Human Saliva as a Liquid Biopsy for Detecting the SARS-CoV-2

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This invited perspective is being shared as a joint venture from a dental clinician cum researcher and a demanding clinical specialty , as our perspective to collaborate and promote the culture of teamwork. On 31st   December 2019, a case of an unknown type of Pneumonia from Wuhan, a city of China, was   reported to the World Health Organization (WHO) head office. The cause of this Pneumonia was identified and spread in the whole city within a few days. On 7th   Jan 2020, the virus was isolated by a Chinese group of researchers and the virus was named as "Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2)", and the illness caused by it is known as "Coronavirus Disease 2019 (COVID-19)", the digit 19, signifies its year of discovery as 2019. In the meantime, this virus was globally spread by travellers to other parts of the world, and   the transmission was confirmed as "human-to-human".   In figure-1, illustration representing the structure of 2019-nCoV, this illustration was created at the Centers for Disease Control and Prevention (CDC), revealing its ultrastructural morphology exhibited by coronaviruses.
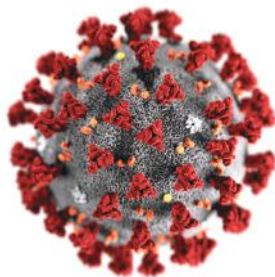
Figure 1: Illustration representing SARS-CoV-2 ultrastructural morphology (Courtesy to CDC, image library).

The clinical features of the infected COVID-19 patients include fever, cough, and shortness of breath, headache, nausea, vomiting, and   chest pain.[1] Only early detection can control this pandemic situation. For that, rapid diagnostic technology was started and reported by taking samples from blood, urine, oral swabs, oropharyngeal swab, and nasopharyngeal swab as well, as its exact source was uncertain.[2-4] All sampling is giving good sensitivity toward virus detection in infected patients but not promising for asymptomatic patients. A recently immunological diagnostic method reported detecting COVID-19 from Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor without the pretreatment and labelling.[5] All mentioned sampling is invasive or scary to the patients. This needed lot of fresh disposable safety gloves for each patient and personal protective equipment (PPE) for the entire health care professional team involved establishing detection. On 12th F ebruary 2020, a breakthrough was reported from Hong Kong on the detection of SAR-CoV-2 from human saliva.[6] In this study, they found a live virus load in the saliva of 12 confirmed infected COVID-19 patients, whereas other samples collected showed insignificant results. This study finding brought a revolution in the field of rapid detection for prevention and controlling the spread of the disease. After that, many studies reported on the human saliva collection for SARS-CoV-2, show detection in the early stages, so the load on the hospitals and quarantine center will reduce. In the USA, Prof Andrew Brooks from Rutgers University got emergency FDA approval for the saliva testing for SARS-CoV-2. This method is non-invasive, technique - simple, secure, cost-effective and does not require a lot of fresh gloves or PPE for all the healthcare professionals.[7] There are a lot of salivary biomarkers reported in the past for the detection and management of oral and systematic diseases such as cardiovascular diseases, arthritis, pulmonary disorders, diabetics, oral cancer, lung cancer, breast cancer, oral lichen planus, dental caries, and periodontal diseases.[8-10] Human saliva contains proteins and peptides such as defensins[11], cathelicidins[12], histatins[13], and many more for helping as biomarkers for early disease detection.[8]

By optimizing salivary biomarkers and immunoglobulins on the microfluidic chip technology, and biosensor will help

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in rapid detection of oral and systematic diseases.[14] A diagnostic validity reported by To K.K.W et al. on the saliva and nasopharyngeal aspirate (NPA) virus specificity by using Xpert® Xpress Flu/RSV kits.[15] A Total of 241 patients was recruited and observed that the saliva and NPA have no significant difference for virus load. This study has proven the comfortability of sampling against NPA collection and low-cost sampling of saliva as well.[15]

