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Buccal swabs as non-invasive specimens for detection of severe acute respiratory syndrome coronavirus-2

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Abstract

Introduction: The current gold standard for detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA involves subjecting nasopharyngeal or oropharyngeal swabs to reverse transcription quantitative PCR (RT-qPCR). However, both sample types need to be collected by trained professionals. Using self-collected buccal swabs as an alternative could simplify and accelerate diagnosis of coronavirus disease 2019 (COVID-19).

Objective: To assess self-collected buccal swab samples as an alternative method for SARS-CoV-2 detection in patients with COVID-19.

Methods: Buccal swab samples were self-collected by 73 patients with COVID-19. Total RNA was extracted using Qiagen kits. RNA encoding the SARS-CoV-2 Env protein and human RNase P as an internal control was amplified using the TRUPCR® SARS-CoV-2 RT-qPCR kit version 2.1 and a Bio-Rad CFX96 Real-Time Detection System.

Result: The sensitivity of RT-qPCR from buccal swabs was 58.9% (43/73; 95% confidence interval [CI] 46.77%–70.27%) and that of RT-qPCR from saliva was 62.90% (39/62; 95% CI

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49.69%–74.84%) taking positive SARS-CoV-2 RT-qPCR from nasopharyngeal swabs as the gold standard.

Conclusion: Self-collected buccal swabs are promising alternatives to nasopharyngeal or oropharyngeal swabs for SARS CoV-2 detection.

Keywords

Oral health, saliva, nasopharyngeal, diagnostics, virus, severe acute respiratory syndrome coronavirus-2, coronavirus disease 2019

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Introduction

With the coronavirus disease 2019 (COVID-19) pandemic firmly and palpably in our midst, there is a very real need for strategies to mitigate the spread of the causative virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV-2 can be recovered from a variety of body fluids.[1] Critically, recovery of the virus from saliva forms the basis for most testing strategies.[2] Nasopharyngeal and oropharyngeal swab (NPS) sampling remain the gold standard for testing.[3] These methods have established track records for the detection of respiratory viruses and have been widely used in pandemic situations.[4]

NPS sampling has unique challenges, especially in the backdrop of the public health emergency we are faced with today. These techniques necessitate proximity between healthcare workers and a potentially infected patient, posing a risk of infection.[5] In addition, NPS sampling induces discomfort and can stimulate the gag reflex in patients undergoing sampling. This can affect compliance and carries a risk of aerosol generation.[6]

Apart from the risks posed to healthcare workers, NPS sampling also requires the use of personal protective equipment (PPE), another critical resource in short supply. In addition, the specific 6-inch long swab required is likely to face shortages as the virus spreads and increased testing capacity is required. Not all patients are good candidates for NPS sampling. For instance, it can be difficult to obtain samples using NPS sampling from children as well as adults with conditions such as trismus, ankylosis of the temporomandibular joint, severe nasal septum deviations, or nasal septum spurs.[7]

In light of these challenges, suitable alternative sampling methods to NPS that can provide comparable diagnostic capacity are urgently needed. The present study was carried out to assess the utility of self-collected buccal swab sampling for diagnosis of SARS CoV-2. We also compared the sensitivity of buccal swab sampling to that of saliva sampling.

Methods

Patients

Following ethical approval from the Institutional Ethics Committee of Postgraduate Institute of Medical Education and Research, Chandigarh, India (Approval No.: INT/IEC/2020/SPL-1542), verbal informed consent was

obtained from all participants. Patients with suspected COVID-19 were recruited from the Communicable Diseases Ward of the Postgraduate Institute of Medical Education and Research, Chandigarh, India, between 7 June 2020 and 25 July 2020. The present study conformed to the STROBE guidelines. Patients were recruited using convenience sampling. Parallel sampling using NPS, buccal swabs, and saliva for SARS-CoV-2 detection was performed for all patients. Samples were stored at the Department of Virology, PGIMER, Chandigarh, India. Reverse transcription quantitative PCR (RT-qPCR) for SARS-CoV-2 detection was first performed only for NPS samples. For NPS samples that were RT-qPCR positive, buccal swabs and saliva samples were also tested by RT-qPCR. Because a convenience sampling methodology was employed initially, the sample size was justified using a post-hoc power analysis.

Data collection. Patient demographic data were recorded.

