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Saliva as an Alternate Specimen Source for the Diagnosis of Coronavirus Disease 2019 in Symptomatic Patients Using Cepheid Xpert Xpress SARS-CoV-2

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Abstract

Background: Rapid and accurate SARS-CoV-2 diagnostic testing is essential for controlling the ongoing COVID-19 pandemic. The current gold standard for COVID-19 diagnosis is real-time polymerase chain reaction (RT-PCR) detection of SARS-CoV-2 from nasopharyngeal swab specimens. The objective of this study is to assess saliva specimens for the diagnosis of COVID-19 by using the Gene Xpert® Xpress SARS-CoV-2 assay.

Methods: In June 2020 we prospectively simultaneously collected saliva samples and a standard nasopharyngeal swab from 60 patients meeting case definition of COVID-19 in the Emergency Department and from in-patients in Rashid Hospital at Dubai Health Authority during the outbreak of COVID-19. Real-time RT-PCR using the Cepheid Xpert Xpress SARS-CoV-2 was performed, and the results of the two specimens were compared.

Results: A total of 60 paired nasopharyngeal swab and saliva specimens were tested. An analysis of the agreement between the two specimens demonstrated 97% observed agreement. 30/28 samples were positive in saliva when compared to the NPS resulting in a positive percent agreement of 93%. 30/32 samples had a negative saliva and NPS. Two samples demonstrated detectable levels of SARS CoV-2 nucleic acid in the saliva, but the NPS were negative, resulting in a negative percent agreement of 94%.

Conclusion: Our data showed that saliva is an acceptable sensitive and specific alternative source for detecting SARS CoV-2 nucleic acid and the use of saliva samples is safer and more convenient for the patient. Nasopharyngeal swab sampling inconsistency may be one of the potential issues for false negatives results.

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

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Introduction

SARS-CoV-2 is one of the epidemic human corona viruses which include SARS-CoV and MERS-CoV. Other human pathogenic corona viruses include HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 that are endemic [1].

One of the most important steps in containing emerging highly infectious viral epidemics is getting access to accurate and rapid diagnostic tools.

In the United Arab Emirates (UAE), as in many other countries, the diagnosis of SARS-Cov-2 relied on Real Time –PCR tests using different platforms [2]. Early in the course of the local epidemic, both oropharyngeal and nasopharyngeal swabs were used to confirm the diagnosis of COVID-19 infection. However, later on, managing physicians relied on nasopharyngeal collected samples only. This was always done by trained medical professionals, usually physicians in most diagnosis and admission facilities.

The collection of these specimen types is a relatively invasive method and requires close contact between healthcare workers and patients, which may pose 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 [3].

With the increasing need for alternative sources, our hospital sought to validate saliva specimens for diagnosis of COVID-19 using the Cepheid Xpert Xpress SARS CoV-2 (Sunnyvale, CA) PCR test. Saliva specimens can be provided easily by asking patients to spit into a sterile bottle. Since no invasive procedures are required and non-aerosol generating, the collection of saliva can greatly minimize the chance of exposing healthcare workers to 2019-nCoV. Previous study demonstrated that saliva has a high concordance rate of greater than 90% with nasopharyngeal specimens in the detection of respiratory viruses, including coronaviruses [4, 5]. In some patients, coronavirus was detected only in saliva but not in nasopharyngeal aspirate, as nasopharyngeal swab sampling inconsistency may be one of the potential issues for false negatives, monitoring an internal control for proper sample collection, may provide an alternative evaluation technique [4]. Saliva has also been used in screening respiratory viruses among hospitalized patients without fever or respiratory symptoms [6]. SARS-CoV-2 can be detected in saliva at high titers [7].

The Xpert® Xpress SARS-CoV-2 assay is a sample to answer real-time RT-PCR test with a run time of approximately 45 minutes. The Xpert test (Cepheid, Sunnyvale, California, U.S.A) received EUA status on March 20, 2020. It is platform integrates specimen processing, nucleic acid extraction, reverse transcriptase polymerase chain reaction amplification of SARS-CoV-2 RNA, and amplicon detection in a single cartridge. Specimens can be tested as soon as they are received as the testing instrument provides random access to individual cartridges. The test detects the nucleocapsid gene (N2) and the envelope gene (E). There are two targets, E and N2, where detection of both targets or N2 alone is considered positive and detection of E alone is considered presumptive positive [8].

Our hospital started using Cepheid Xpert Xpress SARS-CoV-2. With this rapid technology, the length of stay and time in isolation in emergency department were decreased. Moreover, rapid triage decisions were made regarding patient disposition and isolation.

Our aim of this study was to evaluate saliva as an acceptable alternative source for detecting SARS CoV-2 nucleic acid.

Materials and Methods

Study type and population

This is a prospective study was conducted on June 2020 in pathology department at Rashid hospital, Dubai Health Authority, UAE. A total of 60 patients were selected for this study and included symptomatic patients of all adult above the age of 18 years presenting to the hospital with features compatible with COVID-19 infection.

Specimen processing

We compared NPS using 3 mL viral transport media (VTM) with unpreserved saliva samples which were collected in the Emergency Department (ED) and from in-patients in a COVID positive hospital unit in the early stage of infection. The specimens were collected prospectively in the ED, when a patient with suspected COVID-19 is being investigated [9].

