Clinical Review

Saliva in the Diagnosis of COVID-19: A Review and New Research Directions

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Abstract

This review presents literature that highlights saliva's utility as a biofluid in the diagnosis and monitoring of COVID-19. A systematic search was performed in 5 electronic databases (PubMed, Embase, LILACS, Scopus, and Web of Science). Studies were eligible for inclusion if they assessed the potential diagnostic value and/or other discriminatory properties of biological markers in the saliva of patients with COVID-19. As of July 22, 2020, a total of 28 studies have investigated the presence of SARS-CoV-2 RNA in saliva. Several of those studies confirmed reliable detection of SARS-CoV-2 in the saliva of patients with COVID-19. Saliva offered sensitivity and specificity for SARS-CoV-2 detection comparable to that of the current standard of nasopharyngeal and throat swabs. However, the utility of saliva in diagnosing COVID-19 infection remains understudied. Clinical studies with larger patient populations that measure recordings at different stages during the disease are still necessary to confirm the accuracy of COVID-19 diagnosis with saliva. Nevertheless, the utility of saliva as a diagnostic tool opens the possibility of using rapid and less invasive diagnostic strategies by targeting bioanalytes rather than the pathogen.

Keywords: coronavirus, coronavirus infections, biomarkers, COVID-19 diagnostic testing, saliva diagnosis, severe acute respiratory syndrome coronavirus 2

Introduction

In early December 2019, an outbreak of pneumonia with an unknown cause emerged in Wuhan, Hubei (China). The clinical symptoms were similar to a viral pneumonia, including fever, dizziness, and cough (Huang et al. 2020). After sequencing analysis of samples obtained from the respiratory tract, the pathogen was identified as a new coronavirus (coronavirus 2, SARS-CoV-2, COVID-19), causing a severe acute respiratory syndrome due to phylogenetic similarity with SARS-CoV (Huang et al. 2020). COVID-19–infected cases have since grown exponentially, and as a result, the World Health Organization declared a public health emergency of international interest (Coronavirus Disease 2019). More than 18.2 million cases have now been confirmed worldwide, with close to 700,000 deaths. Human-to-human transmission of SARS-CoV-2 majorly relies on respiratory droplets, which are naturally produced by talking and coughing and which contain saliva (Peng et al. 2020).

An effective and safe vaccine that provides herd immunity is yet to be developed (Clemente-Suárez et al. 2020). Thus far, the main strategy for controlling the pandemic depends on testing as many individuals as possible to avoid the risk of transmission to other patients and health care professionals, including transmission from asymptomatic people, who account for approximately 79% of the contagion (Li et al. 2020). Unfortunately, only part of the population has access to rapid testing, while COVID-19 testing should be widely available. Currently available tests are technically demanding and expensive, and some can provide a high proportion of false-negative results on samples from the upper respiratory tract. In addition, respiratory sample collection can cause discomfort to the patients and pose a high risk of transmission to the health professional. Previously, studies showed the validity of affordable and rapid diagnostic salivary tests against other viruses (HIV, Zika, etc.) for use in laboratory and at-home settings (Reynolds and Muwonga 2004). Since high SARS-CoV-2 RNA is detectable in the oropharyngeal cavity, saliva serves as

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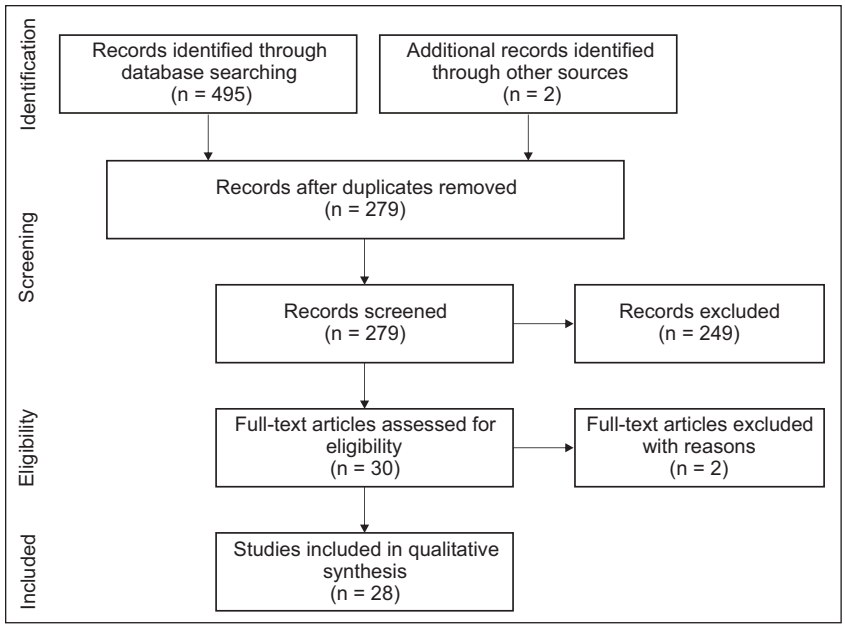

