Clinical Infectious Diseases

BRIEF REPORT

Consistent Detection of 2019 Novel Coronavirus in Saliva

Kelvin Kai-Wang To,[1,2,3,a] Owen Tak-Yin Tsang,[4,a] Cyril Chik-Yan Yip,[1,2,a] Kwok-Hung Chan,[1,a] Tak-Chiu Wu,[5] Jacky Man-Chun Chan,[4] Wai-Shing Leung,[4] Thomas Shiu-Hong Chik,[4] Chris Yau-Chung Choi,[4] Darshana H. Kandamby,[6] David Christopher Lung,[7] Anthony Raymond Tam,[8] Rosana Wing-Shan Poon,[1,2] Agnes Yim-Fong Fung,[1] Ivan Fan-Ngai Hung,[8] Vincent Chi-Chung Cheng,[1,2] Jasper Fuk-Woo Chan,[1,2,3] and Kwok-Yung Yuen[1,2,3]

[1]State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology, Carol Yu Centre for Infection, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China, [2]Department of Microbiology, Queen Mary Hospital, Hong Kong Special Administrative Region, China, [3]Department of Clinical Microbiology and Infection Control, The University of Hong Kong–Shenzhen Hospital, Shenzhen, China, [4]Department of Medicine and Geriatrics, Princess Margaret Hospital, Hong Kong Special Administrative Region, China, [5]Department of Medicine, Queen Elizabeth Hospital, Hong Kong Special Administrative Region, China, [6]Intensive Care Unit, Princess Margaret Hospital, Hong Kong Special Administrative Region, China, [7]Department of Pathology, Queen Elizabeth Hospital, Hong Kong Special Administrative Region, China, and [8]Department of Medicine, Queen Mary Hospital, Hong Kong Special Administrative Region, China

The 2019 novel coronavirus (2019-nCoV) was detected in the self-collected saliva of 91.7% (11/12) of patients. Serial saliva viral load monitoring generally showed a declining trend. Live virus was detected in saliva by viral culture. Saliva is a promising noninvasive specimen for diagnosis, monitoring, and infection control in patients with 2019-nCoV infection.

Keywords. 2019 novel coronavirus; saliva; diagnostics; viral load; transmission; COVID-19.

In 2003, the severe acute respiratory syndrome coronavirus (SARS-CoV) caused a devastating global outbreak with a case-fatality rate of 10% [1]. In December 2019, a SARS-CoV–like coronavirus, the 2019 novel coronavirus (2019-nCoV), has emerged in Hubei Province of China and has spread rapidly in mainland China and to other parts of the world [2, 3]. The 2019-nCoV belongs to *Betacoronavirus* genus lineage B, and is phylogenetically closely related to bat SARS-like coronaviruses [2]. However, the spike, ORF8, and ORF3b proteins differ significantly from other known SARS-like coronaviruses, which may confer differences in pathogenicity and transmissibility from SARS-CoV [4]. Similar to SARS-CoV, the 2019-nCoV can be efficiently transmitted between humans. Cases of familial clustering have been reported [2].

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[a]K. K.-W. T., O. T.-Y. T., C. C.-Y. Y., and K.-H. C. contributed equally to this work.

Correspondence: K. K.-W. To, Department of Microbiology, 19th Floor, Block T, Queen Mary Hospital, Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region (kelvinto@hku.hk).

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Rapid and accurate detection of 2019-nCoV is crucial in controlling the outbreak in the community and in hospitals. Nasopharyngeal and oropharyngeal swabs are the recommended upper respiratory tract specimen types for 2019-nCoV diagnostic testing. However, the collection of these specimen types requires close contact between healthcare workers and patients, which poses a risk of transmission of the virus to the healthcare workers. Furthermore, the collection of nasopharyngeal or oropharyngeal specimens causes discomfort and may cause bleeding, especially in patients with thrombocytopenia [2]. Hence, nasopharyngeal or oropharyngeal swabs are not desirable for serial monitoring of viral load. Sputum is a noninvasive lower respiratory tract specimen, but only 28% of patients with 2019-nCoV in 1 case series could produce sputum for diagnostic evaluation [3].

