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Is your saliva the perfect test for corona virus? - Analysis of Salivary Diagnostics and its Significance in Covid19.

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ABSTRACT

The Current COVID-19 pandemic threatens the medical community with several theories. Because of its higher rate of transmission, the clinical and epidemiological findings of COVID -19 remain vague. On COVID-19, their understandings are expressed from every corner of the world but the meaning of those understandings is still uncertain. The development of timely, cost-effective, reliable and non-invasive diagnostic methodologies is now an important activity for both clinicians and scientists. Detecting early-stage pathologies may have a major effect on patient pain, prognosis, clinical intervention, survival rates and recurrence. As a diagnostic fluid, saliva has tremendous potential and provides an advantage over other biological fluids, as its method of processing does not entail invasive, costly procedures and is useful for monitoring systemic health. The scientific community is in desperate need of an important review of this COVID-19 hypothesis. In this prospective review we will discuss the saliva as a source of markers for local, systemic and infectious disorders and its importance. In addition, we will discuss the current state of salivary diagnostics and related tools, future expectations, and potential as the chosen route for disease identification, control, and prognostics.

Key words: COVID-19–Saliva–Biomarker

1 INTRODUCTION

Coronavirus disease 19 or COVID-19 was first identified in December 2019 and was confirmed in Wuhan, China[1−2]. According to WHO estimates, it has now spread quickly in too many nations, becoming a global pandemic, affecting 4,993,470 people worldwide and causing 327,738 deaths as of 23rd May 2020 [3]. Saliva is an exocrine secretion generated from the salivary glands and has various functions including oral cavity cleaning and defence, antimicrobial effects and digestive aids. Owing to the rapid progress of salivaomics, saliva is now well established as a collection of biological markers ranging from changes in biochemicals, nucleic acids and proteins to the microflora. As a diagnostic fluid, saliva has tremendous potential and provides an advantage over other biological fluids, as its method of processing does not entail invasive, economical procedures and is useful for monitoring systemic health. In the near past, the invention of responsive and reliable salivary diagnostic tools and the formulation of established guidelines after careful testing have

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allowed the use of salivary diagnostics as chair side tests for various oral and systemic diseases[4]. In this article, we will briefly illustrate the potential function of saliva / salivary glands to examine the Coronavirus 19 novel, and suggest other ways in which these studies can be put into motion.

The pandemic of the coronavirus disease (Covid-19) is the world's greatest threat and public health issue since World War Two. The suggested specimen forms for Covid-19 diagnostic testing include nasopharyngeal and oropharyngeal swabs. Collecting these types of specimens involves close contact between healthcare workers and patients, and creates a risk of virus transmission, causes irritation, and can cause bleeding, particularly in patients with conditions such as thrombocytopenia. Therefore, nasopharyngeal or oropharyngeal swabs are not suitable for sequential viral charge control. Specimens of saliva can be quickly collected when the patient is being asked to spit into a sterile container. The saliva collection is non-invasive and significantly minimizes exposure to Covid-19 by healthcare staff. In the identification of respiratory viruses, including coronaviruses, saliva has a high consistency rate greater than 90 percent with nasopharyngeal specimens.

Comparison with SARS-CoV-2 & MERS - CoV: COVID-19, caused by SARS-CoV-2 (severe acute respiratory coronavirus syndrome 2) initially known as 2019-nCoV, belongs to the genus Betacoronavirus and is the third animal coronavirus infection affecting humans[5]. Only with the outbreak of two life-threatening epidemics, SARS (Severe Acute Respiratory Syndrome) in China in 2002-2003 and about decade years later MERS (Middle East Respiratory Syndrome) in the Middle East Countries, was considered the high pathogenic potential of coronaviruses to affect people[6]. Both the coronavirus SARS (SARS-CoV) and coronavirus MERS (MERS-CoV) are known to originate from bats. It has been shown that the genetic code of SARS-CoV-2 is 79.6 percent identical to that of SARS-CoV and 96 percent identical to a coronavirus bat[7]. SARS-Cov, SARS-Cov2 & MERS- Cov triggers variable-severe epidemics of respiratory and extra respiratory manifestations. The mortality rate for SARS-Cov & MERS-Cov was 10 per cent and 35 per cent respectively. Table (Table 1) below summarizes the similarities and disparities in saliva, in terms of diagnosis value of saliva, direct invasion to oral tissues and saliva droplet transmission between SARS-CoV and 2019–nCoV , hopefully explaining the pace of 2019-nCoV transmission. Given the initial zoonotic existence of COVID-19, human-to-human interaction with the usual clinical symptoms of fever, often unproductive cough, malaise, dyspnoea and pneumonia is now rapidly spread[7−8]. Some unusual signs include sputum, hemoptysis, fatigue and gastrointestinal symptoms such as diarrhea, nausea and vomiting[9]. Transmission may also occur early in the disease cycle, often prior to the signs, demonstrating asymptomatic or slightly symptomatic patient's transmission potential[10]. Despite COVID-19's novelty, certain virus characteristics remain unknown yet.

