Review Article

Saliva as a propitious diagnostic biofluid, biomarker, and bodies first line of defense against COVID-19: A review

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Abstract

This review aims to recognize the role of saliva not just as a transmitting agent of COVID 19, but also comprehend its role in the diagnosis, and as a biomarker. A systematic literature search was performed in the PubMed database and eligible studies were included if they addressed the key issues i.e saliva as a diagnostic aid. As of January 10, 2021, a total of 309 articles across the PubMed database were identified of which 28 studies met the inclusion criteria. They were carefully examined for the type of study, sample size, parameters used, sample collection technique, and conclusions drawn. Diagnostic properties of saliva, the role of ACE 2 receptors, antibody formation ability, and antiviral characteristics were also explored. Comparisons among methods of sample collection like nasopharyngeal swabs and oropharyngeal swabs to saliva were also investigated. The observations and important deductions among the different studies were compared. Results indicated that saliva could be a reliable and financially viable option in both testing viral titers as well as marking for bio analytes due to its propitious specificity and sensitivity results reported in most of the studies. However, the inferences drawn from many of these studies should be interpreted with caution due to small sample sizes, inadequate detailing on the sample handling, laboratory processing, and rush in Corona-related publication. Scientific research with larger sample sizes, in diverse populations and age groups, at different phases of disease progression of COVID-19 are essential to reach any conclusion regarding its multi-facet use in the future.

Keywords: Antibodies in SARS-CoV-2, saliva and COVID-19, saliva as a diagnostic tool, SARS-CoV-2

Introduction

Coronoviridae is a large, enveloped, single-stranded RNA virus.[1] The term corona (crown in Latin) means a spherical form with surface projections.[2] Coronavirus is divided basically into four groups Alpha, Beta, Gamma, and Delta. Alpha and Beta forms infect the respiratory tract, gastrointestinal tract, the central nervous system in mammals whereas Gamma and Delta forms mostly infect birds.[3] The Beta form appears to have originated from live animals and the seafood market in China which could have harbored the first virus found in animals. This was later transmitted to humans and started evolving faster in humans. Bats have the same genomic sequence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which could be the accountable cause for COVID-19.[2] In 1030% patients, it leads to severe upper respiratory tract illness including SARS and Middle East Respiratory Syndrome (MERS).[1,2]

SARS-CoV-2 may also cause severe lower respiratory tract infection and ACE-2 receptors are its predominant binding proteins.[1] The virus can survive on various surfaces when it gets favorable humidity and temperature [Table 1].[4,5] It can even

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Table 1: Duration of survival of Coronavirus on various surfaces

Surface	Duration of survival
Plastic	72 h
Stainless steel	48 h
Copper	8 h
Cardboard	24 h
Surgical mask	7 days

endure a temperature of -80°C but can be inactivated by exposure to 75% ethanol, 0.1% sodium hypochlorite, and 0.5% of hydrogen peroxide or on exposure to a temperature of 56°C for 30 min.[6,7]

Usually, the transmission of SARS-CoV-2 occurs by respiratory droplets. The threat is highest from asymptomatic people, who are accountable for up to 79% of infection as they remain active showing no symptoms. Until herd immunity is established either through infection or vaccination, testing remains the only mainstay as our primary defense.

The current diagnostic procedures are based on two principles either direct detection of the virus (or its part) or immunological testing which detects the consequences of infection in the host. These diagnostic aids can be valuable in conditions like symptomatic, at-risk pre-symptomatic individuals, confirmatory testing, differential diagnosis, testing of patients with previous exposure; surveillance at sites of previous/potential outbreaks, and treatment monitoring.

Currently available tests are technique sensitive, time-consuming, expensive, lack specificity, and require trained health care professionals. Salivary biomarkers can be extremely promising in both testing and monitoring patients in real-time which is exceedingly critical in this pandemic era when physicians and primary care providers need consistent diagnostic tools to prioritize patients' access to intensive care. Saliva allows for a fast, easy, affordable, and non-invasive collection of specimens that can be repeated multiple times. This review aims at analyzing the presently available literature regarding saliva and its role in COVID diagnosis and monitoring.

Methods

Study design

This review was planned following PRISMA criteria to evaluate the role of saliva as a dependable fluid in COVID diagnosis. The review included studies published till January 2021 of various types including case-control studies, cross-sectional, prospective studies, case reports, and reviews [Flowchart 1].

Search approach

The search was carried out on January 10, 2021, in the PubMed database, and keywords were chosen with MeSH terms. The MeSH terms included—saliva and COVID-19, saliva and SARS-CoV-2, saliva as a diagnostic tool, oral saliva and COVID, saliva and antibodies in SARS-CoV-2. The search approach did not impose language, year or publication type restrictions. The search strategy that was accepted is as follows:

Selection principles

Studies were considered to be eligible for inclusion if they accessed the data of saliva in diagnostic use or as the presence of a biomarker in salivary samples of COVID-19 positive patients. Studies were excluded if: 1. They were not original research. 2. Not peer-reviewed. 3. Unpublished conference abstracts. Authors jointly decided on inclusion and exclusion criteria and the reference list was made and analyzed. Subsequently, the reference list was checked manually to identify any articles that could have been lost.

