Abstract: The outbreak of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide. Nasopharyngeal swabs are widely used in polymerase chain reaction (PCR) test to detect SARS-CoV-2. However, the collection of nasopharyngeal swabs has a series of drawbacks concerning exposure of healthcare staff, difficulty in collection, and discomfort of patients. Therefore, an alternative noninvasive sample for diagnostic of emerging viral diseases is required. The usefulness of saliva screening tests is compared to conventional swab tests in this report. The results suggest that saliva could be a reliable sample for detecting SARS-CoV-2.

Keywords: coronavirus, COVID-19, PCR, saliva, SARS-CoV-2

Introduction

The outbreak of an epidemic of pneumonia emerged in China at the end of December 2019. The World Health Organization (WHO) named the new coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) disease as Corona virus disease 2019 (COVID-19, https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-19)-and-the-virus-that-causes-it). More than five million confirmed cases of COVID-19 have been reported around the world (May 26, 2020, Johns Hopkins University & Medicine Coronavirus Resource Center, https://coronavirus.jhu.edu/). Detection of SARS-CoV-2 is important to prevent rapid infection extension. Nasopharyngeal swabs are widely used in polymerase chain reaction (PCR) test to detect SARS-CoV-2. The collection of nasopharyngeal swabs has a series of drawbacks concerning exposure of healthcare staff, difficulty in collection, and discomfort of patients. Saliva specimens can be obtained noninvasively as the patient spits into a sterile bottle. The saliva collection minimizes the exposure of healthcare staff. Niedrig et al. [1] analyzed the usefulness of alternative noninvasive samples for the diagnostic of emerging viral diseases. They concluded that saliva has the potential to be used as an alternative sample. Saliva is advantageous in terms of ease of collection and safety of healthcare staff. The purpose of this report is to compare the usefulness of saliva screening tests to conventional swab tests. A reference search was conducted up to May 18, 2020 using PubMed.

Comparison of PCR test samples for the screening of COVID-19

Saliva and nasopharyngeal swabs as test samples of PCR are compared in Table 1. Azzi et al. [2] reported that SARS-CoV-2 was detected in all 25 patients’ first and after 4 days saliva swabs. All patients were classified as severe or very severe COVID-19. Saliva was collected using the drooling technique. To et al. [3] also reported that SARS-CoV-2 RNA in saliva of all patients except for three who were intubated, was detected. They performed a cohort study including 23 patients with laboratory-confirmed COVID-19. Ten patients had severe COVID-19, of whom all required oxygen supplementation, and 13 patients had mild disease. An early morning saliva sample from the posterior oropharynx was collected for sampling.

One hundred and twenty-one self-collected saliva or healthcare worker-administered nasopharyngeal swabs of 44 COVID-19 inpatients were investigated by Wylie et al. [4]. Thirty-nine of the saliva samples and 46 of the nasopharyngeal swabs were detected as positive. Eight cases had positive saliva and negative nasopharyngeal samples, and three cases had negative saliva and positive nasopharyngeal samples. The validity of saliva as a sample of screening test of SARS-CoV-2 was also investigated [5]. Overall, 39/622 patients had PCR-positive in nasopharyngeal swabs, and 33 of 39 patients had SARS-CoV-2 detected in saliva. These studies indicate that SARS-CoV-2 is detected from saliva as efficiently as in nasopharyngeal swabs.

On the other hand, SARS-CoV-2 was detected in four saliva samples of 31 COVID-19 patients, with oropharyngeal swabs detecting positive [6]. Three cases with positive detection in saliva were severe patients on ventilator support. Saliva was collected from the opening of the salivary gland canal of the cleaned oral cavity to avoid contamination by other secretions from the respiratory tract. The low positive ratio of this study might be due to the procedure for collecting saliva.

Several studies indicated that saliva samples had higher viral load or lower cycle threshold value compared to nasopharyngeal swab samples [4,5]. The sensitivity of saliva samples in detecting SARS-CoV-2 was also observed. Salivary results of two patients showed positive on the same days when the pharyngeal or respiratory swabs showed conversion [2]. Less variability in the repeated SARS-CoV-2 test with saliva sample was addressed [4].

These results suggest that saliva could be a reliable sample for detecting SARS-CoV-2. Based on laboratory and clinical examinations, PCR kits for salivary samples have already been approved by the Food and Drug Administration (FDA) in the USA and are being made available around the world. Recently, the FDA has granted emergency use authorization (EUA) for a test biomaterial for SARS-CoV-2 with saliva (April 13, 2020, https://www.rutgers.edu/news/new-rutgers-saliva-test-coronavirus-gets-fda-approval). Furthermore, FDA has issued EUA to Rutgers Lab for at-home saliva collection of SARS-CoV-2 test (May 8, 2020, https://www.rutgers.edu/news/fda-approves-first-home-saliva-collection-test-coronavirus).

Kits for detecting SARS-CoV-2 with saliva are shown in Table 2.

Discussion

Retroactively from April 1, 2020, the Bureau of Social Welfare and Public Health, Tokyo Metropolitan Government authorized, the Nippon Dental University Hospital as one of the designated medical facilities, i.e. a hospital for recent arrivals and people who have been exposed to a person with confirmed COVID-19. Although no dental hospitals or dental clinics in Japan can accept COVID-19 patients for medical treatment, this was a pioneering designation for dental hospitals and dental clinics in Japan.