Figure 1. Flow diagram of the article search.

an excellent diagnostic fluid for controlling COVID-19 spread (To, Tsang, Leung, et al. 2020). This review highlights recently published studies that utilized saliva for diagnosis and/or monitoring of COVID-19.

Methods

Study Design

A review with a systematic search in the literature was performed following the applicable recommendations of the PRISMA criteria (Preferred Reporting Items for Systematic Review and Meta-analyses; Moher et al. 2009). This review was carried out to evaluate the available data regarding the use of saliva as a reliable tool for COVID-19 diagnosis and monitoring, including study types such as case reports and series, as well as case-control, cross-sectional, and prospective observational studies, published up to July 2020.

Search Strategy

The searching process was conducted on July 22, 2020, in 5 electronic databases (PubMed, Embase, LILACS, Scopus, and Web of Science) as well as the gray literature (hand search in the reference lists). General controlled vocabulary (MeSH terms) and keywords were chosen, and the searches had no language, year, or publication type restriction. The search strategy, adapted to each database, is detailed as follows:

- Query [1] Search: ((((((((((coronavirus [MeSH Terms]) OR (coronavirus[Text Word])) OR (coronavirus infections[MeSH Terms])) OR ("coronavirus infection"[Text Word])) OR (COVID-19[Text Word])) OR ("SARS-CoV-2"[Text Word])) OR ("SARS-CoV2"[Text Word])) OR ("nCoV"[Text Word])) OR ("2019-nCoV"[Text Word])) AND (y_10[Filter])) AND ((((saliva[MeSH Terms]) OR (salivary proteins and peptides[MeSH Terms])) OR ("oral fluid"[Title/Abstract])) OR (saliva [Title/Abstract]) AND (y_10[Filter])) Filters: in the last 10 years; and query [2] Search: "biomarker's"[All Fields] OR "biomarkers" [MeSH Terms] OR "biomarkers"[All Fields] OR "biomarker"[All Fields]

Selection Criteria

Studies were eligible for inclusion if they assessed the potential diagnostic value or other discriminatory properties of biological markers in the saliva of patients with COVID-19. Studies were excluded if they were 1) not original research (reviews), 2) conference abstracts, 3) written in non-Latin alphabet, or 4) not peer-reviewed. The eligibility criteria were resolved by a consensus among the authors. The reference lists of the selected articles were analyzed manually to identify articles that could have been lost during searches in the electronic database.

Data Extraction

Data were extracted and reviewed, including author, year of publication, sample type, methods of SARS-CoV-2 identification (reverse transcription polymerase chain reaction [RT-PCR], viral cultures, or other), and main findings. All authors analyzed each selected study and critically reviewed the main findings.

Results

Study Selection

The search retrieved 495 articles across the 5 electronic databases, and 2 were manually identified. After removal of duplicates, 279 articles remained. A comprehensive evaluation of the titles and abstracts resulted in the exclusion of 249 studies and the identification of 30 articles as being potentially relevant. A full-text review was conducted on the 30 articles retrieved in the second phase of the selection. This process led to the exclusion of 2 studies such that 28 articles were retained for final analyses. The reason for exclusion was lack of differentiation between sputum and saliva during data analysis and results presentation (Park et al. 2020; Zheng et al. 2020). A flowchart describing the process of identification, inclusion, and exclusion of studies is shown in Figure 1.

Study Characteristics

All selected studies were published in 2020 and conducted in 10 countries: China, Korea, Italy, Japan, Australia, Canada, Thailand, United Kingdom, United States, and Vietnam

Table. Studies That Investigated the Presence of SARS-CoV-2 in Human Saliva.

