ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2020; 6(2): 593-599

www.oraljournal.com
Received: 25-02-2020
Accepted: 27-03-2020

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De-mystifying asymptomatic carriers of SARS-CoV-2 and saliva as a new plethora for diagnosis

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Abstract
Diagnostics can play an important role in the containment of COVID-19, enabling the rapid implementation of control measures that limit the spread through case identification, isolation, and contact tracing (i.e., identifying people that may have come in contact with an infected patient). We explore the various symptoms and the challenges we face in dealing with asymptomatic carriers of SARS-CoV2 and the various modalities of sample collection and the latest developments in Point-of Care testing. The use of saliva for diagnostic purposes opens the possibility of using other tools other than the direct detection of the pathogen, such as the use of proteomic, metabolomics, detection of antibodies, especially IgA, cytokines, chemokines, techniques in order to search for markers enabling the use of rapid diagnostic devices. Currently, vaccine development is underway and has to be developed at a pandemic speed.

Keywords: SARS-CoV-2, salivary biomarkers, COVID-19, RT-PCR, surveillance, pandemic

Introduction
An emergent pneumonia outbreak originated in Wuhan city, Hubei province of China in the late December 2019 and rapidly spread to other countries [1]. The World Health Organization declared a public health emergency of international concern over this global pneumonia outbreak on 30th January 2020 [2, 3]. The infectious agent of this viral pneumonia happening in Wuhan was finally identified as a novel coronavirus (2019-nCOV), after Chinese researchers quickly isolated a new virus from the patient and sequenced its genome (29,903 nucleotides) as the seventh member of the family of coronaviruses that infect humans [4]. On 11th February 2020, WHO named the novel viral pneumonia as "Corona Virus Disease (COVID19)", while the International Committee on Taxonomy of Viruses (ICTV) suggested this novel coronavirus name as "SARS- CoV-2" due to the phylogenetic and taxonomic analysis of this novel coronavirus and declared it as a pandemic on 11 March, 2020 [5].

The typical initial clinical symptoms of the patients who suffered from the novel viral pneumonia were fever, cough, and myalgia or fatigue with abnormal chest CT, which revealed varied opacities (denser, more profuse, and confluent) in comparison to images of healthy lungs and the less common symptoms were sputum production, headache, haemoptysis, and diarrhoea [6, 7]. Since 2019-nCoV can be passed directly from person to person by respiratory droplets, emerging evidence suggested that it may also be transmitted through saliva and fomites. In addition, the asymptomatic incubation period for individuals infected with 2019-nCov has been reported to be ~1–14 days, and after 24 days individuals were reported, and it was confirmed that those without symptoms can spread the virus [7]. It is estimated that a SARS-CoV-2-infected person will infect approximately three new people (the reproductive number, R_o is averaged to be 3.28) [8].

Characteristics of 2019 novel coronavirus
Coronaviruses belong to the family of Coronaviridae, of the order Nidovirales, comprising large, single, positive sense RNA as their genome [9, 10]. Currently, there are four genera of coronaviruses: α- CoV, β-CoV, γ-CoV, and δ-CoV [10]. Most of the coronavirus can cause the infectious diseases in human and vertebrates. The α-CoV and β-CoV mainly infect the respiratory, gastrointestinal, and central nervous system of humans and mammals, while γ-CoV and δ-CoV mainly infect the birds [9, 11-13].

Usually, several members of the coronavirus cause mild respiratory disease in humans; however, SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) explored in 2002–2003 and in 2012, respectively, caused fatal severe respiratory diseases [14-16]. The SARS-CoV and MERS-CoV belong to the β-CoV [16, 17]. 2019-nCoV explored in Wuhan also belongs to the β-CoV according to the phylogenetic analysis based on the viral genome. Exploratory data established via whole-genome sequencing and subsequent bioinformatics analyses revealed that 2019-nCoV is phylogenetically related to SARS-CoV [4]. Although the nucleotide sequence similarity is less than 80% between 2019-nCoV and SARS-CoV (about 79%) or MERS-CoV (about 50%), 2019-nCoV can also cause the fetal infection and spread more faster than the two other corona- viruses [18-20]. The genome nucleotide sequence identity between a coronavirus (BatCoV RaTG13) detected in the bat Rhinolophus affinis from Yunnan Province, China, and 2019-nCoV, was 96.2%, indicating that the natural host of 2019-nCoV may also be the Rhinolophus affinis bat [21]. The current hypothesis is that the first transmission occurred between bats and a yet-to-be-determined intermediate host animal.