On May 7, 2020, President Yoshitake Yokokura (Japan Medical Association, JMA) gave a press conference and announced that the JMA called
Table 1 Comparison of saliva and nasopharyngeal swab as the PCR test samples for SARS-CoV-2 detection

| Number of patients | Saliva | Nasopharyngeal swabs | Collection method of saliva | Saliva detection rate (%) | Remarks | Reference |
|--------------------|--------|----------------------|-----------------------------|--------------------------|---------|-----------|
| 25                 | 25     | 25                   | Drooling                    | 100                      | There were two patients with positive saliva results on the same day when their pharyngeal or bronchoalveolar swabs proved to be negative. | 2        |
| 23                 | 20     | 23                   | Coughing out                | 87                       | Salivary viral load was higher during the first week after symptom onset and subsequently declined with time. | 3        |
| 38*                | 35     | 30                   | Self-collecting            | 92                       | Twenty-nine were able to simultaneously compare self-collected saliva with nasopharyngeal samples collected by health-care workers (38 samples), but saliva samples also had higher viral load. Eight cases had positive saliva and negative nasopharyngeal samples, and three cases had negative saliva and positive nasopharyngeal samples. | 4        |
| 39                 | 33     | 39                   | Drooling                    | 85                       | The viral load of nasopharyngeal swabs is higher than that of saliva. Both NPS and saliva samples had a positive correlation between the median cycle threshold values and days since onset. | 5        |
| 13**               | 4      | 13**                 | Directly from salivary gland duct | 31                      | For critically-ill patients, saliva has a higher potential for detection of SARS-CoV-2. | 6        |

*Analysis to only patient-matched nasopharyngeal and saliva samples (38 for each sample type). **Saliva and oropharyngeal swabs samples were collected at the same time. In this study, 13 cases tested positive for oropharyngeal swab nucleic acid detection.

Table 2 Saliva-based detection kits for SARS-CoV-2

| Kit for extracting RNA from saliva | Manufacturer | PCR kits | Manufacturer | Reference |
|-----------------------------------|--------------|----------|--------------|-----------|
| QIAamp Viral RNA Mini kit         | Qiagen, Hilden, Germany | Luna Universal qPCR Master Mix | New England Biolabs, Ipswich, MA, USA | 2 |
| EMAG                             | biMérieux, Mucy, France | LightCycler Multiplex RNA Virus Master Kit | Roche Diagnostics, Mannheim, Germany | Cheng VCC et al. Infect Control Hosp Epidemiol 41: 493, 2020. 3 |
| MagMAX                           | Thermo Fisher Scientific, Waltham, MA, USA | LightMix Modular SARS Wuhan CoV E-gene mix | Roche Diagnostics | TIB Molbiol, Berlin, Germany |
| Qiaqen EZ1 platform              | Qiagen, Hilden, Germany | – | – | 4 |
| Commercial test kit instructions | BioGerm, Inc., Shanghai, P. R. China | – | – | 5 |
| MagNA Pure 96 96 NA small volume kit | Roche Diagnostics, Mannheim, Germany | PowerChek 2019-nCoV Real-time PCR Kit | Kogene Biotech, Seoul, Korea | Han MS et al. Clin Infect Dis, Apr 16, doi: 10.1093/cid/ciaa447, 2020. |
| MagNA Pure 96                    | Roche, Basel, Switzerland | A China Food and Drug Administration approved commercial kit specific for SARS-CoV-2 detection | Bioie, Shanghai, China | Zheng S et al. BMJ, Apr 21, doi: 10.1136/bmj.m1443, 2020. |
| Thermo Kingfisher platform       | Fisher Scientific, Hampton, NH, USA | – | – | 6 |
| –                                | – | SARS-CoV-2 Direct Detection RT-qPCR Kit* | Takara Bio Inc., Kusatsu, Japan |
| –                                | – | 2019 Novel Coronavirus Detection Kit* | Shimadzu Corp., Kyoto, Japan |
| –                                | – | SARS-CoV-2 Detection Kit* | Toyobo Co., Ltd., Osaka, Japan |

*Tuberculosis Infectious Disease Division, Health Bureau, Ministry of Health, Labour and Welfare, Japan, 0602-1, June 2, 2020.

upon the Japanese Government to approve the PCR test with saliva (17:32, May 7, 2020, TBS News, Tokyo, Japan). President Yokokura emphasized that salivary samples, already approved in the USA, would be obtained from patients more easily and safely as compared with nasopharyngeal samples.

The Ministry of Health, Labour and Welfare, Japan approved on (June 2, 2020) PCR examinations using saliva through fast-track approval. As a result, the insurance medical fee payment funds in Japan, including the Health Insurance Claims Review & Reimbursement Services, approved payment for the PCR test screening COVID-19 with saliva samples from June 2020. Several hospitals with dental clinic in Japan are currently performing pre-clinical COVID-19 PCR tests by means of a saliva-based detection kit.

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Conflict of interest
The authors have no conflict of interest to declare.

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