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Oral Fluids—A Diagnostic Tool for COVID-19: A Review

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Abstract

Global outbreak of coronavirus disease 2019 (COVID-19) in December 2019 has affected millions of people around the world. This virus binds to angiotensin-converting enzyme-2 receptors present in the pharynx, nose, oral cavity, salivary glands, tongue, etc. Saliva has been shown to have viral loads of COVID-19 as it reported to be 2019-novel-coronavirus nucleic acid positive. This article is based on the association of oral fluids and their role in diagnosis of coronavirus infection.

Keywords
- COVID-19
- saliva
- gingival crevicular fluid

Introduction

The global pandemic of coronavirus disease 2019 (COVID-19) has affected close to 1% of the world's population to date. Coronavirus patients, according to the World health Organization, present with dry cough, tiredness, fever, diarrhea, sore throat, headache, discoloration of fingers and toes, loss of taste or smell, aches and pains, rash on the skin, and conjunctivitis. This infection spreads from human to human. The virus transmission is either direct or indirect. Direct transmission is through droplet infection, sneeze, or cough, or through contact, such as saliva, ocular contact, or contact of mucous membranes of the nose and eyes.[1,2]

Many published articles and reviews have stated the mouth as the principal source of infection and also the importance of saliva in diagnosis of the disease. Compared with nasopharyngeal swabs, saliva is said to be more sensitive to coronavirus nucleic acid detection.[3]

Oral cavity is intimately related to the pharynx. Hence respiratory infections can harbor and multiply in the oral cavity and vice versa. Gingival sulcus has been said to be a microbiological niche of various respiratory diseases.[4,5]

This article aims to present a literature review of the association of oral fluids in diagnosis of coronavirus infection.

Role of Saliva

Coronavirus has been found in various human secreta such as saliva, feces, and urine. Angiotensin-converting enzyme-2 (ACE-2) receptors, the principal receptors for coronavirus, are found in high numbers in minor salivary glands of humans.[6] Hence in severe forms of infection due to high viral loads of the virus, it is detected in saliva, and the destruction of salivary glands is seen in later stages of the disease.[7]

Hyposalivation is the causative factor of dry mouth.[8] Patients having COVID-19 infection present with hyposalivation and dry mouth.[7] Additionally, there is decrease in salivary flow rate with increase in age. Hyposalivation leads to reduction of antiviral properties and proteins in saliva, which makes the patient more susceptible to infections.[9] Hyposalivation can be due to medication (e.g., diabetic or hypertension medication) or other systemic diseases, inflammatory processes, and infections. COVID-19 has also been found to be more severe in patients above 50 years of age.[10-12] Hence, there might be a possible correlation between the two.

Antibody response to COVID-19 has been widely studied in blood samples of patients. Total immunoglobulin G (IgG) levels, but not IgA or IgM levels, were found to be higher in COVID-19 patients compared with controls. This study

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provides evidence that the IgG response to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike persists in the saliva and the serum, and that this response can be correlated between the two biofluids, particularly for IgG.[13] Isho et al in their study stated that IgA and IgM degrade faster as compared with IgG. They proved that the same level of IgG in saliva and serum was detected since the onset of symptoms for a minimum of 3 months.[13]

Viral ribonucleic acid (RNA) could still be detected for 20 days or even longer in deep throat saliva specimens of one-third of included patients, suggesting the viral RNA could stay for a longer period of time instead of dying out after antibody application.[14]

Salivary Fluid in the Identification of Coronavirus

Saliva is a popular noninvasive diagnostic tool for various diseases.

The three approaches for collecting salivary sample that have been used to date are swabs, sputum, and direct collection from the salivary duct.[15-18] Recommended tests for detection of COVID-19 is nasopharyngeal and oropharyngeal swabbing; the major disadvantage of these techniques is that they are invasive and there is poor patient cooperation. On the other hand, saliva collection is noninvasive, hence has better patient cooperation, especially when multiple tests are required for monitoring viral load. Due to the risk of bleeding during nasopharyngeal or oropharyngeal swabbing, saliva collection can be done especially in patients with bleeding and clotting disorders (e.g., thrombocytopenia). Saliva had 90% consistency rate comparable to nasopharyngeal swabs in detection of respiratory infections.