Table 1: Comparison of clinical symptoms and incubation time of human coronavirus [22, 23]

Human Coronavirus	Symptoms	Incubation Time
HKU1	Fever, running nose, cough, dyspnoea	2–4 days
SARS-CoV	Fever, myalgia, headache, malaise, chills, non-productive cough, dyspnoea, respiratory distress, diarrhoea (30–40% of patients)	2–11 days
MERS-CoV	Fever, cough, chills, sore throat, myalgia, arthralgia, dyspnoea, pneumonia, diarrhoea and vomiting (one third of patients), acute renal impairment	2–13 days
2019-nCoV	Malaise, fever (98%), dry cough (76%), Sputum production (28%) dyspnoea (55%), diarrhoea (3%), myalgia/fatigue (44%), headache (8%), Haemoptysis (5%)	1-14 days

Clinical manifestations

To a large extent, the clinical similarities of 2019-nCoV infection with SARS-CoV infection are substantial. The incubation period of 2019-nCov has been estimated to be 1–14 days, and it has been shown that asymptomatic individuals may also be involved in the spread of this virus [22-24]. Since the possibility of the transmission from asymptomatic carriers has been raised currently, checking body temperature only may not be enough to screen asymptomatic carriers. The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis. According to a recent report, temperature-based screening in the airport can detect only 46% of 2019-nCoV carriers and the others were found during the self-isolation period after immigration [25]. The primary non-specific reported symptoms of 2019-nCoV infection at the prodromal phase are malaise, fever, and dry cough. According to India's apex medical research agency, ICMR, 80% of the COVID positive patients are asymptomatic and has shown positive results in rapid antibody test (IgG) and the rRT-PCR test.

CDC suggests that COVID-19 patients commonly present with

- Dry/ Productive Cough
- Shortness of breath or difficulty breathing
- Fever
- Chills
- Muscle pain
- Sore throat

The most commonly reported signs and symptoms are fever (98%), cough (76%), dyspnoea (55%), and myalgia or fatigue (44%) [24, 26].

