Potential Implication of Saliva-Based Molecular Diagnostic in SARS-CoV-2

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Abstract. SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a new type of virus with positive single-stranded RNA virus that derived from the genus *Coronaviridae* causes coronavirus disease 2019 (Covid-19). On 11 March, 2020 WHO declared Covid-19 as pandemic due to the first outbreak emerged December 2019 in Wuhan, China. It's been almost a year the world lives with this pandemic covid-19, the global cases confirmed is 103 million worldwide. The massive covid-19 testing has proven to control pandemic and stop the spread of Covid-19. Reverse-transcriptase polymerase chain reaction (RT-PCR) is gold standard for molecular diagnostic testing Covid-19 it can be obtained from nasopharyngeal swab or oropharyngeal swab. However RT-PCR sampling from nasopharyngeal or oropharyngeal swab is very challenging due to invasive methods for patients and high risk exposure for health care. Therefore another method diagnostic approach is indeed. Here we have discussed the advantages and disadvantages of salivary sampling compared to those of the nasopharynx and oropharynx based on published journals. Several studies showed RNA viral load in saliva test is comparable with sputum and saliva-based diagnostic test potentially promising as molecular diagnostic testing in Covid-19 due to the accuracy, non-invasive method, and cost-effective, nonetheless more evidence for saliva test in Covid-19 is needed.

Keywords: "SARS-CoV-2", "Saliva test", "Diagnostic", "Covid-19"

1. Introduction

Coronavirus disease (COVID-19) is a disease caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2)[1], Angiotensin-Converting Enzyme 2 (ACE-2) is the main receptor for SARS-CoV-2 to invade host cells, this interaction also mediated by Transmembrane Protease Serine 2 (TMPRSS2). TMPRSS2 is a viral membrane fusion to enter the host cell by attaching glycoprotein spike (S) protein to the surface of the virus at the ACE-2.[2,3] ACE-2 receptor expressed in multi-organs such as lungs, eyes, the cardiovascular system, skin, nervous system, and digestive tract so that the clinical symptoms are very varied[4] and a person can become infected without symptoms[5]. The COVID-19 outbreak first appeared in Wuhan, China in December 2019, then spread around the world as case reports increased daily at the beginning of 2020. On March 11, 2020, World Health Organization (WHO) announced that COVID-19 to be a global pandemic worldwide, the impact of this pandemic caused a global crisis which can affect socio-economic life. Therefore, one way to control this pandemic is by massive-scale testing to help detect SARS-CoV-2 quickly[6], Basically, SARS-CoV-2 is type of coronavirus with a single-stranded RNA genome with a length of approximately 26-32 kb[22]. Four types of Coronavirus have been identified, namely the α, β, γ, δ types. Types with human coronaviruses (HCoVs) detected in the α coronavirus are NL63 and HCoV-229E, while β coronavirus includes SARS-CoV, MERS-CoV, HCoV-HKU1, and HCoV-OC43 types[23]. The international virus classification commission named this Novel β-CoV virus as "SARS- CoV-2" after viral genome sequences from pneumonia patients in Wuhan in December 2019 turned out to have 88% similarities with SARS genome from at-SL-CoVZC45 and bat-SL-CoVZXC21. In the fight against COVID-19, an accurate examination to determine the status of a patient whether positive or negative is very important as the first step in community tracing. Until now the detection of COVID-19 uses a molecular-based diagnostic tool,

