Saliva as a Biological Sample for COVID-19 Diagnosis?

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ABSTRACT

The COVID-19 pandemic, caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), has become a severe global health problem affecting almost every country in the world. Compared to other coronaviruses, SARS-CoV-2 is considered to be more infectious thereby leading to a rapid spread of this disease across the world. The effective control of this disease relies on timely diagnosis, proper isolation, contact tracing of the infected people and segregation of vulnerable group from potential contamination. Currently, the gold standard diagnostic test for COVID-19 is real-time reverse transcriptase polymerase chain reaction (RT-PCR) using nasopharyngeal swab (NPS). However, NPS collection has several shortcomings. Besides requiring an active involvement of healthcare personnel and personal protective equipment (PPE), NPS collection is uncomfortable for the patient as it can induce coughing, gagging, vomiting and even bleeding. Evidence from current studies indicates that saliva has a potential to be useful as an alternative biological sample for COVID-19 diagnosis. Indeed, saliva as a biological sample offers several advantages over NPS. Saliva collection is better accepted by patients, it can be self-collected and does not require PPE and active involvement of healthcare personnel. Moreover, preliminary results indicate that the sensitivity and specificity of saliva for COVID-19 diagnosis is similar to that of NPS. This summarizes recent observations in the field and discusses the potential use of saliva for COVID-19 diagnosis.

KEY WORDS

Diagnosis, Saliva, SARS-CoV-2, Real time PCR

INTRODUCTION

Pandemic incidents are rare in the history of mankind. Before COVID-19, the deadliest pandemic, last seen immediately after the World War I in 1918, was a flu pandemic which claimed 50-100 million lives worldwide.[1] The pandemic lasted for 2 years, affected one-quarter of the world's population and took more lives than did world war I. Almost 100 years later, there is another outbreak, the pandemic known as COVID-19 (Corona Virus Disease -2019), which is caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). SARS-CoV-2 is believed to have been transmitted to human from bat, and is genetically similar to both 2003 SARS-CoV and 2013 MERS-CoV.[2,3] It is difficult to imagine that an outbreak of a viral infection in the twenty-first century, when science and technological advances have led to artificial intelligence gradually taking over human tasks, would cause the whole world to stand still for several months.

The pneumonia caused by SARS-CoV-2 was first reported in December 2019 in the city of Wuhan, China.[4] In the third week of July 2020, the death toll has already exceeded 648,000 and the number of infected totals over 16.2 million.[5] There are no signs that the spread of COVID-19 and accompanying death toll will end soon. WHO has already predicted that the COVID-19 pandemic will last for 18-24 months.[6] According to a recent press release from United Nations Development Program (UNDP), this outbreak has pushed millions of people into poverty, hunger and malnutrition.[7] The global economy has been hit hard by this crisis and socioeconomic impact is extensive.

Because of the highly contagious nature of the SARS-CoV-2 virus, controlling of disease has become challenging. Though the virus shares a high degree of genetic similarity with SARS-CoV and MERS-CoV, there is some speculation that it may have mutated into a more contagious form than its predecessors, which is clinically supported by the

recent global outbreak in a short period of time.[8,9] Though the mode of transmission of SARS- CoV- 2 is from human to human, there are still controversies and conflicting opinions on the pathophysiology of disease process.[10-12]