Study selection

The search retrieved a total of 309 articles across the PubMed database [Flowchart 2]. After removal of the duplicates and evaluation of the abstracts and titles was carried out, concentrating on the diagnostic property of saliva and formation of antibodies. After the exclusion of 206 articles, 103 articles were reserved. Reviews and meta-analyses were excluded if there was inadequate data on diagnosis or use as a biomarker. This resulted in the exclusion of 68 articles and 30 articles were selected. Subsequently, three other articles were excluded as it was difficult to differentiate between sputum and deep throat salivary samples based on the method used in the study.

Data retrieval

Retrieval and reviewing of collected data for the year of publication, author, nature of the study, type of sample, kind of microbiological assay used, and significant deductions were assessed [Table 2]. Individual studies were comprehensively evaluated and critically analyzed by the authors separately for all the available pieces of evidence.

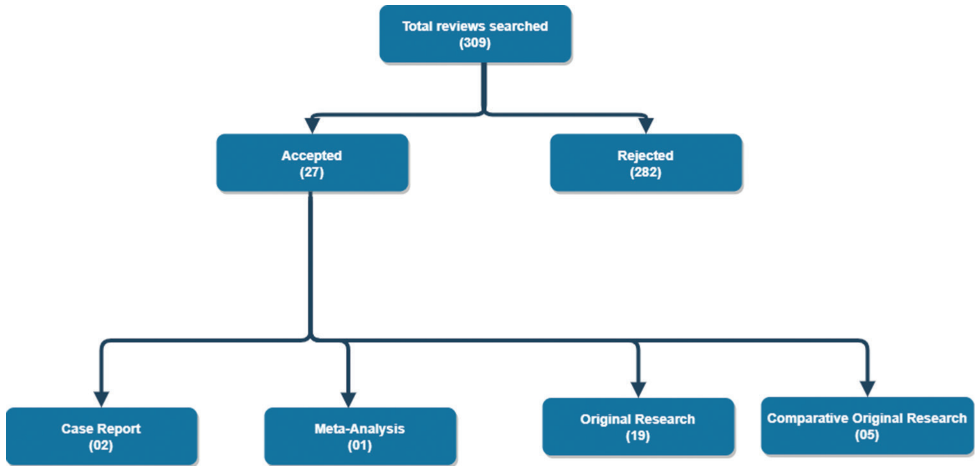
Results

Study attributes

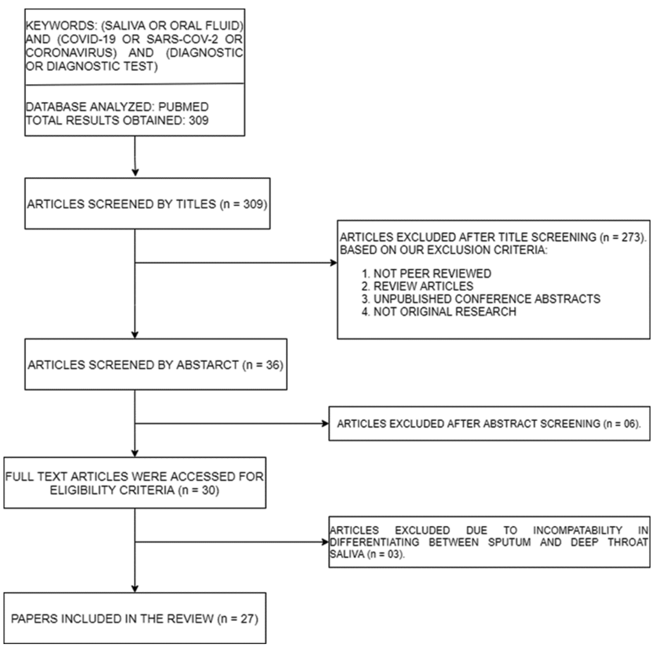
All the selected studies were published before 2021, were written in the English language, and conducted in 11 countries: China, Japan, Iran, USA, Taiwan, Israel, Italy, Malaysia, Canada, Singapore, and India. The majority indicated the use of saliva as a diagnostic tool and compared it with the other frequently employed methods. No study was undertaken on neonate or pediatric patients. The sample size ranged from 1 to 564 with a total of 3544 for the review. The majority of the studies suggested the use of saliva in diagnosing the presence of SARS-CoV-2 infection or the presence of biomarkers against the viral activity.

