Short Communication

Detection of SARS-CoV-2 RNA Using RT-qPCR in Saliva Samples and Nasopharyngeal, Lingual, and Buccal Mucosal Swabs

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ABSTRACT: Coronavirus disease 2019 is diagnosed based on the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in nasopharyngeal swabs or saliva samples using reverse-transcription quantitative polymerase chain reaction. Nasopharyngeal swabs should be collected by medical professionals who are covered with full personal protective equipment (PPE), while saliva samples can be collected by patients themselves without any PPE. However, collecting saliva is difficult for people who are unable to follow instructions, including infants or unconscious patients. Owing to the high viscosity of saliva, special attention is required to handle saliva samples in laboratories. To solve these problems, we compared lingual and buccal mucosal swabs (oral swabs) with nasopharyngeal swabs and saliva samples. Among 13 patients who had a positive result for SARS-CoV-2 RNA in their nasopharyngeal swabs, 8 and 10 patients had a positive result for SARS-CoV-2 RNA in their saliva (concordance rate, 61.5%) and oral swabs (76.9%), respectively. Among the eight patients with a positive result for SARS-CoV-2 RNA in saliva, seven (87.5%) had SARS-CoV-2 detected in their oral swabs. We could not obtain saliva samples from four patients, but we found perfect concordance of SARS-CoV-2 positivity between the nasopharyngeal and oral swabs. Therefore, oral swabs can be used for SARS-CoV-2 RNA detection.

Pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China, by the end of December 2019 (1,2). Since then, it has rapidly spread worldwide. Currently, coronavirus disease 2019 (COVID-19) is diagnosed based on SARS-CoV-2 RNA detection in nasopharyngeal swabs or saliva samples using reverse transcription quantitative polymerase chain reaction (RT-qPCR) (3,4). Nasopharyngeal swabs should be collected by medical professionals who are covered with full personal protective equipment (PPE) in an isolated room to avoid contact with airborne droplets generated during sneezing while sampling. In contrast, saliva can be collected easily by patients themselves, without the need for PPE and special techniques, which is advantageous when compared with collecting nasopharyngeal swabs. However, collecting saliva

is difficult for people who are unable to follow the instructions given by medical staff, including infants, patients with motility disorders or dementia, and unconscious patients. The preparation of PCR samples from saliva in the laboratory is more challenging because of the viscosity of saliva was higher than that of nasal swabs. To solve these problems, we attempted to detect SARS-CoV-2 RNA using lingual and buccal mucosal swabs (oral swabs) and compared them with saliva samples and nasopharyngeal swabs.

Saliva samples and nasopharyngeal, lingual, and buccal mucosal swabs were simultaneously collected from each patient diagnosed with COVID-19 at Ashigarakami Hospital (10 patients) and University of Yamanashi Hospital (8 patients). This study was approved by the Institutional Ethics Board, and written informed consent was obtained from all patients. All COVID-19 patients had mild-to-moderate disease, with no patient requiring ventilator use. The median age of the study patients with COVID-19 was 44.5 years (range, 22–67 years), and the median number of days for sampling was 5.4 days (range, 0–12 days) after symptom onset. Nasopharyngeal samples were collected by passing the swabs through the nostril until they reached the posterior nasopharynx and rotating them before removal. Lingual and buccal mucosal

Received February 15, 2021. Accepted June 22, 2021.

J-STAGE Advance Publication July 30, 2021.

DOI: 10.7883/yoken.JJID.2021.091

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Table 1. Results of SARS-CoV-2 RT-qPCR using nCoV-DK kit in nasopharyngeal swab, saliva, lingual, and buccal mucosal swab

nCoV-DK kit (N = 14)		Nasopharyngeal swab	
		Positive	Negative
Saliva	Positive	8 (61.5%)	0
	Negative	5	1
Oral swab	Positive	10 (76.9%)	0
	Negative	3	1
	Lingual swab	Positive	9 (69.2%)
	Negative	4	1
Buccal mucosal swab	Positive	8 (61.5%)	0
	Negative	5	1
Total		13	1

The percentage in parentheses indicates concordance rate for nasopharyngeal swab.

samples were collected by scraping the tongue and buccal mucosa, respectively, using swabs. The swab sample was suspended in a tube predisposed with 1 mL of a virus-transporting medium. In contrast, saliva was collected by the patients themselves, wherein the saliva was collected into a sterile 15-mL conical tube using a thick plastic straw.