Sample collection. Buccal swabs were self-collected by patients using sterile nylon tipped swabs. Patients were asked to place the swab in between the forefinger and thumb and insert the flocked swab into one side of the mouth between the cheek and the upper gum. Patients maintained the same precautions as those used for saliva collection. The swab was pressed firmly and twirled against the inside of the cheek using an up and down motion from front to back and back to front. The patients were asked to avoid excess saturation of the swab with saliva. The samples were then sealed in a vial containing viral transport medium (VTM) (HiViral^{TM} HiMedia Laboratories Pvt. Limited, Nashik, India). Saliva collection was accomplished by expectoration of 0.5 to 1 mL of unstimulated whole saliva into sterilized sputum containers.[8]

Samples were transported on ice in triple layer packaging to the Department of Virology, PGIMER, Chandigarh, India for subsequent processing and detection of SARS CoV-2 RNA by RT-qPCR.

RNA extraction and RT-qPCR. Sample processing was performed in a class II A2 biosafety cabinet following all safety precautions including wearing of PPE. RNA was extracted from 140 µL of each sample using RNEasy Mini Kits (Qiagen, Hilden Germany) and eluted in 30 µL of 10 mM Tris containing 1 mM ethylenediaminetetraacetic acid.

The eluted RNA was tested for SARS-CoV-2 using the TRUPCR® SARS-CoV-2 RT-PCR kit version 2.1 (3B BlackBio Biotech, Bhopal, India) per the manufacturer's instructions. The kit targets the SARS-CoV-2 *E* gene and uses the human *RNase P gene* as an internal control. RT-qPCR was performed using the CFX96 Real-Time Detection System (Bio-Rad, Hercules, CA, USA) RT. When the cycle threshold (Ct) values of both target genes were >38, the results were deemed negative. When the Ct values of both target genes were ≤38, the results were considered positive. When one target gene had a Ct value of ≤38, while the other had a higher Ct value (>38), the sample was retested. Samples showing repeated discrepancies were considered negative.

Statistical analysis

Descriptive and inferential statistical analyses were performed using IBM SPSS version 23 (IBM Corporation, Armonk, NY, USA). Because data were normally distributed, parametric tests (e.g., Kolmogorov–Smirnov test, Shapiro–Wilk test) could be used. Inferential statistics, such as the independent sample Student's t test and Pearson

correlation analysis, were used. Because sample size was not calculated *a priori*, G* power version 3.1 (HHU Dusseldorf, Dusseldorf, Germany) was used to conduct a post-hoc power analysis. Provided an alpha value of 5%, the post-hoc power analysis of the study results revealed a minimum power of 82% and a maximum of 100%.

Results

The demographic and disease course data for all 73 patients are presented in Table 1. Patients ranged in age from 13 to 82 years (mean 37.7 years, standard deviation 15.8 years). The ages of symptomatic patients ranged from 23 to 65 years, and those of asymptomatic patients ranged from 13 to 82 years. The male:female sex ratio was 1.2:1.

Fifty-six (76.7%) patients had no pre-existing medical conditions. The 17 patients who reported past medical histories had conditions such as diabetes (8), epilepsy (1), hypothyroidism (1), hypertension (9), coronary artery disease (2), and systemic lupus erythematosus (1). Three patients were obese.

RT-qPCR results from NPS samples was used as the gold standard to gauge the diagnostic capability of RT-qPCR from buccal and saliva samples. We included both asymptomatic (n=59) and symptomatic (n=14) patients whose NPS samples were positive for SARS-CoV-2 by RT-qPCR in our study. During sample processing we observed that the quantity of saliva from 11 patients was insufficient. These samples were not analyzed further. Of 73 buccal swabs, 43 (58.90%) were positive for SARS-CoV-2 by RT-qPCR and of 62 saliva samples, 39 (62.90%) were positive for SARS-CoV-2 by RT-qPCR.

In the 30 patients whose buccal swabs were negative, 10 had positive saliva samples. Conversely, buccal swabs tested positive for 6 of the 23 patients in whom saliva was negative by RT-qPCR. The mean Ct values of NPS, buccal swabs, and saliva samples were 24.92 ± 6.51, 31.06 ± 4.09, and 28.16 ± 4.48, respectively (Table 2, Figure 1). Statistically significant differences were observed between the Ct values for different sample types. A slight positive correlation was observed between NPS and buccal swab Ct values ($r = 0.342$; $p = 0.025$) and between NPS and saliva Ct values ($r = 0.359$; $p = 0.025$). A moderate positive correlation was observed between buccal swab and saliva Ct values ($r=0.464$; $p=0.011$). Sampling by NPS was found to be most sensitive, followed by saliva, with buccal swabs being the least sensitive.

Although our study was cross-sectional in design, we attempted to correlate Ct values with the day of illness. Using the available data, we found that Ct values were inversely proportional to the day of illness (Table 1). This result pointed to a waning of viral shedding and could potentially explain why SARS-CoV-2 was not detected in three buccal swab samples and one saliva sample collected on days 4 and 5.