Overall view

The most used method of testing the salivary sample is the reverse transcriptase polymerase chain reaction (RT-PCR). Other methods included reverse transcriptase direct polymerase



Flowchart 1: Total articles searched types of studies selected



Flowchart 2: Identification, screening, analysis, and selection of articles during the search process

chain reaction, reverse transcribed colorimetric loop-mediated isothermal amplification), Raman Spectroscopy, rapid antigen test, Cobas SARS-CoV-2, laboratory develop test RT-PCR (LDT RT-PCR), high-performance LAMP, AU-FBG sensor probe, that is, GO decorated by QI Aamp viral RNA kit.

Integrated Results

As of January 10, 2021, a total of 27 studies mentioned the presence of SARS-CoV-2 in salivary samples. The method of salivary sample collection was mentioned by some authors but mostly, the general term saliva was used without detailing the technique. A comparison was made of studies where different salivary collection and testing methods were used for confirming the results. Studies that described the efficiency of the salivary sample and compared it to nasopharyngeal swabs (NPS) or oropharyngeal swabs were also scrutinized.

Synthesis of the results

The samples collected were either self-collected or collected by a healthcare worker. The salivary samples used were unstimulated saliva, sputum sample, deep throat sample, drooling saliva, oral swabs, and posterior oropharyngeal saliva. None of the studies compared each of these techniques to the other.

Results of review

As of now till January 20, 2021, there have been 94,963,847 confirmed cases of COVID-19, including 2,050,857 deaths, reported to World Health Organization (WHO). Chest computed tomography shows a pathognomic ground-glass appearance.[35,36] The specimen is collected from the upper respiratory tract and hence NPS and oropharyngeal swab collections are considered to be a gold standard. Owing to its invasive nature, collecting the specimen requires close contact between the patients and the health care workers. Furthermore, in patients receiving anticoagulant therapy or having thrombocytopenia, it may be painful and may induce bleeding and hence in such conditions, nasopharyngeal/oropharyngeal sample collections are not desirable. These conditions support non-invasive collection methods, that is, by asking the patients to spit saliva in the sterile container and then check for the viral load.[8]

Saliva: As a prompt promoter of COVID-19

The saliva secreted by a normal adult in a day is approximately 600 mL.[37] Saliva is associated with more than 700 microbial species which may cause various diseases. Infection from blood may spread to the saliva via gingival crevicular fluid and through salivary glands.[38] The major transmitting element of COVID-19 is salivary droplets.[39] The viral load of saliva varies from 9.9×10^2 to 1.2×10^8 copies/mL in different studies.[5,8,37,40] Droplets larger than 60 μm did not play a major role in disease transmission as they are large and settle rapidly on land from air whereas smaller ones can cause a short range of transmission