We performed RT-qPCR using two methods: 2019 Novel Coronavirus Detection Kit (nCoV-DK; Shimadzu Corporation, Kyoto, Japan) and conventional direct PCR. nCoV-DK eliminates the steps required for RNA extraction and purification using Ampdirect technology (5). The swab or saliva sample was dissolved in an equal volume of sample buffer, and the dissolved solution was heated at 90°C for 5 min. These samples were directly used for the RT-qPCR assay. The primer sets used for nCoV-DK were 2019-nCoV N1 and 2019-nCoV N2, which detect a part of the nucleocapsid genes recommended by the US Centers for Disease Control and Prevention. For conventional direct PCR, viral RNA was extracted from clinical specimens using magLEAD 6gC (Precision System Science Co., Ltd., Matsudo, Japan). Then, SARS-CoV-2 RNA was detected using AgPath-ID™ One-Step RT-PCR Reagents (AM1005; Applied Biosystems, Tokyo, Japan) (6). The primer sets used for the conventional direct RT-qPCR were N and N2, which detect a part of the nucleocapsid genes recommended by the National Institute of Infectious Diseases in Japan. The sample volume required for one reaction was 5 µL for the nCoV-DK method and 80 µL for the conventional method. The detection sensitivities of both methods were approximately 10 copies per reaction, determined using a common internal standard. In each case, when both the primer set and internal control showed an increase in fluorescence within 40 cycles, the sample was determined to be positive for SARS-CoV-2.

Saliva samples could not be collected in 4 of 18 patients because of the low level of consciousness. We analyzed 14 full-set samples (saliva and nasopharyngeal, lingual, and buccal mucosal swabs) using nCoV-

DK and found that SARS-CoV-2 was detected in 13 nasopharyngeal swabs, among which SARS-CoV-2 was detected in 8 saliva samples (concordance rate, $8/13 = 61.5\%$) and 10 oral swabs (in either lingual or buccal mucosal swabs) (concordance rate, $10/13 = 76.9\%$) (Table 1). Three patients had a positive result for SARS-CoV-2 only in nasopharyngeal swabs but not in saliva or oral swabs. Sampling in two of three patients was performed after more than 12 days since symptom onset. A low concordance rate between the saliva samples and nasopharyngeal swabs after 10 days since symptom onset has been reported previously (4), which was also noted for oral swabs. Notably, no patient showed positive results for SARS-CoV-2 in saliva or oral swabs and negative results in nasopharyngeal swabs, suggesting that nasopharyngeal swabs are the most sensitive samples to detect SARS-CoV-2 RNA.

We performed conventional direct PCR, which involves viral RNA extraction, and compared it with nCoV-DK between the different samples. nCoV-DK showed higher positive rates, regardless of the sample type (data not shown); however, the reason for this remains to be elucidated. We found it challenging to handle saliva samples with high viscosity during RNA extraction, but it was easy to handle oral swabs.

Among the eight patients who tested positive for SARS-CoV-2 in the saliva samples, SARS-CoV-2 was also detected in oral swabs in seven patients (7/8) (Table 2). The overall concordance rate between saliva samples and oral swabs was moderate, as judged by Cohen's kappa coefficient ($\kappa = 0.553$; 95% confidence interval, 0.112–0.994). We could not obtain saliva samples from four patients. For these patients, we found a perfect concordance rate between the nasopharyngeal and oral swabs (Table 3).

Our study has several limitations, including the analysis of a small number of samples and paying more focus on the positive concordance rate. Although a large-scale study is required for verifying and drawing conclusions, it is possible to use oral swabs instead of saliva to detect SARS-CoV-2 RNA. Oral swabs not only

Table 2. Comparison of saliva and oral swab for SARS-CoV-2 qRT-PCR using nCoV-DK kit

nCoV-DK kit (N = 14)		Saliva		Total
		Positive	Negative	
Oral swab	Positive	7 (87.5%)	2	9
	Negative	1	4 (66.7%)	5
Total		8	6	14

The percentage in parentheses indicates concordance rate for oral swab.

Table 3. Results of nasopharyngeal and oral swab from patients who were unable to collect saliva

nCoV-DK kit (N = 4)		Nasopharyngeal swab		Total
		Positive	Negative	
Oral swab	Positive	3 (100%)	0	3
	Negative	0	1 (100%)	1
Total		3	1	4

The percentage in parentheses indicates concordance rate for oral swab.

have the same advantages as those of saliva samples but can also be collected from patients who cannot follow instructions, such as infants and unconscious patients.

Conflict of interest None to declare.

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