Journal of Orofacial and Health Sciences 2024;11(4):168–171

Content available at: https://www.ipinnovative.com/open-access-journals

DJ COLLEGE OF DENTAL SCIENCES & RESEARCH

Journal of Orofacial and Health Sciences

Journal homepage: https://www.johs.in/

INNOVATIVE PUBLICATION

Review Article

Saliva as a diagnostic tool for COVID-19 (Novel Coronavirus)

Rubeena Anjum[1], Azra Kouser[1]*, Pradakhshana Vijay[1], Ruchika Raj[1], Wajiha Khanam[1], Ayeda Jehan[1]

[1]*Dept. of Oral & Maxillofacial Pathology & Oral Microbiology, Indira Gandhi Govt. Dental College, Jammu, Jammu and Kashmir, India*

ARTICLE INFO

Article history:
Received 10-08-2024
Accepted 20-09-2024
Available online 16-11-2024

Keywords:
Coronavirus
SARSCoV2 virus
Saliva
Pandemic
ACE2
Nasopharyngeal

ABSTRACT

The coronavirus disease (COVID-19) is an emerging illness that has rapidly spread across the globe. First identified in late December 2019, this new strain had not previously been detected in humans. The novel coronavirus isolated by researchers was subsequently named the 2019 novel coronavirus (2019-nCoV). COVID-19 can be diagnosed through various methods, with RT-PCR being the gold standard for these tests. The most common sampling techniques involve swabs from the nose, throat, or mouth. Saliva, a critical biological fluid containing several diagnostic biomarkers, plays an important role in detecting SARS-CoV-2 and other coronaviruses. Its use offers numerous benefits and helps researchers better distinguish between symptomatic and asymptomatic patients.

1. Introduction

An outbreak of coronavirus disease (COVID-19) is emerging and rapidly spreading worldwide.[1] A public health emergency of international concern (PHEIC) was declared over COVID-19, which is the sixth time WHO has declared a PHEIC since the International Health Regulations took effect in 2005. This new strain of disease was firstly reported in the late December of 2019 and has not been previously identified in human. The novel coronavirus isolated by researchers afterward was named as 2019 novel coronavirus (2019-nCoV).[2] Coronaviruses are enveloped RNA viruses, and two strains of them—severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)— are zoonotic in origin and known to cause fatal respiratory diseases as 2019-nCoV. Due to wide distribution and genomes recombination of coronaviruses, 2019-nCoV is the successive but novel coronavirus and shown to have a higher rate of infection.[3–5] Early diagnosis of coronavirus and effective prevention of transmission are core tasks in control of 2019-nCoV epidemic.

2. Review of Literature

There are 26 species of coronaviruses within the order Nidovirales, which can be divided into four genera. The Coronaviridae family encompasses the coronaviruses. Its genus includes two of the most common human pathogens. Coronaviruses such as SARS-CoV, SARS-CoV-2, and MERS-CoV all belong to this genus. Efforts to control SARS-CoV-2, the novel coronavirus causing COVID-19 pandemic, depend on accurate and rapid diagnostic testing. Nasopharyngeal swabs are commonly used in respiratory virus diagnostics; they show relatively poor sensitivity for SARS-CoV-2 detection in early infection and are inconsistent during serial testing.[2–6] Moreover, collecting nasopharyngeal swabs causes discomfort to patients due

* *Corresponding author*.
E-mail address: azrarubbani@gmail.com (A. Kouser).

https://doi.org/10.18231/j.johs.2024.034

to the procedure's invasiveness, limiting compliance for repeat testing, and presents a considerable risk to healthcare workers, because it can induce patients to sneeze or cough, expelling virus particles.[7] The procedure is also not conducive to large-scale testing, because there are widespread shortages of swabs and personal protective equipment for healthcare workers, and self-collection of nasopharyngeal swabs is difficult and less sensitive for virus detection.[8,9] These challenges will be further exacerbated as the COVID-19 pandemic intensifies in low-income countries. A more reliable and less resource-intensive sample collection method, ideally one that accommodates self-collection in the home, is urgently needed.