Reference[a]	No.[b]	Sample Type	Methods	Main Findings Regarding Salivary Analysis
To, Tsang, Yip et al. 2020 (Hong Kong, China)	12	Coughed-out saliva	RT-qPCR and viral culture	SARS-CoV-2 was detected in coughed-out saliva of 11 of 12 (91.7%) patients with COVID-19. Serial saliva specimens showed declines in salivary SARS-CoV-2 RNA levels after hospitalization and positive viral culture of live viruses in the saliva of 3 patients.
Zhang et al. 2020 (China)	15	Oral swab	RT-qPCR	Of 15 patients with COVID-19 evaluated, 8 (53.3%) had positive oral swabs.
Cheng et al. 2020 (Hong Kong, China)	1	Saliva (not specified)	RT-qPCR	Viral load of 3.3×10^6 copies/mL (pooled nasopharyngeal and throat swabs) and 5.9×10^6 copies/mL (saliva) of 1 patient.
Chen, Zhao, et al. 2020 (China)	13	Pure saliva secreted from submandibular/sublingual salivary gland duct, collected with cotton swabs	RT-PCR	SARS-CoV-2 was detected in saliva in 4 of the 13 cases analyzed; three-quarters of patients with positive detection in saliva were critically ill and receiving ventilator support.
Fang et al. 2020 (China)	32	Saliva (not specified)	RT-PCR	The positive rate of saliva was 78.1% (25/32). Saliva in non-ICU and ICU patients took 13.33 ± 5.27 and 16.50 ± 6.19 d (mean $\pm$ SD) to converse to negative.
To, Tsang, Leung et al. 2020 (Hong Kong, China)	23	Early morning saliva sample from the posterior oropharynx (coughed-out saliva)	RT-qPCR	20 of 23 (87%) patients who had SARS-CoV-2 detected in NPS or sputum also had SARS-CoV-2 detectable in saliva. Salivary viral load was highest during the first week after symptom onset and subsequently declined with time. In 1 patient, viral RNA was detected 25 d after symptom onset.
Azzi, Carcano, Gianfagna, et al. 2020 (Italy)	25	Drooled saliva and salivary swab	RT-qPCR	SARS-CoV-2 was detected in the first salivary swab of all 25 patients.
Han, Seong, Heo, et al. 2020 (South Korea)	1	Saliva (not specified)	RT-qPCR	Decrease in viral load in saliva of a neonate 6, 9, and 12 d after symptom onset.
Williams et al. 2020 (Australia)	39	Passive drooling	RT-PCR	33 of 39 patients with a positive NPS (84.6%) had SARS-CoV-2 detected in saliva. SARS-CoV-2 was detected in 1/50 patients with negative NPS (2%).
Yang et al. 2020 (China)	1	Saliva (not specified)	RT-qPCR	Persistent viral RNA positivity during recovery period of a patient with SARS-CoV-2 infection with high titers in saliva.
Azzi, Carcano, Gasperina, et al. 2020 (Italy)	2	Drooled saliva	RT-qPCR	Two cases of COVID-19 showed negative respiratory swabs but positive salivary samples at the same time.
Pasomsub et al. 2020 (Thailand)	200	Saliva sample, void of coughing	RT-qPCR	200 pairs of samples were collected. The sensitivity and specificity of saliva sample RT-PCR were 84.2% and 98.9%, respectively. An analysis between the specimens demonstrated 97.5% observed agreement.
McCormick-Baw et al. 2020 (USA)	49	Saliva, not sputum	Xpert Xpress SARS-CoV-2 PCR test (Cepheid)	47/49 samples were positive in saliva when compared with NPS, resulting in a positive percent agreement of 96%. 105/106 samples had negative saliva and NPS. A single sample demonstrated detectable levels of SARS-CoV-2 nucleic acid in the saliva, but the NPS was negative (1/106), resulting in a negative percentage agreement of 99%.
Yoon et al. 2020 (Korea)	2	Saliva (not specified)	RT-qPCR	Viral load in saliva: patient 1 = 6.63 log10 copies/mL, patient 2 = 7.10 log10 copies/mL). SARS-CoV-2 was detected up to hospital day 6 (illness day 9 for patient 2) from the saliva of both patients.
Hung et al. 2020 (Hong Kong, China)	16	POPS	RT-qPCR	Overall trend of lower Ct values from specimens collected in the early morning, with a gradual decrease of viral load toward nighttime (statistical significance only when compared with the specimens collected at bedtime).
Iwasaki et al. 2020 (Japan)	10	Self-collected saliva specimens by spitting into the tube	RT-qPCR	SARS-CoV-2 was detected in 8/10 patients in NPS and saliva samples and in 2/10 patients in either sample only. Viral load was equivalent at earlier time points but lower in saliva than in NPS at the convalescent phase.
Chau et al. 2020 (Vietnam)	27	Saliva (not specified)	RT-PCR	SARS-CoV-2 RNA was detected in 20/27 (74%) with available saliva: 7/11 (64%) in the asymptomatic group and 13/16 (81%) in the symptomatic group ($P = 0.56$).