Table 2: Studies investigating the role of saliva in SARS-CoV-2 diagnosis

Reference	Sample size	Sample type	Methods used	Inferences derived
TO KK-W *et al.* 2020 (Hongkong)[8]	12	Saliva	RT-PCR	Saliva is a non-invasive specimen used in COVID-19 diagnosis. 1 mL of minimum essential medium (MEM) at 2×10^5 cells/mL in culture tubes were incubated at 37°C in a carbon dioxide incubator for 1-2 days until confluence for inoculation with a median viral load of the first available saliva specimens was 3.3×10^6 copies/mL. Viral load increased with each passing day.
Xu J *et al.*[9] 2020 (China)	Not mentioned	Human organs	GTEx portal	The cause of infection is both the salivary gland and saliva. The expression of ACE-2 in minor salivary glands was higher than that in lungs (lung medium PTM: 1.010, minor salivary gland medium PTM: 2.013). Rate- up to 91.7% for salivary samples.
Wan Y *et al.*[10] 2020 (Wuhan)	Various Genomic sequence	Glutamine Asparagine Threonine Leucine Phenylalanine serine	Not mentioned	S487 T mutation adds a favourable interaction at the RBD-human ACE-2 interface. It increases viral binding to human ACE-2 and plays role in human-to-human transmission. It provides information to understand the genomic sequence in humans compared to bats. Close monitoring of patients is essential for early recognition of the emergence of novel mutations at 501 positions.
Xu H *et al.*[11] 2020 (Wuhan)	13 organs	695 paranormal tissues	FANTOM5 CAGE dataset	ACE-2 receptors are concentrated in lymphocytes of salivary glands, lungs, and digestive tract. Among 32 adjacent normal tissues in the oral cavity, 13 tissues are in the tongue, 2 at the base of the tongue, 3 on the floor of the mouth, and 14 tissues did have no definite site and were just put into the category of the oral cavity.
Chen J *et al.* 2020 (Hongkong)[12]	20 samples	27 tissues	GTEx portal	ACE-2 receptors are highly concentrated in salivary glands in young Asian females compared to males. When studied in mice, cytokine storm in SARS-CoV-2 severe symptom patients, showed a decline of ACE-2, which further harmed CD4+T cells and Treg cells. Lower estrogen levels contributed to higher ACE-2 expression in Asian females than males. ACE-2 expression was induced by estrogen plus androgen block or even estrogen alone. The decline of sex hormones contributed to ACE-2 expression decrease with an increase in age.
Chen L *et al.*[13] 2020 (Wuhan)	31	Saliva from opening of salivary glands	RT-PCR	ACE-2 receptors are concentrated in the salivary glands and the major symptoms were dry mouth and amblygeustia. Three positive cases were critically ill and on ventilator support, providing high potential (75%) for detection of 2019-nCoV in the saliva. The two major oral-related symptoms, dry mouth (46.3%) and amblygeustia (47.2%), were found in a high proportion in the COVID-19 patients.
Song J *et al.*[14] 2020 (China)	71	ACE2 and TMPRSS2 gene on salivary glands in mice and other organs	GTEx dataset	ACE-2 was highly expressed in the testis, small intestine, and adipose tissue whereas, lower expression was seen in the spleen and blood. TMPRSS2 was highly expressed in the pituitary gland and prostate whereas lesser expressed in the spleen, heart, adipose tissue, and blood. ACE-2 and TMPRSS both are moderately expressed in oral mucosa and salivary glands and hence SARS-CoV-2 may be concentrated in salivary glands due to the presence of ACE-2 receptors with Pearson's correlation coefficient R=0.35, P=0.01, N=55 positive correlation.
Iwabuchi H *et al.*[15] 2020 (Japan)	323	Follow up survey	Follow up survey	Hyposalivation may be a risk factor for acute severe respiratory syndrome when studied on 323 individuals. Out of the 278 patients completing the study, the incidence of acute respiratory infection was 60.4%, while hyposalivation was present in 96 subjects (35.5%). Improvement in hyposalivation may improve the prevention possibility of acute respiratory infection.
Wyllie AL *et al.*[16] in 2020 (USA)	121	Saliva	RT-PCR	Serial salivary samples exhibit a progressive decrease in SARS-CoV-2 titres on taking serial salivary samples. When tested with NPS and salivary samples, five earlier negative NPS were found positive on getting retested but no change in results with salivary samples was reported.
Wang W-K *et al.*[17] 2020 (Taiwan)	17	Saliva	RT-PCR	No decrease in SARS-CoV-2 titers of salivary samples. Saliva remains positive even after 25 days after the first symptom appears.
Ben-Assa N *et al.*[18] 2020 (Israel)	182	Throat Nasal swabs Self-collected saliva	RT-LAMP RT-PCR	The efficacy of RT-LAMP was equal to that of RT-PCR after more than 40 min had passed. The Human pop7 gene was taken as a control. It showed an equal positive ratio for both salivary and NPS samples.
Wei S *et al.* 2020 (USA)[19]	24	Saliva	RT-PCR RT-LAMP	The sensitivity of RT-LAMP is 97-100% after 30 min had passed to RT-PCR. Efficacy of both salivary and NPS samples was equal with 5/24 positive patients, that is, 20.34%.

Contd...

Table 2: Contd...