2.1. Deep throat saliva

A study from To et al. showed that deep throat saliva has high diagnosis rate of 2019-nCoV.[10,11] Twelve positive patients were confirmed based on epidemiological history, clinical criteria, and laboratory detection of 2019-nCoV in nasopharyngeal or sputum specimens, and saliva were collected by coughing out a few days after hospitalization.[11] Using real-time reverse transcription quantitative polymerase chain reaction (RT-PCR) by testing the S gene of 2019-nCoV, saliva specimens were positive for 2019-nCoV out of 12 patients (91.67%).[11,12] Those 33 patients who are negative for laboratory test of 2019-nCoV were all negative in saliva examination. In addition, six patients offer serial saliva, and five out of them showed a declining trend of virus as hospitalization is going on. Live virus was detected in three patients of the above six patients by viral culture.[11]

A study conducted by the same research group used self-collected deep throat saliva samples from COVID-19 patients to test for 2019-nCoV RNA and analyze the temporal profile of the viral load. In this study, saliva mixed with nasopharyngeal and bronchopulmonary secretions was collected by coughing up in the morning. Among the 23 COVID-19 patients included in the study, 20 showed detectable levels of 2019-nCoV RNA in their saliva. The temporal profile revealed that the viral load in saliva peaked during the first week after symptom onset and then gradually declined.[1]

2.2. Expression of ACE2 in oral tissues

Xu et al. analyzed public bulk RNA sequencing (RNA-seq) data from normal tissues adjacent to oral carcinomas and discovered that ACE2, the receptor used by SARS-CoV-2 to enter cells, is expressed in oral buccal and gingival tissues.[13] Additionally, their analysis of single-cell RNA-seq data from patients' oral tissues revealed that ACE2 is highly enriched in the epithelial cells of the tongue, as well as in epithelial cells, T cells, B cells, and fibroblasts of the oral mucosa.[14]

Saliva is produced in the salivary glands and flows through ducts into the oral cavity. Liu et al. conducted a study on rhesus macaques and found that ACE2, the receptor for SARS-CoV-2, is also expressed in the epithelial cells lining the ducts of minor salivary glands.[15] These ACE2-expressing epithelial cells can be found in various regions, including the sinonasal cavity, oral cavity, pharynx, larynx, trachea, and lungs, totaling approximately 800–1,000 cells and contributing nearly 1% of daily saliva production.[16] The same research group also established animal models by intranasally inoculating functional pseudovirus. Their findings revealed that the ACE2+ epithelial cells in the ducts of minor salivary glands are targeted as host cells as early as 48 hours after infection.[17]

In addition to the evidence from animal studies, Chen et al. analyzed data from various human databases, including GTEx, HPA, FANTOM, and consensus datasets.[5] Their analysis revealed the expression of the ACE2 receptor in human granular cells within the salivary glands. This suggests that ACE2+ cells in the salivary glands could potentially serve as target cells for 2019-nCoV, leading to the theoretical possibility of these cells producing infectious saliva in a sustained manner.[18]

The diagnosis of COVID-19 is made through a nasopharyngeal swab. Initially, the test was carried out on patients with severe symptoms and on the subjects who had come into contact with them in the previous days. Today, only patients with severe symptoms undergo the test, while asymptomatic patients go completely undetected.[19]

Sputum and oropharyngeal secretions have recently been suggested as a possible target for the molecular diagnosis of COVID-19.[12] and salivary droplets represent the main source of the human-to-human transmission of the SARS-CoV-2 infection when social distance is less than 2m.

The use of saliva as a diagnostic sample has several advantages: since saliva can be easily provided by the patient,[13] it does not require specialized personnel for its collection. In addition, the comfort of the procedure is significantly higher if compared with the nasopharyngeal swab or sputum procedure. However, before considering saliva a promising tool to detect SARS-CoV-2, it is imperative to confirm the presence of the virus in this fluid.