The billion-dollar question raised now, "Is the Oral cavity a bed for SARS-CoV-2 and is it?". So, the answer will be "Yes", because of the expression of the angiotensin-converting enzyme 2 (ACE2) receptors in the oral tissues and its help to SARS-CoV-2 spike proteins to bind and infect the salivary glands as well.[16,17] Recently, Chen et al. observed 2019-nCoV nucleic acid in the saliva, collected directly from the opening of the salivary gland.[18] Another study reported from Italy, a total of 25 patients were recruited in the saliva sampling after the confirmation of SARS-CoV-2 infection.[19] A conspicuous observation was about two patients not positive to pharyngeal or bronchoalveolar swabs, but they were positive on salivary results. So, this should be evident that there is a strong relation of saliva to help in the detection of SARS-CoV-2 in symptomatic or asymptomatic patients.[20] An observational cohort study was reported for the monitoring of the viral load with the use of posterior oropharyngeal (deep throat) saliva in 12 patients.[4] This self-collected sampling method help also to proven the prevention of virus contamination between patient s and health workers for monitoring viral load.[4] As a consultant Oral & Maxillofacial Surgeon, we have made policy matter, to mandatory use injection glycopyrrolate, known to improve sialorrhea for all emergency trauma and life-threatening spreading odontogenic infection cases to decrease salivary secretions and its associated COVID-19 viral load in the oral cavity. This, we hope, will yield great benefits to minimize the chances of infection.[20,21,22]

Since Dental Profession is predominantly focused on addressing Oral health issues, and the recent discoveries about saliva as a lethal carrier for COVID-19 should be seen as "a paradigm shift". We also feel, who knows what long term profound impact this virus will have on future clinical dental practices. To summarize, saliva is a reliable biopsy for the rapid and early diagnostics in this COVID-19 pandemic situation. Therefore, it is essential to consider more studies on the immunological and virological levels to understand more about the benefits of saliva for future point-of-care (POC) technology.[23]

REFERENCES

1. 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 [Internet]. 2020 Feb 28 [cited 2020 Apr 28];NEJMoa2002032. Available from: *http://www.nejm.org/doi/10.1056/NEJMoa2002032*

2. Chen C, Gao G, Xu Y, Pu L, Wang Q, Wang L, et al. SARS-CoV-2-Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19. Ann Intern Med. 2020 Mar 30; *https://doi.org/10.7326/M20-0991*

3. Zhang W, Du RH, Li B, Zheng XS, Yang X Lou, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect. 2020;9:386-89. *https://doi.org/10.1080/22221751.2020.1729071*

4. To KK, Tak O, Tsang Y, Leung W, Tam AR, Wu T, et al. Articles Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;3099:1-10.

5. Seo G, Lee G, Kim MJ, Baek S-H, Choi M, Ku KB, et al. Rapid Detection of COVID-19 Causative Virus (SARS-CoV-2) in Human Nasopharyngeal Swab Specimens Using Field-Effect Transistor-Based Biosensor. ACS Nano [Internet]. 2020; Available from: *http://www.ncbi.nlm.nih.gov/pubmed/32293168* *https://doi.org/10.1021/acsnano.0c02823*

6. To KK-W, Tsang OT-Y, Chik-Yan Yip C, Chan K-H, Wu T-C, Chan JMC, et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis [Internet]. 2020;(Xx Xxxx):4-6. Available from: *http://www.ncbi.nlm.nih.gov/pubmed/32047895* *https://doi.org/10.1093/cid/ciaa149*

7. Khurshid Z, Asiri FYI, Al Wadaani H. Human Saliva: Non-Invasive Fluid for Detecting Novel Coronavirus (2019-nCoV). Int J Environ Res Public Health. 2020;17:10-4. *https://doi.org/10.3390/ijerph17072225*

8. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of Salivary Biomarkers in Oral Cancer Detection. Adv Clin Chem. 2018;86:23-70. *https://doi.org/10.1016/bs.acc.2018.05.002*

9. Khurshid Z, Zafar M, Khan E, Mali M, Latif M. Human saliva can be a diagnostic tool for Zika virus detection. Vol. 12, Journal of Infection and Public Health. Elsevier Ltd; 2019. p. 601-04. *https://doi.org/10.1016/j.jiph.2019.05.004*