Reference	Sample size	Sample type	Methods used	Inferences derived
Azzi L *et al.* 2020 (Italy)[20]	140	Saliva	RT-PCR Rapid salivary test	The sensitivity of Rapid Salivary testing is equal to that of RT-PCR which is 93%.
Nagura-Ikeda M *et al.* 2020 (Japan)[21]	103	Saliva	LDT-RT-PCR Cobas SARS-CoV-2 Direct RT-PCR RT-LAMP RAT	The sensitivity of LDT RT-PCR: 81.6%, Cobas SARS-CoV-2: 80.6%, direct RT-PCR: 76.7-78.6%, RT-LAMP: 50.5-70.9%, and RAT: 11.7%.
Lai CKC *et al.*[22] 2020 (Hongkong)	563	Deep throat sample Nasopharyngeal Sputum Dried blood spot	RT-PCR	The best way is sputum collection as the positive rates and viral RNA copies are higher in sputum and lower in deep throat saliva. The viral RNA copies in deep throat saliva were 3.54, in NPS is 4.63 and in sputum is 5.03. High viral RNA copies were found in the sputum sample compared to the deep throat salivary sample. Deep throat saliva showed a positive ratio of 68.7%, NPS 80.9%, and sputum 89.4%. A higher positive ratio was seen in sputum when tested with the RT-PCR method.
Valentine-Graves M *et al.* 2020 (USA)[23]	153	Saliva Oropharyngeal Dried blood clot	RT-PCR	Overall acceptability of saliva and oropharyngeal samples were 84-86% compared to a dried blood clot which was 90%
Procop GW *et al.* 2020 (USA)[24]	224	Saliva nasopharyngeal	RT-PCR	The midday or early morning sample efficiency of salivary samples remains the same. Total 38/216 samples were found positive with both nasopharyngeal and salivary sample with an exception of one sample, which was positive with the salivary sample and negative with a nasopharyngeal sample.
Rao M *et al.* 2020 (Malaysia)[25]	217	Saliva nasopharyngeal	RT-PCR	Saliva is a better alternative that can be self-collected compared to NPS swabs which can create a risk to the health care workers. In COVID-positive patients, nasopharyngeal sample 84/160 (52.5%), and salivary sample 149/160 (93.1%) gave positive results.
Iwasaki S *et al.* 2020 (Japan)[27]	76	Saliva nasopharyngeal	RT-PCR	In the initial days of infection, viral load is the same in NPS and salivary samples whereas it reduces gradually in later days in both. Of the 10 positive patients 2/10 (20%) with the nasopharyngeal sample, and 8/10 (80%) with the salivary sample gave positive results.
Kandel C *et al.* 2020 (Canada)[28]	432	Saliva	RT-PCR	Saliva is a noninvasively collected sample that can be taken to avoid risk to healthcare workers. The sample demonstrated a sensitivity of 0.91 and 0.93 for saliva and NPS.
Aita A *et al.* 2020 (Italy)[30]	43	Saliva	RT-PCR	Saliva is a utility fluid that helps in measuring IgA against SARS-CoV-2 positive patients where the ratio of positive and negative for saliva was the same. There was a difference of only one sample which tested positive with salivary sample and negative with NPS, that is, nasopharyngeal positive 7/43 (16.27%) and for salivary sample positive in 8/43 (18.60%). IgA antibody is found positive for 18/27 patients, that is, 66.67% of the cases.
Tajima Y *et al.* 2020 (Japan)[26]	1	Saliva	RT-PCR	600 μL saliva was collected, the titers of antigen were found more in early morning samples compared to midday samples
Azzi L *et al.* 2020 (Italy)[20]	2	Saliva Nasopharyngeal	RT-PCR	Salivary samples gave better results compared to nasopharyngeal samples on the 26th day. RT-PCR of the salivary sample was positive and the nasopharyngeal sample was negative initially, which after 2 days gave the same results.
Tan SY *et al.* 2020 (Singapore)[31]	500	Saliva	RT-PCR	Self-swab or saliva has a lower efficiency than the health care workers but the combination of self-swab and saliva was equivalent to health care worker sample. Salivary sample: 74.3%, self-swab: 75.1%, health care worker: 82.8%, and saliva+self-swab: 86.5%, and for self-collected to health care worker samples, self-collected sample was 8.5% less than other samples and for saliva was 9.5%.
Varadhachary A *et al.* 2020 (USA)[32]	38	Saliva	RT-PCR	IgA in saliva acts as a biomarker to identify patients at increased risk for clinical deterioration of COVID-19 symptoms. IgA antibodies were formed in the salivary samples where 35/38 patients had IgA antibodies (92.15%).
Desai S *et al.* 2020 (India)[49]	201	Saliva	Raman spectroscopy	The sensitivity of viral SARS-CoV-2 RNA was 106-1011 viral RNA copies/mL in saliva which can be detected by this method, and further, follow-up tests need to be performed to confirm the positivity.

Contd...

Table 2: Contd...

Reference	Sample size	Sample type	Methods used	Inferences derived
Samavati A *et al.* 2020 (Malaysia)[33]	06	Saliva	AU/FBG sensor probe was used with GO decorated to detect viral RNA and further tested by RT-PCR method	Wavelength increases with an increase in time and the sensitivity increases. This helps in accurate, easy, and remote sensing of COVID-19 patients. 1.6×103 copies/mL after 10 seconds by RT-PCR by QI Aamp kit tested for salivary samples.

PTM: transcripts per kilobase of exon model per Million mapped reads; RT-LAMP: Reverse transcribed colorimetric loop-mediated isothermal amplification; RT-PCR: Reverse transcription-polymerase chain reaction; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; 2019-nCoV: 2019 novel coronavirus; RBD: receptor-binding domain; CD4+T cells: cluster of differentiation 4+T helper cells; RAT: Rapid Antigen Test; NPS: Nasopharyngeal Swab; Ig: Immunoglobulins; AU/FBG: Fibre Bragg grating; GO: Graphene oxide; HF: Hydrogen fluoride; ACE-2: Angiotensin-converting enzyme 2; TMPRSS2: transmembrane serine proteases 2; GTEx: Genotype-Tissue Expression; LDT-RT-PCR: laboratory-developed test Reverse transcription Polymerase Chain Reaction; FANTOM5: FANTOM 5 project; CAGE: Cap Analysis of Gene Expression

up to 1 m.[40] Transmission through salivary droplets can occur when a person sneezes, coughs, breathes. Coughing can generate approximately 3000 salivary droplets which is equivalent to a 5-min talk whereas sneezing produces around 40,000 droplets which can cover several meters in the air.[41,42]