(continued)

Table. (continued)

Reference[a]	No.[b]	Sample Type	Methods	Main Findings Regarding Salivary Analysis
Han, Seong, Kim, et al. 2020 (South Korea)	11	Saliva (not specified)	RT-qPCR	Positivity in saliva samples was 80% in week 1, 33% in week 2, and 11% in week 3.
Mak et al. 2020 (Hong Kong, China)	45	Throat saliva	BIOCREDIT COVID-19 Ag test, RT-PCR	Mean Ct was 19.65; 18/45 tested positive in Ag test.
Tajima et al. 2020 (Japan)	1	Self-collected saliva specimens by spitting into the tube	RT-PCR	SARS-CoV-2 RNA detected in saliva for 37 d after onset. Early morning saliva specimens showed better sensitivity than daytime saliva specimens.
Chen, Yip, et al. 2020 (Hong Kong, China)	58	POPS	Xpert Xpress SARS-CoV-2 (Cepheid)	84.5% (49/58) tested positive in NPS and saliva, 10.3% (6/58) in NPS only, and 5.2% (3/58) in saliva only.
Wong et al. 2020 (Hong Kong, China)	95	POPS	RT-PCR	POPS and NPsp positivity of 61.5% and 53.3%, respectively. Better positive percentage agreement was observed in POPS-NPsp obtained within 7 d of symptom onset.
Jamal et al. 2020 (Canada)	91	Self-collected saliva specimens by spitting into the tube	RT-qPCR	Sensitivity was 89% for NPS and 72% for saliva ($P = .02$). NPS was more sensitive than saliva for SARS-CoV-2 detection especially if the patient was later in illness. 11% of patients in this study with at least 1 positive specimen were positive only in their saliva.
Leung et al. 2020 (Hong Kong, China)	95	DTS (posterior oropharyngeal)	RT-PCR	The rates of SARS-CoV-2 detection for DTS (53.7%) and NPS (47.4%) samples were comparable.
Zhu et al. 2020 (China)	994	Saliva (not specified)	RT-qPCR	SARS-CoV-2 RNA levels in saliva peaked soon in 1 wk after symptom onset, ranging from around 10^4 to 10^8 copies/mL during this time, then steadily declined.
Azzi, Baj, et al. 2020 (Italy)	119	Saliva by the drooling technique.	RST and RT-PCR	The sensitivity of the RST was 0.93 and specificity was 0.42. 57% of the false-positive cases had their saliva positive when analyzed with real-time RT-PCR, which means that the virus was actually present and that the NPS was less sensitive in these cases.
Nagura-Ikeda et al. 2020 (Japan)	103	Self-collected saliva specimens by spitting into the tube	RT-qPCR, direct RT-qPCR, RT-LAMP, rapid Ag test	Viral RNA was detected in 50.5% to 81.6% of the saliva specimens by molecular diagnostic tests, and an Ag was detected in 11.7% of the specimens by a rapid Ag test. Detection of viral RNA in saliva was significantly higher in specimens collected within 9 d of symptom onset.
Bosworth et al. 2020 (United Kingdom)	15	Saliva (not specified)	RT-qPCR	5/15 samples positive in saliva. The Ct values were lower (by approximately 3 to 5 Ct) vs. results on the patient's throat swab.

Ag, antigen; Ct, cycle threshold; DTS, deep throat saliva; ICU, intensive care unit; NPS, nasopharyngeal swabs; NPsp, nasopharyngeal specimens; PCR, polymerase chain reaction; POPS, posterior oropharyngeal saliva; RST, rapid salivary test; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; RT-qPCR, reverse transcription quantitative polymerase chain reaction.
[a]Chronologic order.
[b]Number of study participants who donated saliva.

(Table). All were written in English. The majority of the studies evaluated salivary COVID-19 markers in adults. One study (Han, Seong, Heo, et al. 2020) and 2 studies (Han, Seong, Kim, et al. 2020; Wong et al. 2020) analyzed data from a neonate and from pediatric patients, respectively. Sample sizes ranged from 1 to 994, reaching a total of 2,095 for this review. All studies investigated the presence of SARS-CoV-2 in human saliva.