3. The SARS-CoV-2 Virus Has the Following Three Processes Present in Saliva

The upper and lower respiratory tracts act as entry points for SARS-CoV-2, with the virus reaching the oral cavity via liquid droplets. SARS-CoV-2 can also exploit gingival crevicular fluid as a route into the human body. The fluid content of saliva aids the virus's entry through the mouth. Infections in both major and minor salivary glands can further facilitate viral entry, as the release of particles through salivary ducts provides a pathway for SARS-CoV-2

to invade the system. Detecting COVID-19 using saliva has shown a 90% success rate, offering an effective diagnostic method for researchers and healthcare professionals.[20,21].

The size of saliva droplets is critical in the transmission of SARS-CoV-2. Larger droplets, with a diameter greater than 60 μm, travel shorter distances and settle quickly, lowering the risk of transmission to nearby individuals. In contrast, smaller droplets, less than 60 μm in diameter, can spread to anyone within a one-meter radius. Droplets smaller than 10 μm, known as aerosols, are capable of long-range transmission. These droplets are generated during coughing, speaking, and sneezing, with size, distance, and quantity varying among individuals. A single cough can release around 3,000 salivary droplet nuclei, while sneezing can produce as many as 40,000 droplets. Even during normal breathing, exhaled droplets can travel more than one meter through the air.[22]

3.1. Importance of saliva for the diagnosis of COVID-19

The presence of ACE2 receptors in the salivary epithelium means that infection with SARS-CoV-2 may lead to symptoms of acute sialadenitis, including pain, irritation, and inflammation in the primary salivary glands. SARS-CoV-2 infection can also result in an increased concentration of salivary amylase in peripheral circulation, as the virus stimulates the salivary glands to produce more amylase. In severe cases, this condition can progress to chronic sialadenitis in the salivary glands.

SARS-CoV-2 can enter saliva through three main routes:

1. **Lower and Upper Respiratory Tract**: The virus can be inhaled and subsequently deposited in the oral cavity via liquid droplets.
2. **Blood:** The virus may enter saliva through the bloodstream, affecting salivary gland function
3. **Gingival Crevicular Fluid and Salivary Glands**: The virus can utilize gingival crevicular fluid as a pathway, and infection in the salivary glands can also contribute to the presence of the virus in saliva.

These routes facilitate the transmission and detection of SARS-CoV-2 in saliva.

On March 19, 2020, the World Health Organization (WHO) recommended that RT-PCR testing be conducted to identify SARS-CoV-2 using samples from the upper and lower respiratory tracts. For early detection of viral infection, swabs from the nasopharynx and oropharynx are used as upper respiratory specimens. Lower respiratory specimens can include samples from bronchoalveolar lavage and endotracheal aspirates; however, these procedures pose a risk of aerosol and droplet transmission to healthcare professionals.

3.2. Limitations of saliva as a diagnostic sample

While saliva offers several advantages for detecting SARS-CoV-2, it also presents notable disadvantages. The content and volume of saliva can vary significantly between individuals, and various medications may influence its composition and quality. Additionally, there is a lack of uniformity in the levels of different biomarkers present in saliva. Furthermore, the presence of proteolytic enzymes, such as amylase and proteases, can degrade salivary proteins, thereby reducing the reliability and predictability of saliva as a diagnostic sample.

4. Major Drawbacks Associated With the Salivary Diagnosis Process

The mutation of the COVID-19 virus poses a significant challenge for salivary diagnostic methods These mutations can alter the virus's structure, making it more difficult for medical practitioners to accurately diagnose infections using saliva samples. Additionally, the saliva collection process may not effectively limit the spread of the virus, especially in children.

Collecting saliva from infants can be particularly problematic due to their lack of dexterity and cooperation, which complicates the process and may lead to unhygienic conditions. Proper precautions are essential to minimize the risk of contamination during sample collection. Therefore, healthcare and testing professionals must exercise extreme care when handling salivary samples, as improper handling can increase the risk of viral contamination and hinder effective diagnosis.

5. Conclusions

Saliva is an essential biological fluid that contains various biomarkers with diagnostic potential. While it offers an alternative method for detecting COVID-19, only a few researchers have focused on standardizing salivary samples for this purpose. This review aims to provide an overview of the processes involved in salivary diagnostics.