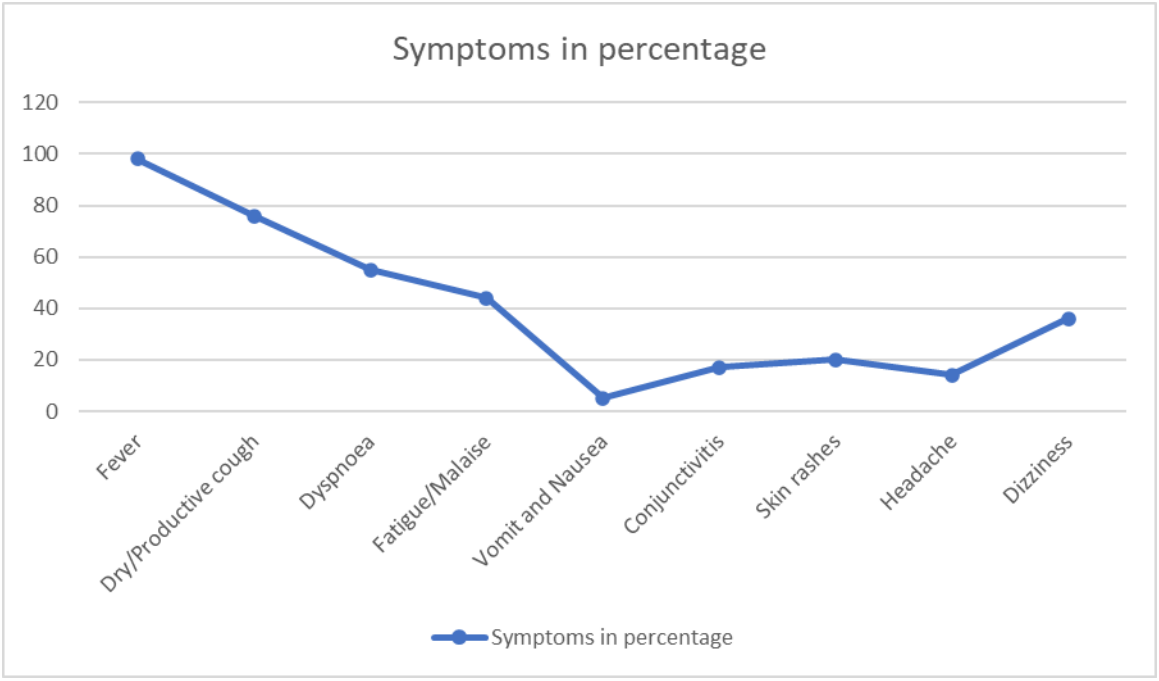

Fig 1: Depiction of the most commonly occurring symptoms in percentage among the COVID-19 positive patients

The clinical and epidemiological characteristics of COVID-19 continue to be investigated as the virus further transmits through the human population. While reliable estimates of the reproduction number and the death risk associated with

COVID-19 are crucially needed to guide public health policy, another key epidemiological parameter that could inform the intensity and range of social distancing strategies to combat COVID-19 is the asymptomatic proportion, which is broadly defined as the proportion of asymptomatic infections among all the infections of the disease [27]. Indeed, the asymptomatic proportion is a useful quantity to gauge the true burden of the disease and better interpret estimates of the transmission potential. Currently, there is no clear evidence that COVID-19 asymptomatic persons can transmit SARS-CoV-2, but there is accumulating evidence indicating that a substantial fraction of SARS-CoV-2 infected individuals are asymptomatic. Asymptomatic transmission of SARS-CoV-2 is the Achilles' heel of Covid-19 pandemic control through the public health strategies WHO has currently deployed. Symptom-based screening has utility, but epidemiologic evaluations of Covid-19 positive cases are not ultimate without the inclusion of asymptomatic cases. Estimation of the asymptomatic ratio-the percentage of carriers with no symptoms-will improve understanding of COVID-19 transmission and the spectrum of disease it causes, providing insight into epidemic spread.

Chemosensory manifestations: Anecdotal reports suggest smell and taste loss are potential early symptom or subclinical markers of Covid-19 infection. A preliminary study from Iran showed a significant increase in new- onset anosmia since the Covid-19 outbreak [28]. An Italian report of 59 hospitalized Covid-19 patients found that 33% reported a chemosensory disorder [29]. Olfactory and gustatory impairment was reported in a study conducted in United States in April 2020 where (40/59) 68% and (42/59) 71% of Covid-19- positive patients, respectively, compared to 16% and 17% of Covid-19-negative patients ($\chi 2$ test $p<0.001$) [30].

Increasing evidence suggests that a lost sense of smell, known medically as anosmia, may be a symptom of COVID-19. This is not surprising, because viral infections are a leading cause of loss of sense of smell, and COVID-19 is a caused by a virus.

A statement written by a group of ear, nose and throat specialists (otolaryngologists) in the United Kingdom reported that in Germany, China, Italy, two out of three confirmed COVID-19 cases had a loss of sense of smell; in South Korea, 30% of people with mild symptoms who tested positive for COVID-19 reported anosmia as their main symptom. "The British Association of Otorhinolaryngology (ENT UK) said the new symptom was found in the "absence of other symptoms" of coronavirus, and patients experiencing it could be "hidden carriers" of the highly contagious disease." On March 22nd, the American Academy of Otolaryngology–Head and Neck Surgery recommended that anosmia be added to the list of COVID-19 symptoms used to screen people for possible testing or self-isolation [31-33].

Many countries across Asia have reported the loss of smell (anosmia) and taste (ageusia) in COVID-19 patients. Some patients have also reported dysgeusia, referring to a change in taste in the mouth. Mao *et al*. [34] analysed the frequency of neurological manifestations in 214 patients with coronavirus disease 2019 (COVID-19), identifying anosmia in 11 (5.1%) patients and ageusia in 12 (5.6%) patients and is only the second study available at the time of writing this article. Nevertheless, this topic is of interest in oncology since patients receiving some cancer treatments such as chemotherapy or immune therapy often experience similar symptoms as side-effects [33].