The most used tool to detect SARS-CoV-2 in saliva samples was reverse transcription quantitative polymerase chain reaction (RT-qPCR). Other methods included viral culture, RT-PCR, Xpert Xpress SARS-CoV-2 polymerase chain reaction test (Cepheid), direct RT-qPCR, RT-LAMP (reverse transcription loop-mediated isothermal amplification), and antigen test. Regarding the sampling methods, drooled saliva, coughed-out saliva, oral swab, glandular secretion, posterior oropharyngeal saliva, and throat saliva were among the terms cited. Descriptive characteristics of the studies are shown in the Table.

Synthesis of the Results

As of July 22, 2020, a total of 28 studies detected the presence of SARS-CoV-2 RNA in saliva (Table). Saliva samples were collected in different ways, such as cough saliva, posterior oropharyngeal saliva, saliva swab, and unstimulated saliva. The most commonly used term was *saliva*, without detailing the sample collection technique. However, no study directly compared those types of sampling.

Detection of SARS-CoV-2 in saliva was first reported in 11 patients (91.7%) with laboratory-confirmed COVID-19 infection from Hong Kong (To, Tsang, Yip, et al. 2020). In this study, serial saliva specimens showed declines in salivary SARS-CoV-2 RNA levels after hospitalization and during the patient's recovery. Positive viral culture of live viruses was also found in the saliva of 3 patients (To, Tsang, Yip, et al. 2020). Soon after, Cheng et al. (2020) reported a SARS-CoV-2

viral load of up to 5.9×10^6 copies/mL in 1 patient's saliva, and Zhang et al. (2020) showed that 8 (53.3%) patients still carried virus following several days of medical treatments. In spite of the low level of evidence produced by these series with small sample sizes, they were the first ones to consistently detect the new coronavirus in saliva.

The reported viral load of saliva specimens ranged from 9.9×10^2 to 1.2×10^8 copies/mL (Azzi, Baj, et al. 2020; Cheng et al. 2020; Han, Seong, Heo, et al. 2020; Han, Seong, Kim, et al. 2020; Iwasaki et al. 2020; To, Tsang, Leung, et al. 2020; To, Tsang, Yip, et al. 2020; Yoon et al. 2020; Zhu et al. 2020). In addition to viral load, the efficiency of saliva collection was investigated and compared with oro-/nasopharyngeal swabs for viral detection. Nine studies reported the sensitivity and/or specificity of RT-qPCR-analyzed saliva specimens as compared with the gold standard diagnosis of throat and nasopharyngeal swabs (NPSs; Azzi, Carcano, Gianfagna, et al. 2020; Jamal et al. 2020; Leung et al. 2020; Nagura et al. 2020; Pasomsub et al. 2020; Tajima et al. 2020; To, Tsang, Yip, et al. 2020; Williams et al. 2020; Zhu et al. 2020), which varied considerably from 66% to 91.7% and from 97% to 100%, respectively. Combined with those results, the cost savings for posterior oropharyngeal saliva collection were analyzed in 1 study and compared with conventional NPS, with the costs of equipment estimated as US $8.24 per 100 saliva specimens as compared with US $104.87 per 100 NPSs (Wong et al. 2020).

Additionally, the use of saliva specimens for diagnosis of COVID-19 via a faster technique than the RT-PCR approach (Xpert Xpress SARS-CoV-2) was successfully validated in saliva as compared with match NPS samples (McCormick-Baw et al. 2020). Xpert Xpress SARS-CoV-2 is a rapid real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2, with a run time of approximately 30 to 51 min and with 2 targets, the viral E and N2 gene regions. Detection of both targets and/or N2 alone defines viral presence, while detection of E alone is considered a presumptive positive result. Using this same technology, Chen and colleagues (Chen, Yip, et al. 2020) showed no significant difference in the detection rate between NPS and saliva, and the results from the Xpert assay had 100% concordance with RT-PCR.

Interestingly, when different techniques were used to analyze saliva samples, RT-qPCR, direct RT-qPCR, and RT-LAMP had good sensitivity, while the rapid antigen test presented low sensitivity (Nagura-Ikeda et al. 2020). In agreement, Mak et al. (2020) showed that the antigen test was less sensitive than RT-PCR to detect SARS-CoV-2 in saliva. Noteworthy, the saliva of symptomatic and asymptomatic patients was monitored, and the presence of viral RNA was detected in >50% of the asymptomatic patients as well as the symptomatic patients before onset of symptoms (Nagura-Ikeda et al. 2020). The saliva samples of health care workers were tested, and those who had positive saliva specimens indicated that they later developed symptoms compatible with COVID-19 (Bosworth et al. 2020).