Educational materials were distributed to the ED nursing staff and the nurses on the COVID unit to encourage proper saliva collection. Also, it was highly recommended that patients not have any food, drink, tobacco or gum for 30 minutes prior to collection. Saliva was collected in sterile, leak-proof container. Five mL of saliva was requested; however, specimens were considered acceptable if approximately 1 mL saliva was submitted.

The idea of population screening emerged to confirm the clearance of the city and to prove or refute the mentioned suggestion of developing COVID19 epidemic and mistaken as usual flu.

Cepheid Xpert Xpress SARS-CoV-2 test

The GeneXpert® 106 Dx System (Cepheid, Sunnyvale, CA) is an integrated diagnostic device that performs automated specimen processing and real-time RT PCR analysis. The Xpert test consists of two main components: The Xpert plastic cartridge, which contains liquid sample-processing and PCR buffers and lyophilized real-time RT-PCR reagents; and the GeneXpert instrument, which controls intra-cartridge fluidics and performs real-time RT-PCR analysis.

The Physicians have collected pairs of specimens, one NPS and one saliva from patients meeting case definition with the diagnosis of COVID19, and tested by Gene Xpert assay.

The liquid, non-viscous components of each specimen were drawn into the disposable pipettes (300 µl) issued with Xpert SARS-CoV-2 cartridges and directly will be transferred to the sample chamber of the assay cartridge. The lid is then closed and the cartridge is loaded onto the GeneXpert platform, which performs automated sample processing, and real time RT-PCR for viral RNA detection. The NPS was collected in the standard fashion and, similarly, testing was performed according to the manufacturer's instructions. The median day of sample collection for confirmed patients was 10 days from symptom onset.

Results

A total of 60 paired NPS and saliva specimens were tested. The overall positivity was 30/60 (50%).

58/60 (97%) samples were in overall agreement. 30/28 samples were positive in saliva when compared to the NPS resulting in a positive percent agreement of 93%. 30/32 samples had a negative saliva and NPS. Two samples demonstrated detectable levels of SARS CoV-2 nucleic acid in the saliva, but the NPS were negative, resulting in a negative percent agreement of 94%.

The average cycle threshold values are summarized and compared in **Table 1.**

Discussion

This is the first study in UAE for the diagnosis of COVID-19 using Gene Xpert® Xpress SARS-CoV-2 assay from saliva specimens; it has generated valuable information regarding the reliability tool of saliva to detect SARS-CoV-2.

Table 1: Average cycle threshold values for targets E and N2 in nasopharyngeal and Saliva specimens (n = 30) from Xpert Xpress SARS-CoV-2 PCR assay.

	E	Ct Range	N2	Ct Range
Nasopharyngeal	22.3 ± 17.4	0-39.7	24.2 ± 19.4	0-43.6
Saliva	27.8 ± 13.4	0-41.2	28.5 ± 16.2	0-44.7

In our study, we have demonstrated that COVID-19 could be detected in the saliva specimens of 30 of the 60 patients studied.

Our results showed that an important pre-analytical variable for SARS-CoV-2 testing is proper nasopharyngeal collection which may have been a contributing factor for the discrepant saliva positive/nasopharyngeal swab negative sample.

We found that using saliva as a diagnostic tool of choice instead of nasopharyngeal or oropharyngeal samples has several advantages; First, saliva specimens can be provided by the patient him/herself easily without any invasive procedures and without the need of a healthcare provider collecting the samples. Therefore, the use of saliva specimens could reduce the risk of healthcare exposure to infectious particles which can be attributed to the cases of COVID-19 infection among healthcare workers which have been found in our hospital. In addition, it is known that patients with viral pneumonias tend to have dry cough and less purulent sputum which makes lower respiratory tract samples not easily obtainable. Moreover, the serious infectious risks associated with sputum induction or Broncho alveolar lavage techniques. Second, saliva samples can be collected from children, adolescents and geriatrics or those with naso-facial anomalies without discomfort. Since our hospital is a major trauma center and there is a high turnover of poly-trauma cases including patients with maxillofacial injuries making nasopharyngeal or oropharyngeal sample collection not-practical or even contraindicated.

Third 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. Forth, 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. Finally, a more cost-effective approach is to test saliva instead of NPS, since the collection of saliva is relatively low cost, it could reduce the cost of special kit needed for the NSP and decrease personal protective equipment usage.

Conclusion

Our data showed that saliva is an acceptable sensitive and specific alternative source for detecting SARS CoV-2 nucleic acid and the use of saliva samples is safer and more convenient for the patient. Nasopharyngeal swab sampling inconsistency may be one of the potential issues for false negatives results.

Saliva is a reliable tool to detect SARS-CoV-2 and the role of saliva in COVID-19 diagnosis could not be limited to a qualitative detection of the virus, but it may also provide information about the clinical evolution of the disease. Salivary diagnostics may play a pivotal role in detection of Covid-19 and can offer mass screening of the population. Further studies are needed to assess virus clearance using saliva testing, and to evaluate the potential diagnostic of Covid-19 in saliva and its impact on transmission of this virus, which is pivotal to develop rapid diagnostic tests and effective strategies for prevention.

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