Ocular Manifestations: SARS-CoV-2 is capable of causing ocular complications such as viral conjunctivitis in the middle phase of illness. Precautionary measures are recommended when examining infected patients throughout the clinical course of the infection. However, conjunctival sampling might not be useful for early diagnosis because the virus may not appear initially in the conjunctiva [35, 36].

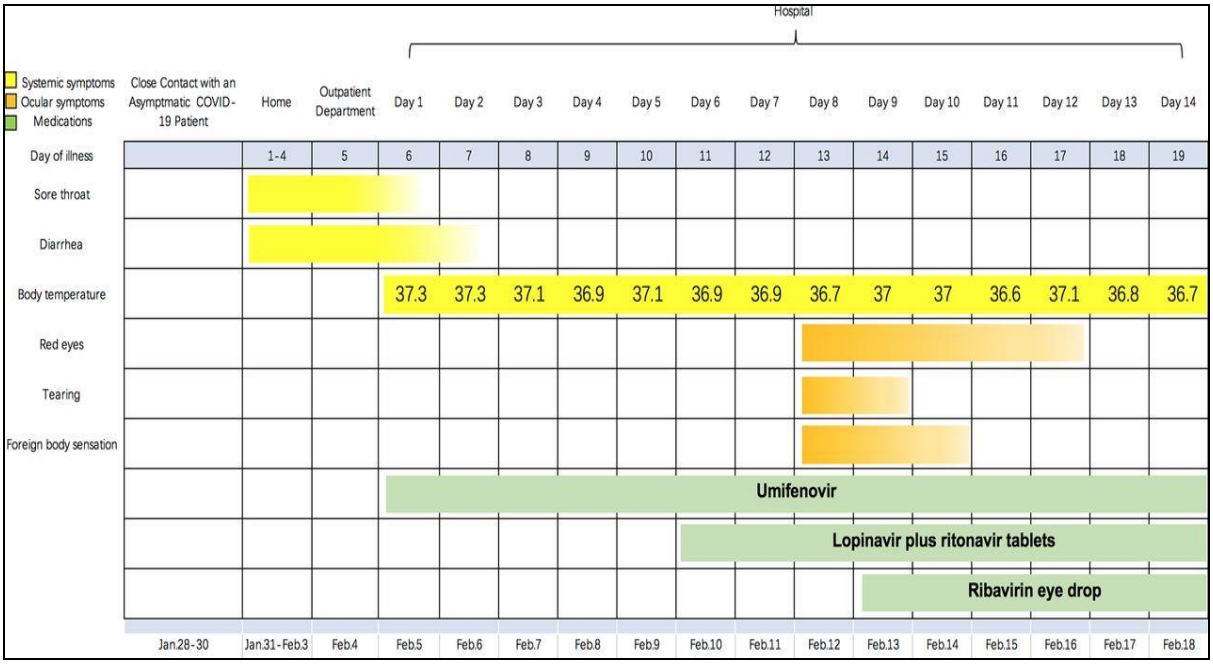

Fig 2: Timeline of occurrence of most common systemic symptoms and ocular symptoms according to day of illness and medication recommended by CDC.

Cutaneous and mucosal lesions related to Covid-19 have been described and reported by dermatologists. Several COVID-19 patients reported severe itchy hives and rashes, whereas, others indicated a burning sensation on their skin and mucosa. French dermatologist union, Le Syndicat National des Dermatologues-Vénéréologues (SNDV) revealed the potential new symptoms in a press release that was released on the April 6, 2020, after the SNDV communicated to a group of

over 400 dermatologists that, they identified signs of skin and mucosal lesions, both associated with and not associated with Covid-19. According to them, these include the appearance of pseudo-frostbite of the extremities, sudden appearance of persistent redness; and sometimes painful, temporary, hive-like lesions. Analysis of a number of cases reported to the SNDV shows that these signs may be associated with Covid-19. The rashes have appeared in different shapes and sizes and have manifested anywhere on the body [37].