Reverse-Transcriptase Quantification Polymerase Chain Reaction (RT-qPCR) is the gold standard tool for the diagnosis of COVID-19 with the nasopharynx and oropharyngeal swab test sampling technique[7]. Sampling with the swab test technique has challenges for patients and health workers because it is invasive so an alternative sampling method is needed at RT-PCR. Taking nasopharyngeal and oropharyngeal swabs from patients is not that easy and needs trained technicians. In addition, nasopharyngeal and oropharyngeal swabs are very uncomfortable for patients, viral transport media is also needed to maintain nasopharyngeal and oropharyngeal swabs to keep until the diagnostic is performed. Together with many difficulties faced in obtaining swabs from nasopharynx and oropharynx, several studies have tried to use other sources for the detection of SARS-CoV-2, these body fluids include urine, feces, tears, and saliva. Many studies confirm that saliva was able to be a potential diagnostic source and a basis for detection of a disease and substitution of commonly used standard biological fluids such as serum and urine. Centers for Disease Control (CDC) has recommended the use of saliva as a sampling method for RT-PCR examination. Although WHO has not yet recommended it, the use of saliva as a sampling site for SARS-CoV-2 diagnostics have various potential implications. Therefore, this article aims to review the use of the saliva sampling method as molecular diagnostics by comparing the swab test method.

2. Methods

This research design is a descriptive study with narrative review method. The literature search strategy in this study is to search for literature published internationally sources published based online journals from Google Scholar, PubMed, and Science Direct keywords “SARS-CoV-2”, “Saliva test”, “Diagnostic”, “Covid-19” publication of 2019 - 2020, in English. After screening 7 literature will be reviewed.

3. SARS-CoV-2 Diagnostics

The doctor will be able to determine the COVID-19 positive-negative status of a patient through molecular diagnostics based on genomic material presence, viral RNA from the analyzed sample. The gold standard used requires trained personnel in taking swabs from the nasopharynx and oropharynx, expensive equipment and materials for extraction and thermal cyclers, as well as high laboratory levels. Gold standard detection using rRT-PCR as a standard diagnostic procedure reference from the experience gained with SARS-CoV in 2003. The procedure is including; the isolation of the RNA as genetic material, purification, reverse-transcription into cDNA, amplification, detection and quantification[11].

Another diagnosis is using reverse transcription loop-mediated isothermal amplification (RT-LAMP). In principle, RT-LAMP amplifies nucleic acids in one simple step and has been widely used to diagnose infectious diseases by bacteria and viruses. In common RT-PCR, temperature fluctuation is required to amplify genetic material, whereas in RT-LAMP there is no need for temperature fluctuation, it only uses constant temperature between 60°C and 65°C. Although both must be preceded by reverse-transcriptase first to convert RNA into cDNA[11].

4. Saliva-based Diagnostic Test

Saliva is a biological fluid that contains a very diverse range of proteins, hormones, antibodies, and so on[24]. An average person is able to secrete 600 ml of saliva with pH range between 6.6 and 7.1. This liquid is colorless and has capacity in releasing antimicrobials in its task of protecting oral health. Saliva also protects the teeth of the oro-esophageal mucosa[25]. Saliva specimens have long been believed to have high sensitivity and specificity in detecting respiratory viruses.

The molecular-based diagnostic test is a test by detecting the molecular structure of viruses such as the genome and proteins in the virus[8]. Sampling in the molecular test is taken through sputum,

nasopharyngeal and oropharyngeal swabs, bronchoalveolar fluid, and aspiration of the lower respiratory tract, this sampling method makes the patient uncomfortable because it is invasive. Several reports have shown that the survival system for some virus strains is still be detected in saliva up to 29 days after infection therefore this fact suggest that it is possible to detect virus from saliva as a non-invasive platform[26].

The idea of using saliva as a detection source has actually been used since the SARS outbreak in 2003. In addition, the similarity of the family to SARS-CoV, Covid-19 is also transmitted very highly from one person to another at close distance with the absence of protection such as a mask, through a droplet.

In an experiment conducted by the Public Health Laboratory Services Branch in Hong Kong, the experiment was aimed to compare the test results taken from saliva and nasopharynx with RT-qPCR as the gold standard in the diagnosis of COVID-19. The results showed that out of 12 positive patients, 11 patients (91.7%) were detected, while 33 non-covid patients all also showed negative using saliva-based diagnostic[27].