Discussion

RT-qPCR of NPS samples is generally regarded as the gold standard for detection of SARS-CoV-2. In addition to putting healthcare workers at risk of infection and necessitating the use of PPE, this method of sample collection can also be uncomfortable for patients. NPS sampling can also induce cough, sneezing, or gag reflex during sample collection. NPS does not have any specific contraindications; however, sampling can be problematic in patients who have undergone recent nasal surgery or trauma, have a coagulopathy such as thrombocytopenia, have a history of chronic nasal passage blockage, or have a severely deviated nasal septum.[9] Other patient

Table 1. Demographics and disease course of study participants.

Symptomatic/ asymptomatic	Age (years)	Sex	Underlying systemic condition	Symptoms	Day of illness	*E* gene Ct value		
						NPS	Buccal swab	Saliva
Symptomatic	25	M	CAD	Fever, cough	2	16	27.83	IS
	27	M		Patient with ILI	2	17.7	28.75	17.23
	40	M		Cough, breathlessness	2	17.5	37.72	36.65
	23	M		Cough, sore throat	2	14.1	31.67	33.22
	64	M	Diabetes	Sore throat	2	18.4	27.06	22.63
	31	M		Fever, cough, sore throat, diarrhea	2	19.5	28.01	25.69
	64	M	Diabetes, hypertension	Fever, sore throat	2	18.5	35.55	26.6
	25	F		Cough, vomiting	2	18	31.8	26.65
	65	F	Hypertension	Fever, cough	3	17	36.56	27.39
	52	M	Diabetes, hypertension,	Fever, cough, sore throat, body ache	3	17.2	35.27	26.32
	58	F		Fever, cough, body ache, breathlessness	3	28.98	UD	UD
	28	M		Fever, vomiting, sore throat, cough	4	24	UD	33.5
	29	M		Fever, cough, nasal discharge, sore throat, body ache	4	27	UD	UD
	31	F		Sore throat	5	34.8	UD	33.92
Asymptomatic	36	M				25.08	31.24	IS
	13	F				16.7	28.43	32.09
	40	F				15.32	31.29	28.06
	20	M				38.3	UD	UD
	26	M				25.4	UD	28.58
	20	F				34.4	31.61	27.02
	31	M	Epilepsy			24.4	UD	26.71
	16	F				27.96	UD	IS
	24	M				37	UD	UD
	22	F				29	UD	29.01

(continued)

Table 1. Continued.

Symptomatic/ asymptomatic	Age (years)	Sex	Underlying systemic condition	Symptoms	Day of illness	*E* gene Ct value		
						NPS	Buccal swab	Saliva
	20	F				31.54	UD	UD
	25	F				25	33.43	IS
	27	M				39.9	UD	UD
	22	M				22.2	26.27	30.22
	41	M				25	32.30	IS
	32	F				22.2	UD	27.9
	17	F				23.5	UD	26.17
	82	F	Hypertension			22.9	30.64	30.26
	40	M				16.7	24.96	IS
	24	F	Morbidly obese			25.96	31.41	UD
	24	M				32.5	UD	UD
	60	M				32	UD	33.75
	30	F				26.99	31.78	UD
	52	F				34.4	UD	UD
	29	F				19.4	24.8	29.34
	42	M				22.6	23.46	16.38
	18	F				21.4	32.27	28.58
	64	F	Diabetes, hypertension, SLE			22.9	28.84	26.81
	54	F				29.35	UD	IS
	28	F				28.0	UD	UD
	29	F				21	32.34	24.99
	53	M				24	30	IS
	24	F				29	33	UD
	34	F				24	31.6	UD
	37	F				25	UD	IS
	42	F				33	UD	UD
	34	M				36	UD	33.66
	15	M				33	UD	UD

(continued)

Table 1. Continued.

Symptomatic/ asymptomatic	Age (years)	Sex	Underlying systemic condition	Symptoms	Day of illness	*E* gene Ct value		
						NPS	Buccal swab	Saliva
	57	M	Diabetes			28.6	36.31	31.43
	51	F	Hypertension			16.7	UD	33.66
	53	M	Hypertension			25.6	35.61	28.8
	56	M				17	35.24	UD
	25	M				19.9	27.81	24.62
	21	M				22.06	36.7	28.7
	48	F				24.7	UD	UD
	59	M	Diabetes			32.7	UD	UD
	57	M	Obese			31.6	UD	UD
	35	F				28.98	31.15	UD
	45	F	Hypothyroidism, morbidly obese			26.1	UD	UD
	62	M				33.2	UD	UD
	56	F	Diabetes, hypertension			15	31.83	22.49
	38	M				22.15	32.63	25.05
	37	M				32	UD	UD
	65	M	Diabetes, hypertension, CAD			26.8	31.84	IS
	32	M				16.4	27.30	22.48
	34	M				31.1	37.56	35.02
	33	F				12.1	17.54	25.29
	28	M				22.9	34.2	IS
	52	F				21.8	30.14	31.35