To et al conducted a study that reported that deep throat saliva method of diagnosis of COVID-19 is highly sensitive. They tested the *S* gene of COVID-19, in which 11 patients out of 12 tested positive when real-time reverse transcription (RT) quantitative polymerase chain reaction (qPCR) was used.[15]

In another study conducted by To et al, they analyzed the temporal profile of the virus load and tested for viral RNA. In this study they asked the patient to cough out a sample early in the morning. This sample consisted of saliva along with nasopharyngeal and bronchopharyngeal secretions. Total 20 out of 23 patients showed detectable viral RNA in saliva. The temporal profile showed maximum increase of viral load in saliva during the first week of symptomatic phase; thereafter decrease was seen.[16]

In the above study, To et al also analyzed viral RNA loads after termination of treatment. Viral RNA was detected in deep throat saliva after antibody application in one-third of the study sample for 20 days or more. This showed that viral RNA can persist for a long time after antibody application instead of dying. A patient after resolution of symptoms tested negative twice before testing positive again 2 days after the last negative result. This showed that even after recovery of clinical symptoms, patients may express viral RNA in saliva for a longer duration. However, they could not prove whether the virus expressed in saliva after recovery of clinical symptoms was shedding virus or of infectious nature.[16]

The most accurate test for COVID-19 in its acute stage is RT-PCR, as it is very sensitive and specific for the virus.[19] However, there are numerous disadvantages of this technique, such as expensive instruments and chemicals that are needed along with experienced technicians. The time required to perform the procedure and obtain results is also more as compared with other diagnostic tests.

After exposure of an individual to the virus, the antibodies can be diagnosed using various test like enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay.[20]

The first test for COVID-19 was invented in March 2020. Following this, many advances were seen in this field. By July 2020, U.S. Food and Drug Administration (FDA) approved around 11 home kits for the diagnosis of COVID-19. Most of these kits work on the principals of RT-PCR; other technologies used are loop-mediated isothermal amplification, etc. Rapid antigen home kits are also in the process of being developed.

A decrease in the risk of hospital acquired infections and cross-infection for the patient as well as medical professional is the major advantage of salivary tests, as the patients can obtain their own samples for testing. Virus load in sputum and salivary samples is said to be similar. However, the testing using nasopharyngeal swab detects the virus longer than that in a salivary sample. The viral load in the saliva decreases over the duration of the treatment.

The disadvantages of nasopharyngeal swabbing for RT-PCR are the possibility of obtaining false positive results and the need for repeated testing.

In a systematic review and meta-analysis done by Butler-Laporte et al, they found that the diagnostic sensitivity for saliva nucleic acid amplification testing (NAAT) is ~83.2%, which is comparable to that reported for nasopharyngeal swab NAAT. As saliva collection can be done without specialized professionals, saliva is a very good alternative diagnostic tool for COVID-19.[21] A systematic review by Bastos et al concluded that nasopharyngeal swabbing remains the gold standard for COVID-19 testing; however, salivary tests have shown to have equal sensitivity with the added advantage of low cost.[22] A systematic review done by Czumbel et al found that the test sensitivities for SARS-CoV-2 were 91% (confidence interval [CI]: 80–99%) and 98% (CI: 89–100%) for saliva and nasopharyngeal swab samples, respectively. They concluded that nasopharyngeal swabs have slightly higher sensitivity; however, the difference is not significant.[23]

Another mode of testing of COVID-19 is the use of serum samples. Studies have demonstrated the presence of COVID-19 RNA in body fluids such as plasma, using diagnostic tests like RT-qPCR[24-26] or droplet digital PCR.[27,28] In all of these studies the presence of viral RNA in blood has been associated with increased disease severity, as it has mostly been found in patients admitted in the intensive care unit.[24-26] Therefore, detection of COVID-19 RNA plasma can be considered to be an important diagnostic tool for critically ill patients, especially in the intensive care unit, to formulate a better treatment plan for the patient.