Saliva is crucial for detecting the SARS-CoV-2 virus, as it allows for the identification of the virus and other coronaviruses, leveraging its numerous advantages. This method enables researchers to more effectively differentiate between symptomatic and asymptomatic patients. Additionally, the components present in saliva provide healthcare professionals with valuable insights for informed decision-making regarding COVID-19 contamination.

However, the mutation of the COVID-19 virus presents challenges for researchers, indicating that salivary diagnostics alone may not be sufficient for a comprehensive understanding of the virus.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. World Health Organization 2019-nCoV Situation report; 2020. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019.ncov.pdf.
2. Lu R, Zhao X, Li J. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet*. 2020;395(10224):565–74.
3. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle. *J Med Virol*. 2020;92(4):401–3.
4. Rehman SU, Shafique L, Ihsan A. Evolutionary trajectory for the emergence of a Novel Coronavirus SARS-CoV-2. *Pathogens*. 2020;9(3):240.
5. Lupia T, Scabini S, Pinna M. novel coronavirus (2019-nCoV) outbreak: a new challenge. *J Glob Antimicrob Resist*. 2019;21:22–9.
6. Centers for Disease Control and Prevention (CDC) Update: novel influenza A (H1N1) virus infections - worldwide; 2009.
7. Kaufman E, Lamster IB. The diagnostic applications of saliva-a review. *Crit Rev Oral Biol Med*. 2002;13(2):197–212.
8. Tian HY. 2019-nCoV: new challenges from coronavirua. *Zhonghua Yu Fang Yi Xue Za Zhi*. 2020;54(0):235–43.
9. Wang KK, Tsang OY, Leung WS. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020;20(5):565–74.
10. Peng X. Transmission routes of 2019-nCoV and controls in dental practice. *Int J Oral Sci*. 2020;12(1):9.
11. Khurshid Z, Asiri F, Wadaani A. Human saliva: non-invasive fluid for detecting novel Coronavirus (2019-nCoV). *Int J Environ Res Health*. 2020;17(7):2225.
12. Mallapaty S. Why does the coronavirus spread so easily between people? *Nature*. 2020;579:183.
13. Martina E, Campanati A, Diotallevi F. Saliva and oral diseases. *J Clin Med*. 2020;9(2):466.
14. Edwards DA. Inhaling to mitigate exhaled bioaerosols. *Proc Natl Acad Sci USA*. 2004;101(50):17383–8.
15. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426(6965):450–4.
16. Coutard B. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antivir Res*. 2020;176:104742.
17. Zou X. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. 2020;14(2):185–192.
18. Chen WJ. Nasopharyngeal shedding of severe acute respiratory syndromeassociated coronavirus is associated with genetic polymorphisms. *Clin Infect Dis*. 2006;42(11):1561–9.
19. Doremalen NV. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med*. 2020;382(16):1564–7.
20. Lee YH, Wong DT, Loganathan S, Kuppusamy M, Wankhar W, Gurugubelli KR, et al. Angiotensin-converting enzyme 2 (ACE2): COVID 19 gate way to multiple organ failure syndromes. *Respir Physiol Neurobiol*. 2009;22:103548.
21. Hung KF, Sun YC, Chen BH. New COVID-19 saliva-based test: How good is it compared with the current nasopharyngeal or throat swab test. *J Chin Med Assoc*. 2020;83(10):891–5.
22. Li F. Structure, function, and evolution of coronavirus spike proteins. *Annu Rev Virol*. 2016;3(1):237–61.

Author's biography

Rubeena Anjum, Professor and HOD

Azra Kouser, Post Graduate Student

Pradakhshana Vijay, Lecturer

Ruchika Raj, Post Graduate Student

Wajiha Khanam, Post Graduate Student

Ayeda Jehan, Post Graduate Student

Cite this article: Anjum R, Kouser A, Vijay P, Raj R, Khanam W, Jehan A. Saliva as a diagnostic tool for COVID-19 (Novel Coronavirus). *J Orofac Health Sci* 2024;11(4):168-171.