A recent study reported that whenever a healthy person comes in contact with the infected one, the smaller infectious droplets travel a distance and can enter the mouth, eyes, or are inhaled into the lungs. This can be minimized to a degree by wearing a surgical mask and protective eyewear or face shield.[43]

Salivary glands

Early target cell for SARS-CoV-2 includes ACE-2 positive cells/keratin epithelial cells.[38] In the early phase of infection, ACE-2 gene receptors are more frequently found in the salivary gland in comparison to the lungs. Lung medium post-translational modification (PTM [transcripts per kilobase of exon model per Million mapped]) is 1.010 whereas the minor salivary gland medium PTM is 2.013, which suggests salivary glands are a target for COVID-19. SARS-CoV-2 RNA is detected first in saliva even before the lung lesions explaining its presence in asymptomatic infections in saliva. The salivary gland could probably be a major source of virus in saliva and its infection rate can reach up to 91.7%.[8,9] Saliva could be a substrate for viral multiplication explaining its high salivary transferability in asymptomatic patients.[8] Thus salivary glands can be a potential source for transmission which should not be neglected.[10]

ACE-2 receptors act as binding receptors of the SARS-CoV-2 virus:

The potential role of epithelial cells of the oral cavity and salivary glands in the expression of ACE-2 receptors was been analyzed by many authors. Hou Xu *et al.*[11] studied the single-cell transcriptase analysis of normal oral mucosal biopsy expression of ACE-2 receptors and reported tongue had the maximum expression sites of 13 followed by the base of the tongue, the floor of the mouth, and oral cavity. High titers of the virus in saliva collected from salivary gland duct with high expression of ACE-2 receptors in a severely ill patient have been confirmed.[12] In a study carried out at the beginning of the Coronavirus outbreak in China, a close relationship between the genome of rats, that is, RatG13, and humans on ACE-2 receptor was reported, and it was found to be its principal receptor.[44] A high titer of the virus has been seen in saliva collected from the opening of salivary glands duct in severely ill patients.[13] Furthermore, the submandibular gland showed still higher titers in comparison to the parotid gland.[14]

Hyposalivation as an early symptom

According to Iwabuchi *et al.*,[15] hyposalivation could lead to severe acute respiratory infection attributed mainly to two reasons: (a) The mucosal surface of the oral cavity on reduced salivary secretion gets dry and enhances the adhesion and cohesion of the viruses. (b) This salivary reduction may also affect the secretion of antimicrobial proteins and peptides. Farshidfar N *et al.*[45] hypothesized that hyposalivation may expose patients to a high risk of getting infected.

Current diagnostic criteria for COVID-19 infection

According to WHO 2020 recommendation two samples one from the upper respiratory tract, that is, the NSP and oropharyngeal swab, and the second from the lower respiratory tract specimen, that is, sputum or endotracheal aspirate should be taken. The reason is, upper respiratory tract specimens may fail to detect early viral infections.[46]

Saliva as a diagnostic agent has the advantages of being non-invasive, easy to gain, low cost, healthier to use than serum sampling. Furthermore, saliva samples do not clot which is an added benefit.[47] Salivary sample collection can be performed either by salivary swabs, coughing out in a sterile container, or by salivary glands secretion collection by segregator cups. For early diagnosis, a sample from the lower respiratory tract or deep throat is needed.

Contradictory results were found in 12 severely ill patients, where a progressively decrease in SARS-CoV-2 titers was noted.[16] Contrasting to this study, Wang W-K *et al.*[17] reported no decrease in SARS-CoV-2 titers with time. Even after 25 days after the appearance of the first symptom, samples remained positive and titer remained high even after recovery.

Ben-Assa N *et al.*[18] [Table 3] compared the sensitivity of RT-PCR to RT-LAMP where the human pop7 gene was taken as control and reported the effectiveness to be the same for both the techniques. The efficacy of RT-LAMP was similar after 40 min and there was a decrease in false-negative results over time. In another study, the sensitivity of RT-LAMP was found to be 97-100% after 30 min.[19]

Comparison of RT-PCR test to rapid salivary test and RT-LAMP test

The sensitivity of the rapid salivary test was found to be similar to that of RT-PCR which was 93% in a study performed on 140 samples.[20] In another study on 103 positive samples of symptomatic as well as asymptomatic patients the sensitivity of the various tests were found to be LDT RT-PCR-81.6%, Cobas SARS-CoV-2-80.6%, direct RT-PCR-76.7–78.6%, RT-LAMP-50.5–70.9%, and Rat-11.7%.[21] The best mode of sample collection is sputum because the viral RNA copies are higher in sputum and lower in deep throat saliva.[22] [Table 4]. The overall acceptability of saliva and oropharyngeal samples were 84–86% compared to a dried blood clot which was 90%.[23]

The time of sample collection was compared in multiple studies but contradictory results were reported [Table 5]. Early morning samples were reported to have high viral titers in one study whereas another found that the efficiency remained the same even in the midday sample.[16,24,25] Tajima Y *et al*.[26] reported a minimum quantity of 600 μL saliva gave accurate results but found higher viral titers in the early morning samples compared to midday. A similar initial viral load was recorded in both NPS and salivary samples although it reduced gradually in later days.[27] Contradictory to this Wyllie *et al*.[16] stated, there may be changes in the viral titer in NPS but no change was found in salivary samples. Kandel C *et al*.[28] emphasized that viscosity and amount of saliva also play a critical role in testing.