The implication of a daily schedule on the sample collection of posterior oropharyngeal saliva was monitored in 2 studies. Differences during the day were detected, as were higher viral loads early in the morning versus bedtime (Hung et al. 2020). In addition, saliva specimens collected during the day had a lower rate of positive concordance with NPS viral load than saliva collected early in the morning (Tajima et al. 2020).

Discussion

Population-Level Employable Rapid Testing Strategy: Advantages of Salivary SARS-CoV-2 Detection

Experts predict that during this COVID-19 pandemic, the economy will reduce global growth of the gross domestic product in 2020 by half a percentage point (from 2.9% to 2.4%; Gupta et al. 2020). Thus, the need for a noninvasive and safe technique to diagnose COVID-19 quickly and reliably is a burgeoning field, with salivary diagnostics serving as a prime candidate for SARS-CoV-2 monitoring. Saliva is a biofluid that can be obtained with minimal discomfort and adequate safety in the context of the COVID-19 pandemic.

The interest of using saliva as an adjunct test that enhances conventional medical assessment approaches to COVID-19 increased in the last few months (To, Tsang, Yip, et al. 2020). A sensitive assay that readily and accurately identifies viral RNA with noninvasively collected clinical specimens would be optimal for SARS-CoV-2 detection and screening. Studies retrieved from this review reported that the sensitivity of RT-qPCR–analyzed saliva specimens was 66% to 92% for COVID-19 as compared with the standard diagnosis with throat and nasopharyngeal swabs. The difference in sensitivity probably reflects differences in the clinical background and timing of sampling in each study. In fact, several studies reported reduced viral load in saliva with time (Han, Seong, Kim, et al. 2020; Iwasaki et al. 2020; Nagura-Ikeda et al. 2020; To, Tsang, Leung, et al. 2020; To, Tsang, Yip, et al. 2020; Williams et al. 2020; Yoon et al. 2020; Zhang et al. 2020; Zhu et al. 2020). However, the specificity ranging from 97% to 100% suggests reliable detection limits of current assays for detecting the absence of SARS-CoV-2 viral loads in saliva samples. While 1 study showed that saliva could be considered for diagnosis in the early stages of disease due to the low costs (Wong et al. 2020), a cost-effectiveness study should be performed to confirm this recommendation.

There are at least 3 possible trajectories for SARS-CoV-2 to be present in saliva (Sabino-Silva et al. 2020): 1) from the upper respiratory tract, when liquid droplets derived from these tissues could enter into the oral cavity; 2) from blood, when the virus could access the oral cavity via an exudate containing local proteins derived from the extracellular matrix and proteins derived from serum; and 3) from infection of the major and minor salivary glands, with the release of viral particles into the saliva via salivary ducts. It was recently reported that epithelial cells in the oral cavity have shown an abundant expression of ACE2 (angiotensin-converting enzyme 2), a receptor that plays a key role in the entry of SARS-CoV-2 into cells (Xu et al. 2020). Therefore, ACE2-positive cells in

salivary glands are also considered target cells of SARS-CoV-2 (Xu et al. 2020), where it can duplicate, thereby making saliva an ideal specimen for viral detection.

In addition to blood samples, the gold standard diagnosis test for SARS-CoV-2 detection is the RT-PCR of an NPS and/or a sample from the oropharyngeal tract (Jin et al. 2020). However, this test may produce false-negative outcomes when the viral load is low, as often seen in asymptomatic patients. Supplementary IgG/IgM antibodies generated against the virus are analyzed via ELISA, but this has thus far mainly been performed on plasma (Béné et al. 2020), and antibody tests are not always COVID-19 specific (To, Tsang, Leung, et al. 2020).

It was also recently found that the clearance time of the virus varies per body specimen. For example, viral RNA was still detectable in urine or stool specimen, while matched saliva throat swabs from the same patient were already negative (Ling et al. 2020). The latter indicates that the same specimen samples should be collected during the disease state of the patient and that viral containment strategies may have to stay in place during the patient's recovery phase.

In addition, it was noted that collection of oropharyngeal and NPS samples often cause great discomfort to patients and require a trained professional to collect. This collection method further involves very close contact between health professionals and patients, representing a high risk of transmission (To, Tsang, Leung, et al. 2020). Therefore, rapid in-home devices should be created to halt the infectious rate before vaccines or other antiviral treatments become available. In May 2020, the Food and Drug Administration granted emergency use authorization to Rutgers' RUCDR Infinite Biologics and its collaborators for the first diagnostic test for COVID-19 based on at-home collection of saliva specimens (Food and Drug Administration 2020a). Another emergency use authorization for oral fluid specimens was approved for the Curative-Korva SARS-CoV-2 Assay (Food and Drug Administration 2020b).

