, Kostalova L, Tomeckova E. Thyroid hormone metabolism in pediatric cardiac patients treated by continuous povidone–iodine irrigation for deep sternal wound infection. Eur J Cardiothorac Surg 2020;21:1037-41.

36. Yeginsu A, Karamustafaoglu A, Ozugurlu F, Etikan I. Iodopovidone pleurodesis does not effect thyroid function in normal adults. Interact Cardiovasc Thorac Surg 2007;6:563-4.

37. Mady LJ, Kubik MW, Baddour K, Snyderman CH, Rowan NR. Consideration of povidone-iodine as a public health intervention for COVID-19: Utilization as "Personal Protective Equipment" for frontline providers exposed in high-risk head and neck and skull base oncology care. Oral Oncol 2020;105:104724.

38. Dexter F, Parra MC, Brown JR, Loftus RW. Perioperative COVID-19 defense: An evidence-based approach for optimization of infection control and operating room management. Anesth Analg 2020;131:37-42.

39. Parhar HS, Tasche K, Brody RM, Weinstein GS, O'Malley BW, Shanti RM, *et al.* Topical preparations to reduce SARS-CoV-2 aerosolization in head and neck mucosal surgery. Head Neck 2020;42:1268-72.

40. Tessema B, Frank S, Bidra A. SARS-CoV-2 viral inactivation using low dose povidone-iodine oral rinse—immediate application for the prosthodontic practice. J Prosthodont 2020;29:459.