Oral Manifestations of COVID-19

The only descriptive case report available is of a 45 year old female patient who presented with an irregular ulcer on the dorsal side of the tongue. History of the lesion revealed 24 h painful inflammation of a tongue papilla, followed by 24 h of erythematous macula, which evolved into irregular and asymptomatic ulcer. After 10 days, the ulcer completely healed without scar. 3 days after occurrence of the oral lesion, an erythematous plane lesion appeared on the big toe. It was also painful during 48 h and then became asymptomatic. General symptoms were mild asthenia. Considering the general aspects of the lesions, and despite a lack of general status alterations and symptoms, a nasopharyngeal Covid-19 test was performed at Day 8. The test was positive [38]. Another study reported in Spain [39] consisted of development of pain, desquamative gingivitis, ulcers and vesiculobullous lesions. Intraoral examinations should be carried out in patients affected or suspected, always when recommended protection measures are available to develop a better understanding of the disease. Oral lesions have been historically linked to infectious diseases as a prodromal syndrome or oral manifestation; and is of paramount importance in diagnosis and hence oral examination should be conducted to establish further evidence.

Table 2: Diagnosis and Treatment of acute oral mucosal lesions during the epidemic [40].

Type	Disease name	Fever characteristics	Identified with COVID-19	Proposed examination	Proposed treatment
Infectious disease	Acute herpes gingivostomatitis	Moderate to high fever	Yes	Oral mucosal examination	Systemic antiviral drugs; topical drugs
	Herpes zoster	Low fever		Oral mucosal examination	Systemic antibacterial drugs; topical drugs
	Coccigenic stomatitis	Low to moderate fever			
	Necrotic ulcerative gingiva-stomatitis	Low fever			
Allergic disease	Allergic medicamentosus stomatitis	Low fever during prodromal period	Yes	Oral mucosal examination	Antihistamine; Low-dose glucocorticoids; topical drugs
	Erythema mutiforme				
Erosive and ulcerative disease	Erosive oral lichen planus	Not accompanied	No	Oral mucosal examination	Immunosuppressant; Low-dose glucocorticoids; topical drugs
	Major aphthous ulcer	May be low fever	When needed	Oral mucosal examination	
	Herpetiform ulcers				
Bullous disease	Pemphigus vulgaris	Low fever on secondary infection	When needed	Oral mucosal examination, Indirect immunofluorescence	moderate-dose glucocorticoids; Immunosuppressant; topical drugs

Challenges in sample Collection: The traditional collection of upper respiratory tract specimens, such as nasopharyngeal swabs, throat swabs, nasal swabs and lower respiratory tract specimens such as sputum and bronchoalveolar lavage (BAL), has a series of drawbacks regarding collection time, healthcare staff exposure, patient's discomfort, use of specific instruments and mainly, difficulty or impossibility of self-collection, thus being one of the factors limiting the expansion of the tests. Saliva has been shown to be an interesting alternative for detection of viruses as oral shedding is more frequent than viremia. The use of saliva, following proper saliva collection and handling high-quality procedures, has a number of advantages, such as less invasiveness, easy collection, possibility of self-collection, less exposure of healthcare workers, shorter execution time, no need of specific instruments, possibility of serial sampling and development of point-of-care devices. The concordance rate of nasopharyngeal aspirate and saliva was 93% for influenza and respiratory syncytial virus. Saliva can be relevant also for the fact that in these samples can be investigated the virus in active replicative status, that likely is the transmissible form, in the asymptomatic patients [41].

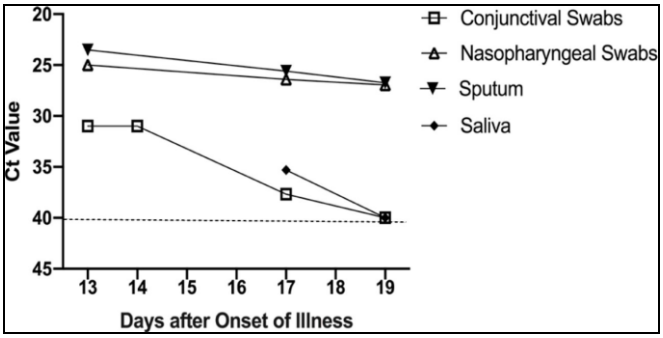

Fig 3: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detected in different types of clinical samples obtained from the case patient. Dynamic alterations of viral loads in nasopharyngeal swabs, sputum, saliva and conjunctival swabs were shown. Cycle threshold (Ct) values were inversely proportional to viral loads. Negative results are shown with a Ct value of 40.