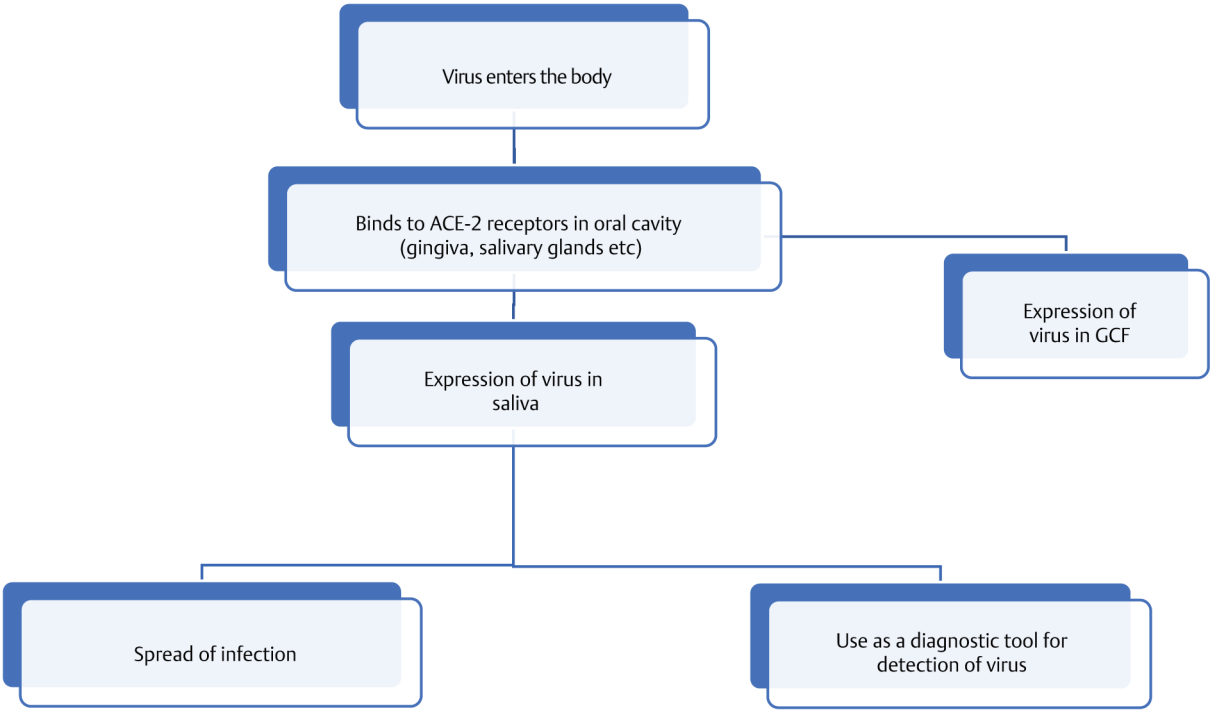

Fig. 1 Transmission of infection

Various new salivary diagnostic tests for COVID-19 are currently under trial and being developed by Nitte University Centre for Science Education and Research, Mangalore, and Medanta Institute of Education and Research in Gurgaon.

A rapid salivary test for COVID-19 that can provide results in less than a second is under trial at Sheba Medical Center, Israel. An at-home COVID-19 test, designed by Stanford researchers to be easy to use and provide results within 30 minutes, will be the focus of a study funded by the Stanford Medicine Catalyst Program. An at-home COVID-19 diagnostic test invented by Manu Prakash, PhD, Associate Professor at Stanford Medicine, is under trial and is expected to give results in 30 minutes and is easy to use. Huergo et al are developing a diagnostic tool for COVID-19 using magnetic bead-based immunoassay, which may be a rapid and low-cost alternative to ELISA in the future.[29]

Rodriguez-Manzano et al have developed a hand-held device for rapid detection of COVID-19, within 20 minutes. The device can be connected to mobiles for epidemiological survaillence.[30]

There are multiple FDA-approved diagnostic tests for COVID-19 available in the market. Few of them are, namely, Curative-Korva SARS-CoV-2 Assay, Ubi SARS-CoV-2 ELISA, EliA SARS-CoV-2-Sp1 IgG Test, RightSign COVID-19 IgG/IgM Rapid Test Cassette.

Role of Gingival Sulcus as a Niche

Oral colonization of respiratory infections has been seen in immunocompromised patients. The gingival sulcus of the oral cavity harbors numerous bacteria as well as virus. The gingival sulcus releases various enzymes such as sialidase, hexosaminidase, fucosidase, and mannosidase, which play a role in modulating the respiratory surfaces and promoting colonization of respiratory microbes.[19] As coronavirus has showed high concentrations in saliva, there might be a strong correlation between the oral cavity and colonization of coronavirus in the gingival sulcus. Furthermore, ACE-2 receptors present in gingiva and salivary glands are the main receptors to which SARS-CoV-2 binds.

These evidences support the use of gingival crevicular fluid for the identification of coronavirus (►**Fig. 1**).

Conclusion

Oral fluids such as saliva as well as gingival crevicular fluid could be used for the detection of coronavirus. Both these methods are not invasive, hence can be used especially in severe cases where multiple tests are done to monitor viral loads. COVID-19 binds to ACE-2 receptors in the body; these receptors are present in the salivary glands, the oral mucosa, and the tongue in the oral cavity. Hence the patient might present with symptoms such as ageusia, hyposalivation, and dry mouth. In severe cases due to increased viral loads in later stages, destruction of the salivary glands may be seen.

Gingival sulcus is a niche for colonization of various oral as well as respiratory bacteria. ACE-2 receptors important in COVID-19 infection are found in the gingiva. It is speculated that as viral loads can be detected in saliva, there is a strong correlation between viral loads in saliva and colonization in the gingival sulcus. Hence, oral fluid may be used as a diagnostic tool; however, further studies are required to prove this correlation.

Conflict of Interest
None declared.

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