Saliva specimens can be provided easily by asking patients to spit into a sterile bottle. Since no invasive procedures are required, the collection of saliva can greatly minimize the chance of exposing healthcare workers to 2019-nCoV. We have previously demonstrated that saliva has a high concordance rate of greater than 90% with nasopharyngeal specimens in the detection of respiratory viruses, including coronaviruses [5, 6]. In some patients, coronavirus was detected only in saliva but not in nasopharyngeal aspirate [5]. Saliva has also been used in screening respiratory viruses among hospitalized patients without fever or respiratory symptoms [7]. SARS-CoV can be detected in saliva at high titers [8].

Given the benefits of saliva testing, we have tested 2019-nCoV in saliva from patients with suspected 2019-nCoV infection based on clinical and epidemiological criteria as outlined by the Centre for Health Protection of Hong Kong. Here, we report the results of the saliva testing.

METHODS

Patient Specimens

In Hong Kong, 2019-nCoV testing was performed by Public Health Laboratory Services Branch in Hong Kong for patients who fulfilled the reporting criteria or enhanced surveillance criteria [9]. A patient is considered to have laboratory-confirmed infection if 2019-nCoV was detected in their nasopharyngeal or sputum specimens.

Saliva was collected by asking the patient to cough out saliva from their throat into a sterile container, and 2 mL of viral transport medium was added as we described previously [5, 6]. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 13-372).

Nucleic Acid Extraction and Real-time Reverse Transcription–Quantitative Polymerase Chain Reaction for 2019-nCoV

Saliva specimens were subjected to total nucleic acid extraction by NucliSENS easyMAG (BioMerieux) as we described previously [5]. Each specimen was mixed with lysis buffer. After extraction, the total nucleic acid was recovered using 55 μL of elution buffer. In-house 1-step real-time reverse transcription–quantitative polymerase chain reaction (RT-qPCR) assay targeting the S gene of 2019-nCoV was performed using QuantiNova SYBR Green RT-PCR Kit (Qiagen) in a LightCycler 480 Real-Time PCR System (Roche), as we described previously [2].

Viral Culture

Viral culture of 2019-nCoV was conducted in a biosafety level-3 facility. Vero E6 cells were seeded with 1 mL of minimum essential medium (MEM) at 2×10^5 cells/mL in culture tubes and incubated at 37°C in a carbon dioxide incubator for 1–2 days until confluence for inoculation. Each saliva specimen was inoculated in duplicate; 1 tube contained tosylsulfonyl phenylalanyl chloromethyl ketone–treated trypsin (0.5 μg/mL) in serum-free MEM and the other tube contained MEM with 1% fetal calf serum. Each tube was inoculated with 0.2 mL of saliva and was incubated in a slanted position so that the inoculum covered the monolayer for 60 minutes at 37°C. Then 1 mL of either MEM or trypsin MEM was added and incubated in a roller apparatus at a speed 12 to 15 revolutions per hour. Virus-induced cytopathic effect was examined daily for up to 7 days.

RESULTS

A total of 12 patients with laboratory-confirmed 2019-nCoV infection in Hong Kong were included. The median age was 62.5 years, ranging from 37 to 75 years. There were 5 female and 7 male patients. At the time of writing, all patients were still hospitalized. Saliva specimens were collected at a median of 2 days after hospitalization (range, 0–7 days) (Figure 1).

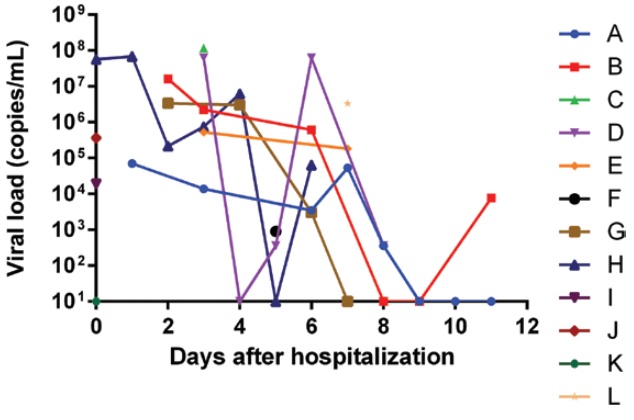

Figure 1. Saliva viral load in patients with 2019 novel coronavirus infection. For this figure, specimens with undetected viral load were assigned a value of 10^1.