Current Diagnostic tests for COVID-19 and its challenges

Molecular techniques are more suitable than syndromic testing and CT scans for accurate diagnoses because they can target and identify specific pathogens. According to the joint report by the World Health Organization (WHO) and China, 104 strains of the SARS-CoV-2 virus were isolated and sequenced using Illumina and Oxford nanopore sequencing from the end of December 2019 to mid-February 2020.

Nucleic Acid Testing: Nucleic acid testing is the primary method of diagnosing COVID-19. A number of reverse transcription polymerase chain reaction (RT-PCR) kits have been designed to detect SARS-CoV-2 genetically. The biggest challenge is the difficulty in optimizing the reverse transcription and amplification steps as they occur simultaneously, which leads to lower target amplicon generation. Currently, the United States Centers for Disease Control and Prevention (CDC) uses a one-step real time RT-PCR (rRT- PCR) assay, which provides quantitative information on viral loads, to detect the presence of SARS-CoV-2. There are three issues that have arisen with RT-PCR. First, the availability of PCR reagent kits has not kept up with demand. Second, community hospitals outside of urban cities lack the PCR infrastructure to accommodate high sample throughput. Lastly, RT-PCR relies on the presence of detectable SARS-CoV-2 in the sample collected. If an asymptomatic patient was infected with SARS-CoV-2 but has since recovered, PCR would not identify this prior infection, and control measures would not be enforced.

Computed Tomography: Due to the shortage of kits and a high false negative rate of RT-PCR, the Hubei Province, China temporarily used CT scans as a clinical diagnosis for COVID- 19. The most common hallmark features of COVID-19 include bilateral and peripheral ground-glass opacities (areas of hazy opacity) and consolidations of the lungs (fluid or solid material in compressible lung tissue). De Wever *et al*. found that ground-glass opacities are most prominent 0−4 days after symptom onset. As a COVID-19 infection progresses, in addition to ground-glass opacities, crazy-paving patterns (i.e., irregular-shaped paved stone pattern) develop, followed by increasing consolidation of the lungs. Based on these imaging features, several retrospective studies have shown that CT scans have a higher sensitivity (86−98%) and improved false negative rates compared to RT-PCR. The main drawback of using CT for COVID-19 is that the specificity is low (25%) because the imaging features overlap with other viral pneumonia. CT systems are expensive, require technical expertise, and cannot specifically diagnose COVID-19 [42].

Emerging Diagnostic Tests

According to the WHO, the immediate priority for COVID-19 diagnostics research is the development of nucleic acid and protein tests and detection at the point-of-care. In order to improve surveillance efforts, serological tests using proteins are needed in addition to nucleic acid tests. These tests have the benefits of detection after recovery, unlike nucleic acid tests. This enables clinicians to track both sick and recovered patients, providing a better estimate of total SARS-CoV-2 infections. Point-of-care tests are cost-effective, hand-held devices used to diagnose patients outside of centralized facilities. These can be operated in areas like community centres to reduce the burden on clinical laboratories.

Lateral flow antigen detection for SARS-CoV-2 is one point-of-care approach under development for diagnosing COVID-19. The lateral flow assay has demonstrated a clinical sensitivity, specificity, and accuracy of 57%, 100%, and 69% for IgM and 81%, 100%, and 86% for IgG, respectively. A test that detects both IgM and IgG yields a clinical sensitivity of 82%.

Another approach for use at the point-of-care is microfluidic devices. These devices consist of a palm-sized chip etched with micrometre -sized channels and reaction chambers. The key advantages of using microfluidics include miniaturization, small sample volume, rapid detection times, and portability.