Salivary Diagnosis

Saliva has long been recognized as a promising biological matrix for early detection of diseases in general. Similar to serum and other body fluids, saliva contains biomolecules such as DNA, RNA, microRNA, protein, and metabolites (Baum et al. 2011). Advantages in saliva sampling have generated great interest in the field of public health (Baum et al. 2011). Due to recent technological improvements, multiple saliva-based biomarkers have been revealed and/or correlated with various diseases (Baum et al. 2011). Combinations of the salivary proteome, transcriptome, metabolome, and microbiome, seen as diagnostic alphabets (*salivaomics*), uncovered saliva as a robust diagnostic vehicle (Wong 2012).

As a result, saliva serves as a superb alternative sample in the diagnosis of respiratory virus infections (Corstjens et al. 2016), particularly for large population-level screenings. As health professionals are not required for the collection, it ends up reducing the risk of hospital transmission to health care workers and other patients. Additionally, the use of salivary samples eliminates the waiting time; therefore, the results would be available in a shorter time, which is essential during a pandemic (To, Tsang, Yip, et al. 2020; Williams et al. 2020).

However, it is currently unclear whether the diagnostic outcomes of saliva for COVID-19 depend on how the samples are collected. It is also important to note that the overall number of participants in the majority of the studies is limited and that these data should be interpreted with caution until larger populations have been evaluated (see Table).

Salivary Biomarkers for COVID-19: New Research Directions

Using saliva as a diagnostic tool opens the possibility of using other strategies besides the direct detection of the viral pathogen, such as the detection of antibodies, cytokines, chemokines, and other bioanalytes, allowing the application of rapid diagnostic devices (Ruhl 2012; Helmerhorst et al. 2018). There are several studies involving quantitative analysis of the biochemical components of saliva in real time. Some of those studies revealed the presence of proteins, glucose, urea, secretory IgA, cortisol, phosphates, among others (Bel′skaya et al. 2018), featuring saliva as a fluid with diagnostic potential for COVID-19 biomarkers discovery.

Due to the recent appearance of the virus, no comprehensive serologic study has been performed on saliva samples regarding the immune response in SARS-CoV-2 infection. The investigation for the presence of antibodies in saliva of large cohorts of individuals could better determine the prevalence of COVID-19, monitor disease progression, and help define previous exposure to SARS-CoV-2 in populations (Béné et al. 2020). In fact, 2 non–peer-reviewed articles were recently published online demonstrating that the temporal kinetics of IgG, IgA, and IgM in the saliva of patients with COVID-19 were consistent with those observed in serum (Pisanic et al. 2020) and that antibodies (IgA, IgG, and IgM) to the SARS-CoV-2 spike glycoprotein could be detected in serum and saliva (Faustini et al. 2020).

In patients with COVID-19, serum concentrations of IL-6 and IL-10 were used as indicators of disease evolution (Wan et al. 2020). Diao et al. (2020) reported that the severity of the disease was correlated with levels of TNF-α, IL-6, and IL-10. Furthermore, alanine aminotransferase, C-reactive protein, neutrophil, lactate dehydrogenase, and serum urea may also be useful in predicting cases with positive RT-PCR results for COVID-19 (Mardani et al. 2020). Of interest, many of the aforementioned bioanalytes are detectable in saliva and could be used to provide early and accurate COVID-19 diagnosis to determine disease prevalence, to define exposure, and to predict the development of severe cases in this disease (Fig. 2).

Challenges and Perspectives of Salivary Diagnosis of COVID-19

Unfortunately, the current understanding of salivary diagnosis in the context of COVID-19 infection is limited. Although good results were observed, due to the rush to publish (Callaway 2020), it is expected that studies evaluating methods

of diagnosis of COVID-19 with saliva have simple experimental designs and small sample sizes. Moreover, our extensive experience with salivary samples obtained from different patients indicates that the type of saliva collection, the preanalytic procedures (e.g., sample handling and processing), and the other variables related to saliva collection and laboratory processing should all be carefully considered while interpreting saliva biomarkers related to COVID-19 infection.