In April 2020, Rutgers University began developing a diagnostic test using saliva samples. This study was carried out to improve testing in patients[9]. Sampling with the saliva method was carried out through the patient spitting on the specimen tube, make sure 30 minutes before taking the sample do not eat, drink, brush teeth and 2 hours previously did not rinse with mouthwash[10,11,12,13]. Nowadays, several countries have implemented salivary sampling method in patients, this is supported by the literature that shows high ACE-2 expression in the oral cavity[19], viral load SARS-CoV-2 at 106-11m / L sputum whereas 108-9m / L saliva both showed comparable viral load[20] and detectable salivary viral loads up to 25 days after the patient developed symptoms[21], but decreased 10% in test results when taking samples were taken over 7 days[15].

5. Comparison of the Saliva-based and the Nasopharynx and Oropharynx-based Test

Research conducted in Canada showed that the estimated sampling procedure using the swab test method was 6 minutes, while the sampling method using the saliva test was 4 minutes. This estimated time included the use of personal protective equipment[14]. Based on the results of this study also showed the results of the sensitivity of RT- PCR using the salivary test method has a sensitivity that is not much different, 3.4% lower than the swab test-taking method. The accuracy of sensitivity in patients who had never been confirmed positive for COVID-19 was 7.9% lower, while for people who had been confirmed positive for COVID-19, it was 1.5% higher than those who took the swab test method[14]. Other studies also show comparisons Both tests are based on clinical symptoms, the accuracy of the sensitivity of the saliva test performed in patients with symptoms was 88% and 87% in asymptomatic patients while using the swab test the accuracy of symptomatic patients was 96% and asymptomatic was 73%[15]. the sensitivity of the saliva test is 60% - 90%, which is where the average sensitivity accuracy is 85% [11].

6. Advantages and Disadvantages of the Saliva-based Test for COVID-19

The advantages of using saliva as a diagnostic source include; Since the beginning of collection, saliva could be obtained from taking sites located outside the hospital, so that open spaces will reduce airborne transmission rate, besides there is no limit to the place of collection can also be categorized as an advantage, that anyone will be able to send saliva directly to the detection center [16, 17].

Compared to sampling from the nasopharynx and oropharynx, saliva sampling is very fast. Sampling from saliva is also an invasive sampling when compared to sampling from the nasopharynx and oropharynx. This collection also does not require reliable personnel to do the swab. It only needs a place to store the saliva and make sure the salivary sample meets good diagnostic criteria[17]. The cost needed

in using saliva as a detection source is also very cheap when compared to detection from nasopharyngeal and oropharyngeal swab, even this is estimated to reduce the cost up to 3.9% cheaper, with the massive diagnostics used in fighting the pandemic, this value will be a more huge price[14].

The advantages of salivary-based samples for COVID-19 detection is the high possibility of contamination from diagnostic tools[10, 18]. In addition, the sampling of saliva also allows for the error of the sampling method in which the timing of eating and drinking should be adjusted to avoid food and drink interfering with detection sensitivity[12].

7. Conclusion

COVID-19 is affecting 219 countries for more than a year. This condition has devastated the health, economy, social and geopolitics of all humans globally. Even though the gold standard of testing for COVID-19 is using swabs from the nasopharynx and oropharynx, limitations are still encountered because this swab source requires trained personnel and the possibility of pain and bleeding by the swab. Because the transmission route of COVID-19 is droplet, it is certain that COVID-19 also be transmitted through saliva, either directly or indirectly, when the patient does not wear a barrier such as a mask and a measured distance. The advantages of the saliva-based test compared to the common swab test; 1) the duration of sampling and sampling is fast, 2) the probability of the price being 3.9% cheaper for the saliva test, 3) the sampling method is not invasive so it does not interfere with patient comfort and sampling can be done independently, thereby reducing the risk of exposure to health workers. Although the saliva test are contamination of the diagnostic tool and incorrect sampling methods such as eating and drinking before carrying out the test still needs to be taken into consideration.

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