Salivary Biomarkers and New Vista for Research

The origin of droplets can be nasopharyngeal or oropharyngeal, normally associated with saliva. Nasopharyngeal and oropharyngeal swabs obtained during the first 5 days symptomatic days has the highest viral loads of all specimen type and tested positive by RT-PCR. In an attempt to reduce false-negatives, diagnosis of COVID-19 can theoretically be performed using salivary diagnosis platforms. Some virus strains have been detected in saliva as long as 29 days after infection indicating that a non-invasive platform to rapidly differentiate the biomarkers using saliva could enhance disease detection. Saliva samples could be collected in patients who present with oropharyngeal secretions as a symptom. Bearing in mind the requirement of a close contact between healthcare workers and infected patients to collect nasopharyngeal or oropharyngeal samples, the possibility of a saliva self-collection can strongly reduce the risk of COVID-19 transmission. Besides, the nasopharyngeal and oropharyngeal collection promotes discomfort and may promote bleeding especially in infected patients with thrombocytopenia. The sputum of a lower respiratory tract was produced by only 28% of COVID-19 patients, which indicates a strong limitation as specimen to diagnostic evaluation. We suggest that there is a minimum of three different pathways for COVID-19 to present in saliva: firstly, from COVID-19 in the lower and upper respiratory tract that enters the oral cavity together with the liquid droplets frequently exchanged by these organs. Secondly, COVID-19 present in the blood can access the mouth via crevicular fluid, an oral cavity-specific exudate that contains local proteins derived from extracellular matrix and serum-derived proteins. Finally, another way for COVID-19 to occur in the oral cavity is by major- and minor-salivary gland infection, with subsequent release of particles in saliva via salivary ducts. It

is essential to point out that salivary gland epithelial cells can be infected by SARS-CoV a short time after infection in rhesus macaques, suggesting that salivary gland cells could be a pivotal source of this virus in saliva. Additionally, the production of SARS-CoV specific secretory immunoglobulin A (sIgA) in the saliva of animal models intranasally immunized was previously shown. Considering the similarity of both strains, we speculate that salivary diagnosis of COVID-19 could also be performed using specific antibodies to this virus [41] It is extremely important to describe this dynamic in asymptomatic and mildly symptomatic patients sent to home quarantine so that the appropriate period of isolation can be determined. This can only be possible by analysing serial saliva samples, which can be easily self-collected. Moreover, such an approach will provide important information on the transmission routes for establishing protective measures not only for the dental community, but also for controlling the current pandemic. The support for research involving the study of saliva in countries with foci of COVID-19 is of paramount importance, which can contribute to the application of diagnostic tests to large populations as well as to the understanding of the biological behaviour of the virus [43].

Conclusion

The use of saliva for diagnostic purposes opens the possibility of using other tools other than the direct detection of the pathogen, such as the use of proteomic, metabolomics, detection of antibodies, especially IgA, cytokines, chemokines, techniques in order to search for markers enabling the use of rapid diagnostic devices. In addition to the diagnosis itself, the study of saliva in cases of COVID-19 will help understanding its pathogenesis, since it has been recently reported that epithelial cells of the oral cavity showed abundant expression of the angiotensin-converting enzyme II (ACE2), a receptor playing a key role in the entry of SARS-CoV-2 into the cells. Development of therapeutics and vaccines is underway, but there are currently no United States Food and Drug Administration (FDA) approved therapeutics or vaccines for the treatment of COVID-19 patients.