The effective control of this disease lies in timely diagnosis, proper isolation, contact tracing of the infected people and segregation of vulnerable groups from potential contamination.[13,14] Although a number of molecular techniques (such as real-time reverse transcriptase polymerase chain reaction (RT-PCR), enzyme immunoassay) and biological samples (such as blood, saliva, gingival fluid, stool, urine) have been used for COVID-19 diagnosis, RT-PCR using nasopharyngeal swab (NPS) as a biological sample is considered to be the gold standard for laboratory diagnosis of COVID-19. Nevertheless, the collection of NPS is associated with a number of limitations related to patient comfort, safety of healthcare personnel (HCP) and usage of personal protective equipment (PPE). NPS collection is less acceptable to patients as it can induce coughing, vomiting or gag reflex and sometimes even cause bleeding at the site of swab collection.[15] This is especially important during disease monitoring where repeat testing is necessary. As NPS collection requires an active involvement of HCP, it involves a high risk of disease transmission to the HCP and patients in the hospital despite the use of PPE. It has been reported that significant numbers of doctors and paramedical staff were infected with SARS-CoV-2 even after taking precautionary measures, leading to a death rate of 1.1% among HCP.[16] In addition, NPS-based diagnosis overburdens the healthcare system as it requires a procedure room, involvement of skilled HCP and other armamentarium such as PPE, viral transport medium (VTM) and swab sticks. Hence, there is an immediate need to identify a reliable alternative biological specimen for COVID-19 diagnosis and monitoring.

Saliva is emerging as a potential alternative to NPS for COVID-19 diagnosis.[17] There is no denial that SARS-CoV-2 is transmitted via droplets and aerosols generated from respiratory tract secretions and saliva.[18,19] If the saliva content has sufficient viral load to cause human to human transmission, then it might be possible to use saliva for the diagnosis of disease as well. Saliva is secreted by three pairs of major (parotid, submandibular and sublingual) salivary glands and numerous minor salivary glands located in oral and lip mucosa. Saliva is composed of water (99%) and a mixture of proteins, enzymes, cytokines, immunoglobulins, mucins, electrolytes, nitrogenous compounds and other organic and inorganic compounds.[20] Several constituents of saliva have been shown to be differentially expressed in disease states and accordingly, there is a high interest in saliva based diagnostics and biomarker studies.[21,22]

Advantage of Saliva over NPS

Saliva as a biological sample for COVID-19 diagnosis bypasses to a large extent the limitations associated with

NPS. Most importantly, saliva can be self-collected by the patients, thereby reducing the risk of disease transmission to health care workers. Saliva itself can preserve virus for a short period of time whereas the viral particles in NPS need to be preserved in VTM.[23] Saliva collection does not need expensive set up and armamentarium, unlike NPS collection.

Source of SARS-CoV-2 virus in saliva

Apart from clinical advantages, there seems to be a sound scientific background for the use of saliva as a biological sample for COVID-19 diagnosis. Saliva is a good reservoir for viruses that originate from oral shedding, and secretions from the lower respiratory tract, nasopharynx and possibly infected salivary glands.[24-26] In parallel, saliva is found to contain live SARS-CoV-2 and the viral load seems to be highest one week after of the onset of symptoms.[27] Two main pathways for the presence of SARS-CoV-2 in saliva have been suggested. First, SARS-CoV-2 can be transported to saliva from nasopharyngeal and lower respiratory tract secretions and droplets (fig. 1).[17] Secondly, SARS-CoV-2 can be secreted to saliva from the infected salivary glands (fig. 1). In line with this suggestion, epithelial cells in the salivary glands have been shown to be the initial targets for SARS-CoV in Rhesus monkeys.[28] In addition, angiotensin-converting enzyme 2 (ACE2), a main surface receptor type for SARS-CoV-2, has been shown to be expressed in human salivary glands.[29] Besides the salivary glands, other locations in the oral cavity such as the tongue and floor of the mouth have been shown to express high levels of ACE2.[30] This observation indicates that the oral mucosa can
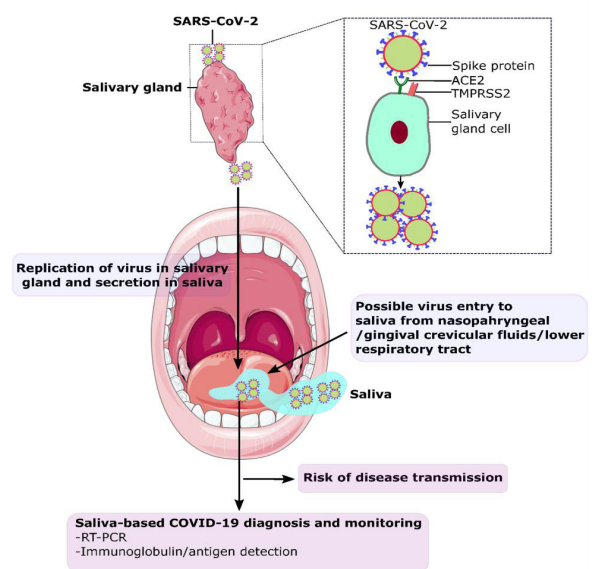