Given that COVID-19 has recently been detected in infected patients' saliva[11], the COVID-19 outbreak is a warning that dental / oral and other health professionals must still be vigilant in protecting against the spread of infectious disease, and it offers an opportunity to assess if a non-invasive COVID-19 treatment of saliva may help identify these viruses and minimize the spread. SARS-cov2 detected in aerosols for 3 hours, copper for up to 4 hours, cardboard for up to 24 hrs and plastic and stainless steel for up to 2-3 days.

Table 1.

Table 1. Comparison of 2019-nCoV and SARS-CoV in terms of saliva

Items	2019-nCoV	SARS-CoV
Diagnostic value of saliva	1. (1) 2. (2) 3. (3)	1. (1) 2. (2) 3. (3)
Direct invasion to oral cavity	1. (1) 2. (2)	ACE2 receptor on host cells of tongue and salivary gland
Infectious saliva droplets	Possible opportunistically airborne transmission	Opportunistically airborne transmission.

2 SALIVA AS A BIOMARKER

The idea of salivaomics was introduced in light of the rapid progress produced in salivary studies. Salivaomics includes the study of metabonomics, genomics, proteomics, transcriptomics and microRNA (miRNA). Wong was responsible for developing a technical knowledge base for salivaomics (SKB) that could regularly handle the salivaomics research data[12]. With the advancement of precise molecular methods and nanotechnology, limitations regarding the use of saliva for diagnosis due to its low concentration of analytes compared to blood were overcome[13].

Similar to SARS-CoV, during close, unprotected contact between infector and infectee, Covid-19 can be effectively transmitted between humans through droplets and fomites. Corona viruses are the ones that have a good understanding of a single-stranded RNA genome[14] (26-32 kb). To date, four subfamily of coronaviruses (α, β, π, ÿ) have been identified with human coronaviruses (HCoVs) found in the genera α coronavirus (HCoV-229E and NL63) and β coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1).[15] Coronavirus S protein was recorded to be a major determinant of virus invasion into host cells. The envelope spike glycoprotein binds to its cellular receptor, SARS-CoV and SARS-CoV-2 to ACE2, CD209L (a lectin type C, also called L-SIGN) for SARS-CoV, and MERS-CoV to DPP4.[16]

Virus genome sequencing of individuals with pneumonia admitted in the hospitals in the month of December, 2019 confirmed the existence in all of them of a previously identified β-CoV strain[17]. This isolated novel β-CoV shows 88 percent identity to the sequence of two extreme bat-derived acute respiratory syndromes (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, and about 50 percent identity to the MERS-CoV sequence.[17]

COVID-19 patients exhibit clinical symptoms including fever, non-productive cough, dyspnea, myalgia, weakness, normal or reduced leukocyte counts, and pneumonia radiographic data. The cytokine storm, the deadly unregulated systemic inflammatory response resulting from the release of large quantities of pro-inflammatory cytokines (IFN-a, IFN-g, IL-1b,IL-6,IL-12,IL-18,IL-33, TNF-a, TGF-b, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9,CXCL10, etc.) is one of the important pathways for acute respiratory distress syndrome ARDS.