References

1. Zhu N *et al*. A novel coronavirus from patients with pneumonia in China, 2019. N. Engl. J Med. https://doi.org/10.1056/NEJMoa2001017, 2020.
2. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. Lancet 2020; 395:470-473
3. Liu T. *et al*. Transmission dynamics of novel coronavirus (2019-nCoV). The Lancet, 2019 Available at www.thelancet.com.
4. Wu F *et al*. A new coronavirus associated with human respiratory disease in China. Nature, 2020
5. Gorbalenya AE *et al*. Severe acute respiratory syndrome-related coronavirus: The species and its viruses—a statement of the Coronavirus Study Group. Pre- print at https://www.biorxiv.org/content/10.1101/2020.02.07.937862v1, 2020.
6. Huang C *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497-506
7. Wang D *et al*. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA https://doi.org/10.1001/jama.2020.1585, 2020.
8. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The Reproductive Number of COVID-19 Is Higher Compared to SARS Coronavirus. J. Travel Med. 2020; 27(2),
9. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol. Biol. 2015; 1282:1-23
10. Gorbalenya A, Enjuanes L, Ziebuhr J, Snijder E. Nidovirales: evolving the largest RNA virus genome. Virus Res. 2006; 117:17-37
11. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat. Rev. Microbiol. 2009; 7:439-450
12. Weiss S, Leibowitz J. Coronavirus pathogenesis. Adv. Virus Res. 2011; 81:85-164
13. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology. 2018; 23:130-137
14. Holmes KV. SARS-associated coronavirus. N. Engl. J Med. 2003; 348:1948-1951
15. Falsey AR, Walsh EE. Novel coronavirus and severe acute respiratory syndrome. Lancet. 2003; 361:1312-1313
16. The Lancet. MERS-CoV: A global challenge. Lance. 2013; 381:1960
17. Al-Tawfiq JA, Zumla A, Memish ZA. Coronaviruses: severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus in travelers. Curr. Opin. Infect. Dis. 2014; 27:411-417
18. De Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat. Rev. Microbiol. 2016; 14:523-534
19. Al-Tawfiq JA, Zumla A, Memish ZA. Coronaviruses: severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus in travelers. Curr. Opin. Infect. Dis. 2014; 27:411-417
20. Bai Y, Nie X, Wen C. Epidemic prediction of 2019-nCoV in Hubei province and comparison with SARS in Guangdong province. The lancet. Available at SSRN: https://ssrn.com/abstract=3531427, 2020
21. Zhou P *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature https://doi.org/10.1038/s41586-020-2012-7, 2020.
22. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497-506
23. Din MA, Bopanna L. An update on the 2019 nCoV outbreak. American Journal of Infection Control, 2020
24. Su S, Wong G, Shi W, Liu J, Lai AC, Zhou J *et al*. Epidemiology, genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol. 2016; 24(6):490-502
25. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J *et al.* Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med https://doi.org/10.1056/ NEJMoa2002032
26. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January Euro Surveill, 2020.
27. Paules CI, Marston HD, Fauci AS. Coronavirus infections-more than just the common cold. JAMA. 2020; 323(8):707-708
28. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395(10223):507–513

29. Bagheri SHR, Asghari AM, Farhadi M *et al*. Coincidence of COVID-19 Epidemic and Olfactory Dysfunction Outbreak. Otolaryngology, 2020.
30. Giacomelli A, Pezzati L, Conti F *et al*. Self-reported olfactory and taste disorders in SARS- CoV-2 patients: a cross-sectional study. Clin Infect Dis, 2020:ciaa330.
31. Association of Chemosensory Dysfunction and Covid-19 in Patients Presenting with Influenza-like Symptoms. Carol H. Yan MD**.** https://doi.org/10.1002/alr.22579
32. Harvard COVID-19 basics. Symptoms, spread and other essential information about the new coronavirus and COVID-19. Updated May 5. https://www.harvard.edu
33. Anosmia and ageusia are emerging as symptoms in patients with COVID-19: What does the current evidence say? Beth Russell E cancer medical science. 2020; 14:98. Published online 2020 Apr 3. doi: 10.3332/ecancer.2020.ed98
34. Mao L, Wang M, Chen S *et al*. Neurological manifestations of hospitalized patients with COVID-19 in Wuhan, China: a retrospective case series study. MedR, 2020.
35. Li J-PO, Lam DSC, Chen Y *et al*. Novel Coronavirus disease 2019 (COVID-19): The importance of recognising possible early ocular manifestation and using protective eyewear Br J Ophthalmol. 2020; 104:297-298
36. Chen L, Liu M, Zhang Z *et al*. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. Br J Ophthalmol Epub ahead of printdoi:10.1136/ bjophthalmol-2020-316304)
37. New emerging dermatological symptoms in coronavirus pandemic. Letter to editor.
38. Oral manifestation of Covid-19 as an inaugural symptom? J Oral Med Oral Surg Letter to editor. 2020; 26(2).
39. Oral vesiculobullous lesions associated with SARS-CoV-2 infection Carmen Martín Carreras-Presa https://doi.org/10.1111/odi.13382
40. Guo Y, Yuan C, We C. Emergency measures for acute oral mucosa diseases during the outbreak of COVID-19. https://doi.org/10.1111/odi.13350.
41. Buddhisha Udugama *et al*. Diagnosing COVID-19: The Disease and Tools for Detection ACS Nano 2020; 14(4):3822-3835 DOI: 10.1021/acsnano.0c02624
42. Sabino-Silva R, Jardim ACG, Siqueira WL. Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. Clin Oral Invest. 2020; 24:1619-1621 https://doi.org/10.1007/s00784-020-03248-x
43. Silva P. SARS-CoV 2: What saliva can tell us? https://doi.org/10.1111/odi.13365