10. Sahibzada HA, Khurshid Z, Khan RS, Naseem M, Siddique KM, Mali M, et al. Salivary IL-8, IL-6 and TNF-a as Potential Diagnostic Biomarkers for Oral Cancer. Diagnostics [Internet]. 2017 [cited 2020 Apr 27];7:21. Available from: *www.mdpi.com/journal/diagnostics* *https://doi.org/10.3390/diagnostics7020021*

11. Khurshid Z, Zafar MS, Naseem M, Khan RS, Najeeb S. Human Oral Defensins Antimicrobial Peptides: A Future Promising Antimicrobial Drug. Curr Pharm Des. 2018;24:1130-7. *https://doi.org/10.2174/1381612824666180403114615*

12. Khurshid Z, Naseem M, Asiri FYI, Mali M, Khan RS, Sahibzada HA, et al. Significance and diagnostic role of antimicrobial cathelicidins (Ll-37) peptides in oral health [Internet]. Vol. 7, Biomolecules. 2017 [cited 2020 Apr 28]. Available from: *https://search.proquest.com/docview/1988597951?pq-origsite=gscholar https://doi.org/10.3390/biom7040080*

13. Khurshid Z, Najeeb S, Mali M, Moin SF, Raza SQ, Zohaib S, et al. Histatin peptides: Pharmacological functions and their applications in dentistry. Vol. 25, Saudi Pharmaceutical Journal. Elsevier B.V.; 2017. p. 25-31.
https://doi.org/10.1016/j.jsps.2016.04.027

14. Khan R, Khurshid Z, Yahya Ibrahim Asiri F. Advancing Point-of-Care (PoC) Testing Using Human Saliva as Liquid Biopsy. Diagnostics [Internet]. 2017 [cited 2020 Apr 28];7:39. Available from: *https://search.proquest.com/docview/1952490728?pq-origsite=gscholar https://doi.org/10.3390/diagnostics7030039*

15. To KKW, Yip CCY, Lai CYW, Wong CKH, Ho DTY, Pang PKP, et al. Saliva as a diagnostic specimen for testing respiratory virus by a point-of-care molecular assay: a diagnostic validity study. Clin Microbiol Infect. 2019;25:372-78.
https://doi.org/10.1016/j.cmi.2018.06.009

16. Xu R, Cui B, Duan X, Zhang P, Zhou X, Yuan Q. Saliva: potential diagnostic value and transmission of 2019-nCoV. Int J Oral Sci. 2020;12:11.
https://doi.org/10.1038/s41368-020-0080-z

17. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci [Internet]. 2020;12:1-5. Available from: *https://doi.org/10.1038/s41368-020-0074-x*

18. Chen L, Zhao J, Peng J, Li X, Deng X, Geng Z, et al. Detection of 2019-nCoV in Saliva and Characterization of Oral Symptoms in COVID-19 Patients. SSRN Electron J. 2020;
https://doi.org/10.2139/ssrn.3557140

19. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, et al. SALIVA IS A RELIABLE TOOL TO DETECT SARS-CoV-2. J Infect [Internet]. 2020 Apr 14 [cited 2020 Apr 15]; Available from: *https://linkinghub.elsevier.com/retrieve/pii/S0163445320302139*

20. Xu J, Li Y, Gan F, Du Y, Yao Y. Salivary Glands: Potential Reservoirs for COVID-19 Asymptomatic Infection. Journal of Dental Research. 2020. p. 22034520918518.
https://doi.org/10.1177/0022034520918518

21. MEL Arbouw, KLL Movig, M Koopmann, PJE Poels: Glycopyrrolate for sialorrhea in Parkinson disease: a randomized, double-blind, crossover trial: Neurology; April 13, 2010; 74 (15)
https://doi.org/10.1212/WNL.0b013e3181d8c1b7

22. Katie T. Blissit et all;: Glycopyrrolate for treatment of clozapine-induced sialorrhea in adults; American Journal of Health-System Pharmacy, Volume 71, Issue 15, August 2014: 1282-1287
https://doi.org/10.2146/ajhp130636

23 Hamid, H., Khurshid, Z., Adanir, N., Zafar, M. S., & Zohaib, S. (2020). COVID-19 Pandemic and Role of Human Saliva as a Testing Biofluid in Point-of-Care Technology. European Journal of Dentistry.
https://doi.org/10.1055/s-0040-1713020