3 PROSPECTIVE

The diagnostic importance of saliva seems to depend upon how specimens are obtained from saliva. Several virus specimens have been found in saliva up to 11 days after infection, suggesting that a non-invasive mechanism for rapidly differentiating biomarkers using saliva will improve the detection of diseases[18]. The existence of the COVID-19 virus in the saliva may derive either from the salivary glands via the ducts or from the GCF (from gingiva) or simply from the lower and upper respiratory tract secretions that mix with the saliva[20]. Only 28 per cent of COVID-19 patients developed sputum from a lower respiratory tract, suggesting a significant limitation as a specimen for diagnostic evaluation[11]. In the identification of respiratory viruses, including coronaviruses, saliva has been shown to have a high consistency rate of over 90 per cent with nasopharyngeal specimens[19]. It has been shown that the ACE2 epithelial cells of the salivary glands are an initial site for SARS-CoV in rhesus macaques, early in the disease cycle[21]. Cellular protease, furin, mRNA and protein levels differ by cell type and were found to be elevated in the salivary glands[22]. Likewise, TMPRSS2 expression was also seen in the salivary glands[23]. Thus, with further studies, consideration and validation should be given to the possibility of the role of salivary gland cells in initial entry, progress of infection and as a source of the virus. This may also be the explanation for the spread of infection between asymptomatic cases, as the organism is confined and continues to spread within the salivary glands and has not yet spread to the respiratory tract.Also consideration should be given to the possibility of salivary glands as storage, actively supporting latent infection which may reactivate later, and this demands further study. Xu et al.[24] stated that ACE2 is overwhelmingly expressed in the oral mucosal epithelial cells, with higher tongue expression relative to the buccal and gingival tissues. Such results suggest high susceptibility to COVID-19 infection is present in the oral cavity.

Saliva is a common and transient virus-transmitting medium. Among saliva particles of varying sizes produced by sneezing, breathing and talking, large droplets easily drop to the floor and only set short-distance transmission[25]. In a favourable climate, saliva may form aerosols and enter a distant host along the airflow[25]. So far, there is no strong evidence to support SARS-nCoV or 2019-nCoV being able to live in air outdoors for a long time to establish transmission of long distance aerosols. Therefore, wearing masks to prevent formation of infectious saliva droplets projecting to the air, thorough indoor air disinfection to block the spread of infectious saliva droplets and keeping a distance from people could slow down the 2019-nCoV epidemic to some extent(Fig. 1).

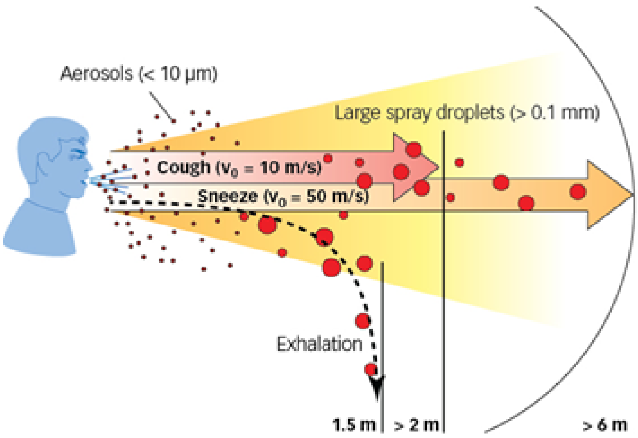

Figure 1. How COVID 19 is transmitted through aerosol particles

Figure 1.

4 CONCLUSION

The holy grail of molecular diagnostics is precisely and accurately early-stage disease identification combined with non-invasive sample processing modes. The use of saliva samples may minimize the risk of nosocomial Covid-19 distribution and is suitable for circumstances where the processing of oro - or nasopharyngeal specimens may be contraindicated. It can also reduce or remove the need to collect samples for healthcare workers. Saliva can play a key role in species-to-species transmission, and salivary lab tests can provide a simple and cost-effective point-of-care tool for infection with COVID-19. In addition, any other receptors or cellular proteases that may shed more light on the pathogenesis of the pandemic disease may open the door for targeted drug therapies. Further research are required to report the potential diagnosis of COVID-19 in saliva and its influence on the transmission of this virus, which is important for improving effective prevention strategies, especially for dentists , surgeons and healthcare providers who generate aerosol performing procedures.

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