Two case studies concluded that although RT-PCR of NPS is a gold standard, salivary samples too gave promising results.[29] [Table 6]

Table 3: Comparison of RT-Lamp to gold-standard RT-PCR in SARS-CoV-2 testing

Author	Sample taken	Salivary sample	RT-PCR	RT-LAMP	Control gene	Time elapsed
Ben-Assa N *et al*.[18] 2020	-Throat -Nasal swabs -Self-collected saliva	Case-1 Total- 99 Case-2 Total-83	Positive-27 Negative-72 Positive-52 Negative-31	Positive-27 Negative-72 Positive-52 Negative-31	Human pop7 gene	After 40 min
Wei S *et al*.[19] in 2020	Saliva	Total-24	Positive-5 Negative-19	Positive-5 Negative-19	Not mentioned	After 30 min

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; RT-LAMP: Reverse transcribed Colorimetric loop-mediated isothermal amplification; RT-PCR: Reverse transcription Polymerase Chain Reaction

Table 4: Comparison of various sample collection techniques with RT-PCR testing

Author	Location	Total patients	Deep throat saliva samples that is, oropharyngeal	Nasopharyngeal swabs	Sputum	Dried blood spot	Positive rates by RT-PCR	Viral RNA copies mean log copy/mL
Lai CKC *et al*. 2020[22]	Hongkong	563	150	309	104	Not mentioned	Deep throat Saliva-68.7% NPS-80.9% Sputum-89.4%	Deep throat Saliva-3.54 NPS-4.63 Sputum-5.03

RT-PCR: Reverse transcription Polymerase Chain Reaction, NPS: Nasopharyngeal swabs

Table 5: Comparative studies to relate the efficacy of salivary samples to nasopharyngeal samples

Author	Samples	Median age	Median days	Nasopharyngeal swab	Salivary samples	Comparison	Efficiency
Procop GW *et al*. 2020 [24]	Total-224 (8 - excluded, 7-indeterminant) Left-216	Not mentioned	Not mentioned	Positive-38 Negative-177	Positive-38 Negative-177	NPS sample negative-1 Saliva-positive	Not mentioned
Rao M *et al*. 2020[25]	Total-217 positive males admitted 8-10 days before Total positive-160	Not mentioned	Not mentioned	Positive-84/160 Negative-133	Positive-149/160 Negative-68	84 samples were positive for NPS and 149 for salivary samples	Saliva-93.1% NPS-52.9%
Iwasaki S *et al*. 2020[27]	Total-76 (positive-10, suspicious-66)	69 years	9 days	Positive-2/10	Positive-8/10	Not mentioned	Not mentioned
Kandel C *et al*. 2020[28]	Total- 432, Reported in the study - 236	42 years	4 days	NPS - 4 positive	Saliva - 7 positive	Not mentioned	Saliva- 91%
Wylie *et al*. 2020[16]	1-121 samples of all participants 2-76 samples of paired NPS and saliva samples)	1-61 years 2-59 years	Not mentioned	NPS samples- 22	Saliva samples- 12	NPS-5 tested negative initially later retesting-found positive. Salivary samples had no changes	Not mentioned
Aita A *et al*. 2020[30]	Total- 43	Not mentioned	Not mentioned	Positive-7 Negative-35	Positive- 7 Negative-35	Saliva sample-1 positive, NPS-1 negative	Not mentioned

NPS: Nasopharyngeal swab

Comparison of self-collected saliva to sample collected by health care worker

Efficacy of self-collected saliva in comparison to that taken in presence of health care workers have shown similar results.[16,25,30] A study carried out on 500 patients (400 were positive) with and without symptoms, the efficiency of saliva was reported to be with 74.3%, self-swab was 75.1% whereas with health care workers was 82.8%, and with self-swab was 86.5%. It concluded that the self-swab or saliva collection method had lower efficiency than the sample collected by health care workers but the combination of self-swab and saliva gave better results.[31]

Saliva: As a defense element

In agreement with previous studies, Farshidfar N *et al.*[45] reconfirmed that saliva contains Cystatin type II which possesses antiviral activity. Cystatins interfere with viral replication and also have antiviral effects as found previously against the herpes virus. Magister and Kos also claimed that Cystatin D takes part in inhibiting the replication of Coronavirus and has an antiviral protective role. Furthermore, salivary microvesicles present in saliva contain at least 20 types of microRNA's that restrict the replication of viruses.[45]