Ct: Cycle threshold; UD: Undetected; IS: Insufficient sample; M: Male; F: Female; NPS: Nasopharyngeal swab; CAD: Coronary artery disease; SLE: Systemic lupus erythematosus; ILI: influenza-like illness.

Table 2. RT-qPCR Ct values for different sample types.

Comparison	Sample	N	Range of Ct values for *E* gene	Median Ct value	Mean ± standard deviation t value	p-value	Correlation coefficient
NPS vs. buccal swab	NPS	43	12.10–34.40	21.8	21.51 ± 4.96	0.001*	0.342 (p = 0.025)
	Buccal swab	43	17.54–37.72	31.61	31.06 ± 4.09		
Buccal swab vs. saliva	Buccal swab	29	17.54–37.72	31.61	30.88 ± 4.68	0.004*	0.464 (p = 0.011)
	Saliva	29	16.38–36.65	27.02	27.29 ± 4.56		
NPS vs. saliva	NPS	39	12.10–36.0	21.8	22.04 ± 5.88	0.001*	0.359 (p = 0.025)
	Saliva	39	16.38–36.65	28.06	28.16 ± 4.48		

N: number of positive samples common to both groups, Ct: Cycle threshold; NPS: Nasopharyngeal swab; RT-qPCR: reverse transcription quantitative polymerase chain reaction; *: statistically significant

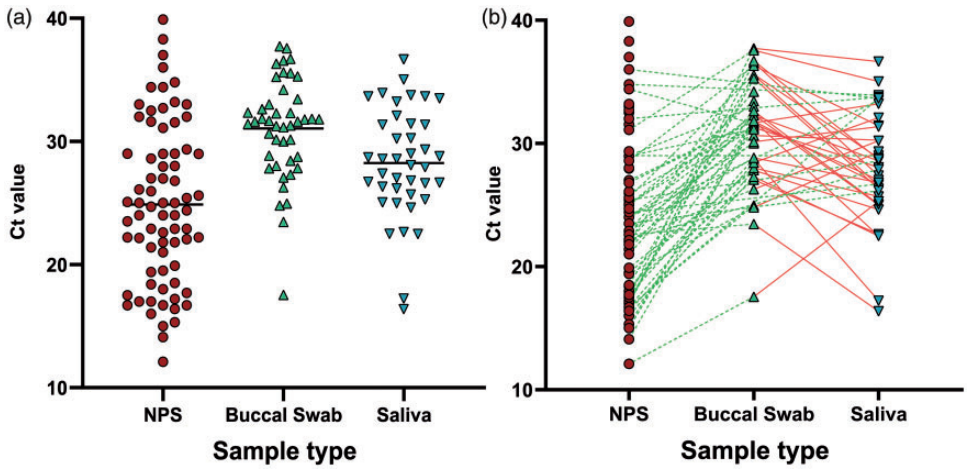

Figure 1. RT-qPCR Ct value comparison for different sample types. (a) A comparison of total positive NPS (n = 73), buccal swabs (n = 43), and saliva samples (n = 39) was conducted. The horizontal line represents the mean Ct value in every group. (b) Connecting lines denote patient matched samples. The green lines represent NPS pairing with buccal swabs or saliva samples while the red lines denote pairing of buccal swab and saliva samples.
NPS, nasopharyngeal swab; Ct, cycle threshold; RT-qPCR: reverse transcription quantitative polymerase chain reaction.

groups such as children and adults with dementia, severe learning disabilities, trismus, or ankylosis of the temporomandibular joint can also not be amenable to NPS sampling.[10]

The present study aimed to determine the utility of non-invasive self-collected buccal and saliva samples for detection of SARS-CoV-2 by RT-qPCR. The use of buccal and saliva samples in diagnostic applications is not new and there is evidence in the literature to justify their potential value in the diagnosis of SARS-CoV-2. Das et al. (2014) and Mesman et al. (2019) used buccal swabs to detect *Leishmaniasis donovani* infection and *Mycobacterium*

tuberculosis.[11,12] Saliva samples also have adequate evidence in the literature for diagnosis of viral infections. Saliva-based bioassays for Zika and Ebola viruses are well established.[13] Other sampling methodologies based on gargles and gingival crevicular fluid have also been reported.[14,15]