The 2019-nCoV was detected in the initial saliva specimens of 11 patients (91.7%). For patient K, the first saliva specimen collected on the day of hospital admission tested negative. The median viral load of the first available saliva specimens was 3.3×10^6 copies/mL (range, 9.9×10^2 to 1.2×10^8 copies/mL).

Serial saliva specimens were available for 6 patients. The viral load was highest in the earliest available specimens for 5 patients (83.3%). For patient H, the viral load was slightly higher on day 1 after hospitalization (6.8×10^7 copies/mL) than on the day of hospital admission (5.7×10^7 copies/mL). For patient B, viral shedding in saliva was still detected on day 11 after hospitalization. In 33 patients whose nasopharyngeal specimens tested negative for 2019-nCoV, all saliva specimens also tested negative. At the time of writing, viral cultures were positive for 3 patients and negative for 2 patients.

DISCUSSION

In this study, we have demonstrated that 2019-nCoV could be detected in the saliva specimens of 11 of the 12 patients studied. Serial saliva specimens showed declines in salivary 2019-nCoV RNA levels after hospitalization. Viral culture demonstrated that live viruses were present in the saliva of 3 patients.

There are several advantages in using saliva specimens for the diagnosis of 2019-nCoV. First, saliva specimens can be provided by the patient easily without any invasive procedures. Therefore, the use of saliva specimens could reduce the risk of nosocomial 2019-nCoV transmission. Cases of 2019-nCoV infection among healthcare workers have been found, with at least 1 reported death [10]. Second, the use of saliva will allow specimen collection outside the hospitals where airborne-infection isolation rooms are not available, such as in outpatient clinics or in the community. In the setting where a large number of individuals require screening, saliva would represent a practical noninvasive specimen type. Third, since healthcare workers are not required to collect saliva specimens, the use of saliva specimens will eliminate the waiting time for specimen collection, and hence the results would be available much sooner. This is especially important in busy clinical settings where the number of available staff is limited.

Among patients with serial saliva specimens available, there was a general decline in viral load for most patients, but 1 patient had viral shedding in the saliva for at least 11 days after hospitalization. The use of saliva is preferred over nasopharyngeal or oropharyngeal specimens for serial viral load monitoring because this would reduce the discomfort to the patient and reduce the health hazards to healthcare workers during repeated sampling. Our experience with SARS in 2003 showed that viral load often peaked at day 10 after symptom onset. Thus, early detection and isolation of cases was strategic for infection control and provides the window of opportunity for antiviral therapy to decrease the peak viral load.

The positive viral culture indicates that saliva contains live viruses that may allow transmission. Respiratory viruses are considered to be transmitted from person to person through direct or indirect contact, or via coarse or fine droplets. Saliva can be emitted through cough, and respiratory droplets containing influenza virus can be found even during normal breathing [11]. Therefore, 2019-nCoV may be transmitted via saliva directly or indirectly even among patients without coughing or other respiratory symptoms. Our findings reinforce the use of surgical masks as a control measure.

SARS-CoV has been shown to infect epithelial cells in salivary gland ducts in rhesus macaques [12]. The presence of 2019-nCoV in patients' saliva suggests the possibility of salivary gland infection. However, it should be noted that saliva specimens not only contain saliva secreted from major or minor salivary glands but also contain secretions coming down from the nasopharynx or coming up from the lung via the action of cilia lining the airway. Further studies are required to delineate the sources of 2019-nCoV in saliva.

Our results have demonstrated the potential for saliva to be a noninvasive specimen type for the diagnosis and viral load monitoring of 2019-nCoV. Because saliva can be provided by patients without any invasive procedures, the use of saliva specimens will reduce the risk of nosocomial transmission of 2019-nCoV and is ideal for situations in which nasopharyngeal specimen collection may be contraindicated.

Notes

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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