Multiple studies have claimed that increase IgA levels and serological free light chains of immunoglobulins in saliva act as a biomarker to identify patients at increased risk for clinical deterioration of COVID-19 symptoms.[31-34] [Table 7] Saliva can be a utility fluid that can measure IgA levels in positive patients.[28] Samavati A *et al.*[33] [Table 8] utilized AU/FBG sensor probe for viral RNA isolation and RT-PCR testing. Wavelength increased with time and the sensitivity improved correspondingly and this helps in accurate, easy, and remote sensing of COVID-19 patients. Newer detection methods based on the molecular analysis of facile detection of the viruses using DNA stabilized nanoclusters are also showing promising results.[48]

Interpretation of the review

This pandemic has highlighted the need to create awareness among dentists as they are high-risk professionals and at the maximum risk of acquiring the infection. It is equally essential for them to continually update themselves for spreading patient and community awareness regarding coronavirus. They need to remain abreast with the latest advancements in the field of isolation, early diagnosis, and sample collection methods and can play a pivotal role in saving lives.

It has become extremely essential to bring out the best and easiest possible way of testing which is accurate and dependable. India being the second-most populous country

Table 6: Comparative studies of Corona positive patients with comorbidities

Author	Region	Age	Days of collecting sample after being tested positive	Comorbidity/other symptoms	Sampling method
Tajima Y *et al.* 2020[26]	Japan	71	37	Allergic Rhinitis	Saliva
Azzi L *et al.* 2020[20]	Italy	71	10	Lipidaemia, obesity, hypertrophy, fever, dyspnoea	RT-PCR of the salivary samples - positive, NPS- negative.
Azzi L *et al.* in 2021[29]	Italy	64	26 2 days later (28)	Hypertension, Dyspnoea, cough, fever	RT-PCR of salivary sample - positive, NPS -negative RT-PCR of salivary sample - positive, NPS - negative

NPS: Nasopharyngeal swab; RT-PCR: Reverse transcription Polymerase Chain Reaction

Table 7: Antibodies formation against SARS-CoV-2 virus

Author	Median days	Time required	Salivary samples	RT-PCR positive	IgA antibody	Serum IgA	Serum IgG	Serum IgM
Varadhachary A *et al.* 2020[32]	61	5-10 min	38	38	Positive-35/38 Negative-3/38	Not mentioned	Not mentioned	Not mentioned
Aita A *et al.* 2020[30]	Not mentioned	Not mentioned	27	27	Positive-18/27	Positive-16	Positive-16	Positive-9/16 Negative-7/16

Ig: Immunoglobulins; RT-PCR: Reverse transcription Polymerase Chain Reaction

Table 8: Additional methods of testing salivary samples for SARS-CoV-2

Author	Method	Total patients	Median age	Sample taken	Positive/Negative	Storage temperature	Isolation of virus	Detection consistency and method
Desai S *et al.* in 2020[49]	Raman spectroscopy	201		1 mL of unstimulated Saliva	Lentiviral RNA test Positive-54 Negative- 131 Enzymatic test positive-16	4°C to (-20) °C		7.05×10^7 TU/mL
Samavati A *et al.* in 2020[33]	AU/FBG sensor probe with GO decorated	Total-6 Female-2 Males-4	58.5	Saliva	Not mentioned	30% HF solution at 15°C	QIAamp viral RNA mini kit	1.6×10^3 copies/mL after 10 seconds by RT-PCR

AU/FBG: Fiber Bragg grating probe; GO: Graphene oxide; RT-PCR: Reverse transcription Polymerase Chain Reaction; HF: Hydrogen Fluoride, QI Aamp -QI Aamp Viral RNA kit was hit hard twice by the infection and needs a testing method suitable for all individuals with the least possible resources. Research outcomes have indicated that due to its novelty, as well as the large spectrum of potential applications saliva could be a reliable and financially viable option in both testing viral titers and marking for bio analytes due to its propitious specificity and sensitivity results reported in most of the studies. Furthermore, hyposalivation leading to burning and redness of mucosa is also one of the common symptoms encountered among the patients who tested positive which could help in the early detection too.

However, the inferences drawn from many of these studies should be interpreted with caution due to small sample sizes, inadequate detailing on the sample handling, laboratory processing, and rush in Corona-related publication. Scientific research with larger sample sizes, in diverse populations and age groups, at different phases of disease progression of COVID are essential to reach any conclusion regarding its multi-facet use in the future.

Conclusion

Saliva not only transmits the virus but also the presence of various biomarkers in it makes it the body's first line of defense against the virus. Saliva is a self-collecting fluid that can be collected and transferred to the laboratory where it can be tested non-invasively and especially easily accepted by small children/elderly patients and differently-abled people where nasopharyngeal sample collection is not acceptable.

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Conflicts of interest

There are no conflicts of interest.

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