Recent studies have reported encouraging findings pertaining to the positivity of both buccal and saliva samples for the detection of SARS-CoV-2.[16,17] One study suggested that buccal swabs were a more tolerable alternative to NPS sampling in pediatric patients with COVID-19. This study had a small sample size and revealed higher Ct values in buccal swabs compared with NPS samples.[16] The present study enrolled patients of a wider age range to include a substantial number of adults. Our data agree with those of previous studies in terms of Ct values and sensitivity of RT-qPCR using buccal swabs. We also found that virus could generally be detected up until the second or third day following the onset of symptoms in symptomatic cases, in agreement with prior studies.

There is evidence to support the shedding and recovery of SARS-CoV-2 in saliva, justifying its use as a sampling methodology.[18,19] There is a gap in the current literature: no direct comparisons have evaluated the specificity and sensitivity of NPS sampling in comparison with buccal swab or saliva sampling. The results of the present study confirmed the feasibility of detecting SARS-CoV-2 in both buccal swab and saliva samples. Compared with NPS sampling, the sensitivity of buccal swabs and saliva samples was 58.90% and 62.90%, respectively. Differences in Ct values among buccal and saliva samples could be attributed in part to differences in sampling methodologies: the buccal swabs were transported in VTM whereas the saliva samples remained undiluted.

The oral cavity has been recognized as a particular region of interest for SARS-CoV-2, with rich expression of angiotensin converting enzyme 2 (ACE2) in different parts of the mouth including the dorsal tongue, gingiva, and buccal mucosa. These regions can be used by the virus as points of entry and can also facilitate viral shedding.[20] The higher expression of ACE2 in salivary glands, and especially the minor salivary glands, compared with the lungs means these glands may be potential reservoirs of SARS-CoV-2. This could explain the detection of the virus in saliva long before the manifestations of pulmonary lesions and provide a plausible explanation for the many asymptomatic infections in some patient groups.[18,20] Our findings justify the exploration of buccal and salivary swabs as potential diagnostic methodologies for SARS-CoV-2 in addition to their obvious practical advantages over NPS sampling.

We observed lower sensitivities for both buccal swab and saliva samples compared with NPS specimens. Both buccal swab and saliva samples had distinct diagnostic performance from NPS samples for high Ct value specimens. Thus, these non-invasive diagnostic modalities may not be suitable for the screening of high Ct value samples. One potential explanation of these data could be low viral spread from primary infected sites, such as the nose, pharynx, and throat, to the saliva and buccal mucosa in these cases. One limitation of the present study was that the buccal and salivary samples were obtained randomly at no defined time points, which could have increased the variability of our results. Future studies should obtain samples at clearly defined time-points to maximize homogeneity in assessment.

Conclusion

Compared with NPS sampling, the sensitivity of RT-qPCR from buccal swabs and saliva was 58.90% and 62.90%,

respectively. The oral cavity and its fluids are of high relevance to the pathophysiology of SARS-CoV-2. Although NPS sampling remains the gold standard , it must be recognized that there are specific patient groups that are not amenable this procedure. These patients require diagnostic testing as well and can benefit from alternative testing modalities such as saliva and buccal swab sampling. Recognition of the efficacy of these methods along with acknowledgement of their limitations may allow them to be repurposed for patient groups not appropriate for NPS sampling.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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Author contributions

Dr. Ritu Gaur: contributed to interpretation of data and critically revised the manuscript
Dr. Dipesh K. Verma: contributed to interpretation of data and critically revised the manuscript
Dr. Ritin Mohindra: contributed to study design, acquisition of data and critically revised the manuscript
Dr. Kapil Goyal: contributed to study design, analysis, and interpretation and critically revised the manuscript
Dr. Shipra Gupta: contributed to conception and design, data acquisition, analysis, and interpretation, and drafted the manuscript
Dr. Vidhi Singla: contributed to data acquisition and critically revised the manuscript
Dr. Vaibhav Sahni: contributed to study design, data analysis and interpretation, and drafted the manuscript
Dr. Arnab Ghosh: contributed to data acquisition and critically revised the manuscript
Mr. RK Soni: contributed to data acquisition and critically revised the manuscript
Dr. Ashish Bhalla: contributed to data acquisition and critically revised the manuscript
Dr. Krishan Gauba: contributed to data acquisition and critically revised the manuscript
Dr. Mini P. Singh: contributed to study conception and design, data analysis and interpretation, and critically revised manuscript
All authors gave final approval and agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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