Figure 1. Illustration showing possible mechanism of viral entry to Salivary gland and sources of SARS-CoV-2 in saliva. The viral spike protein has been suggested to bind with the ACE2 receptor on the surface of salivary gland cells, followed by priming of spike protein with serine protease TMPRSS2 and subsequent entry into the cells. After replication and subsequent packaging, the new virus particles are released from the salivary gland cells into the saliva.

be a target for SARS-CoV-2 entry and further supports the use of saliva for COVD-19 diagnostics.

Current evidence for and against the use of saliva for the diagnosis of Covid-19

Recent studies have shown promising results supporting the use of saliva for COVID-19 diagnosis.[27,31-35] To et al. from Hong Kong showed that the sensitivity of saliva-based SARS-CoV-2 detection was 91%.[34,36] The authors further reported a high viral load in oropharyngeal saliva during the early stage of COVID-19, and suggested that this could be one of the reasons for the highly contagious nature of the disease. Studies by Kojima et al. and Aziz et al. suggested that the sensitivity of saliva-based COVID-19 diagnosis was similar to or even better than that of NPS.[27,33] Kojima and colleagues compared the concordance of self-collected saliva and nasal swab (mid turbinate) with clinician collected nasopharyngeal swab. They found that the sensitivity of self-collected saliva was higher (90%) as compared with the clinician collected nasopharyngeal swab (85%) and self-collected nasal swab for the diagnosis of COVID-19. Azzi et al also found that saliva could be a reliable alternative to NPS with 100% concordance with NPS testing.[27] Similar results were reported by Wyllie et al.[32] In addition, the authors found saliva to be positive for COVID-19 even after the NPS tested negative. This indicates that virus may be present in saliva for a longer duration than in nasopharyngeal swab. According to Han et al. significant viral load was detected for more than 3 weeks in faecal and saliva samples of symptomatic and asymptomatic COVID-19 positive patients.[37] Despite the promising results presented above, a study from Becker et al found the sensitivity of saliva to be 30% less than that of NPS for COVID-19 diagnosis.[38] This underscores that more studies using saliva samples from a large number of

COVID-19 patients with different severity are needed to determine the sensitivity and specificity of saliva-based COVID-19 diagnosis and disease monitoring.

Saliva-based immunoassays

Using serum and plasma samples, recent studies have shown promising results for the detection of immunoglobulins (IgG and IgM) against SARS-CoV-2 in COVID-19 patients.[39,40] Unlike RT-PCR, the immunological testing can identify both current and previous infection status. In the past, salivary immunoglobulins were used for the diagnosis of different viral diseases.[41,42] However, the presence of SARS-CoV-2 specific antibodies has not been investigated in saliva so far. SARS-CoV-specific secretory IgA were detected in the saliva of mice intranasally immunized with SARS-CoV virus like particles.[43] Hence, it is possible that anti- SARS-CoV-2 antibodies might be present in human saliva. However, this suggestion warrants further studies. If saliva can be used instead of blood, widespread immunological testing can be administered at a community level, benefitting rural and resource deprived areas.

CONCLUSION

The use of saliva for the diagnosis of COVID-19 seems promising. There are not only a number of clinical advantages of saliva over other sample types, but the use of saliva also seems to have a reasonable scientific basis. So, the use saliva as an alternative to NPS should be considered. However, more studies using saliva samples from a large number of COVID-19 patients are needed to determine the sensitivity and specificity of saliva-based COVID-19 diagnosis and disease monitoring before saliva-based COVID-19 test is available for clinical use.

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