Asymptomatic patients represent an urgent issue to be addressed by public health policies against COVID-19, but to date there are no reliable procedures that can be used for mass screening. Due to the low cost and high specificity, salivary diagnosis is an option. However, it is fundamental that the salivary load in asymptomatic carriers be analyzed to establish a sensitivity threshold for a future test, as studies reported the presence of viral RNA in the saliva of the asymptomatic patients and presymptomatic patients (Bosworth et al. 2020; Han, Seong, Kim, et al. 2020; Kojima et al. 2020; Nagura-Ikeda et al. 2020).

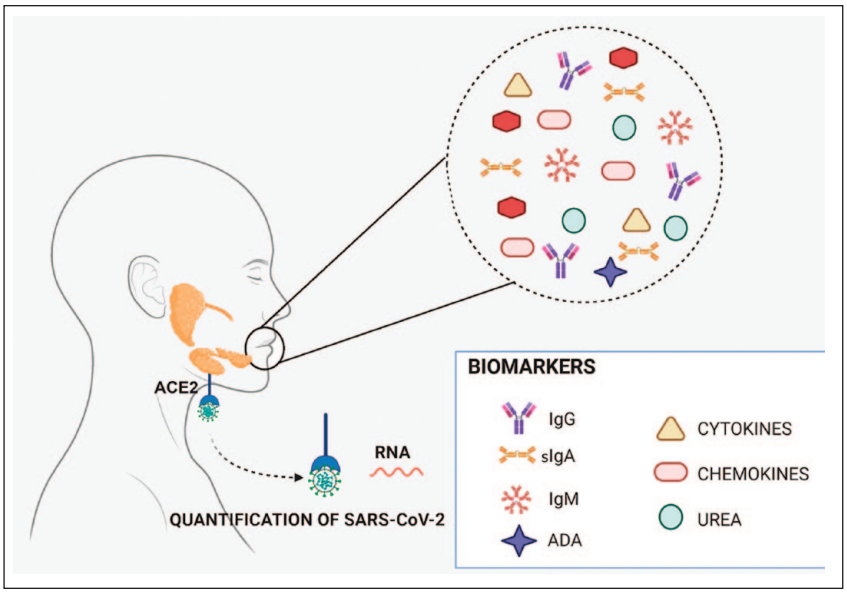

Figure 2. Salivary biomarkers potentially used for COVID-19 diagnosis and disease monitoring. ACE2, angiotensin-converting enzyme 2; ADA, adenosine deaminase; IgG, immunoglobulin G; IgM, immunoglobulin M; RNA, ribonucleic acid; sIgA, secretory immunoglobulin A.

There are, of course, several limitations and challenges when saliva is used for COVID-19 diagnosis. The use of universal terms is much needed in the salivary research field, as well as sample collection protocols ensuring greater standardization. Unfortunately, much information in this regard is lacking in the articles included in the present review. To avoid missing any information, we opted to use the same terms used by the authors. Consequently, some results need to be reviewed with caution since, for instance, posterior oropharyngeal saliva and coughed-out saliva may contain secretions coming from the posterior nasopharynx and the salivary glands and respiratory secretions swept up from the tracheal-bronchial tree. Furthermore, a more standardized sample collection should be planned and performed for the next studies. Additionally, details on sample collection, transportation, processing, and analysis may also affect the outcome of the tests and should be reported in detail. Unfortunately, the lack of information in the studies halts any conclusions about the possible effects of these confounding factors on the accuracy of saliva in the diagnoses of COVID-19.

Conclusion

A reliable detection of SARS-CoV-2 in the saliva of patients with COVID-19 has been confirmed, with diagnostic performance comparable to the current standards (nasopharyngeal and throat swabs). However, there is a lack in understanding salivary biomolecules that could be used for salivary diagnostics in the context of COVID-19 infection. Moreover, studies with larger cohorts are necessary at different stages of COVID-19 infection to confirm the accuracy of saliva's utility in COVID-19 diagnosis. Salivary samples allow other diagnostic strategies, not only the direct detection of the pathogen, but also the investigation of bioanalytes that could be used in rapid diagnostic devices. Ideally, this review can guide efforts worldwide to generate rapid COVID-19 detection and monitoring tools through saliva.

Author Contributions

L.L. Fernandes, V.B. Pacheco, M. Jimenez, N. Dame-Teixeira, D. Heller, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; L. Borges, contributed to data analysis and interpretation, drafted and critically revised the manuscript; H.K. Athwal, contributed to data analysis and interpretation, critically revised the manuscript; F. de Paula Eduardo, L. Bezinelli, L. Correa, contributed to data interpretation, critically revised the manuscript; I.M.A. Lombaert, contributed to data analysis and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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