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Saliva Biofluid: Transmission Source and Potential Diagnostic Specimens for Covid-19 – A Review

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ABSTRACT

Covid-19 was first reported in Wuhan, China, due to the case of pneumonia. This virus spread through droplets from humans with or without symptoms. Angiotensin-converting enzyme 2 (ACE-2), the receptor for Covid-19, facilitates the viral spike (S) protein entry to the target cells. This virus is reported to accumulate in nasal, oral, and oropharyngeal and will further accumulate in the lungs. The number of ACE-2 receptors in salivary glands is higher than those expressed in the lungs. It was suggested that the salivary gland is a reservoir for Covid-19, especially in asymptomatic patients. ACE-2 receptors are also highly enriched on the tongue, gingiva, and the floor of the mouth. Viral RNA was also detected in saliva at high titers. The diagnosis of Covid-19 was proved using a nasopharyngeal and oropharyngeal swab. Sampling specimens requires direct contact between medical personnel and patients; thereby, it can facilitate healthcare-associated infections. Specimens collected from this area causes discomfort for the patient and may cause a risk of bleeding. In addition, those swab specimens are not desirable for monitoring the viral load of Covid-19. In contrast, taking saliva specimens is easier to carry out by patients, as well as more comfortable and non-invasive. Thereby, it can potentially reduce the risk of transmission to medical personnel. Collected information suggested that saliva is a great potential candidate for Covid-19 diagnosis and monitoring. This review aims to collect information on the potential of saliva as a diagnostic tool for Covid-19 and the impact of its transmission.

Keywords: *Covid-19, saliva, salivary diagnostic, SARS-CoV-2, transmission, viral load*

1. INTRODUCTION

Covid-19 is an acute atypical respiratory disease that was first reported in Wuhan, Hubei Province, China, in December 2019. This disease is caused by a novel coronavirus, which is termed as Severe Acute Respiratory Syndrome 2 (SARS-CoV-2). Genetically, this virus has homology about 79% with SARS-CoV and 50% with Middle East respiratory syndrome coronavirus (MERS-CoV). However, it was reported that Covid-19 is more severe compared to those two diseases [1]. The transmission rate of Covid-19 is very fast, and currently, it was reported that it has spread in more than 200 countries in the world. On March 11th, WHO announced this disease as a pandemic [2].

The transmission route of this disease is reported through airborne (droplets/ aerosol), direct contact, and indirect contact. The airborne spread can be classified as splatter, droplets, and aerosol, both solid and liquid particle, which has diameter >50 μm, >10 μm, and < 5 μm, respectively. All of them can be produced by a sneeze, coughing, or respiratory secretion from the nose or mouth. The salivary droplets are the primary source of the particle out hale from the mouth [3]. Saliva is an oral biofluid that has a function to help mastication, make the food wet, therefore, helps the food easy to swallow. Salivary glands produce saliva from major and minor salivary glands. The composition of saliva is 99% water, and 1% is organic and inorganic materials. Salivary droplets originate from saliva itself, which contains bacteria, fungi, viral, epithelial desquamation, crevicular fluid, food debris, and even blood. The composition in the saliva is reflected in the systemic conditions in the body. Many studies reported the utilization of saliva as a medium to diagnose cardiac, diabetes, kidney failures, infections due to microorganisms, including viruses [4]. Salivary droplets that are secreted from the mouth have a heterogeneous size, which can remain in the air and travel or moving depending on the airflow direction. Since saliva is a biological fluid in the oral mouth, which can easily spread out of the body, it should be considered as the primary source of transmission of Covid-19 [5]. This review aims to collect information on the potential of saliva as a diagnostic tool for Covid-19 and the impact of its transmission.

2. COVID-19 STRUCTURE

Coronavirus is an enveloped positive-strand that has a length of ~30 kb and 50-200 nm in size. According to the genomic structure, this virus is classified into 4 genera, namely; α, β, γ, and δ. SARS-COV-2 belongs β coronaviridae family, which is one family member with SARS-CoV and MERS-CoV [6]. The main structure of this virus consists of Spike (S) protein, E protein, M protein, and Nucleocapsid, which store RNA inside it (Figure 1).

Spike (S) protein has a function to facilitate the virus to enter the host cells. When it enters the host cell, spike (S) protein will be cleavage into S1 and S2. Spike1 (S1) has a role in binding to the plasma membrane of the host cell, while S2 facilitates fusion into the host cell. The membrane M glycoprotein has the function of supporting the shape of the virus. Envelope (E) protein, which is the smallest protein, has a function for the production and maturase of this virus. The nucleocapsid is structurally attached to the nucleic acid and is located in the endoplasmic reticulum-golgi apparatus [7].

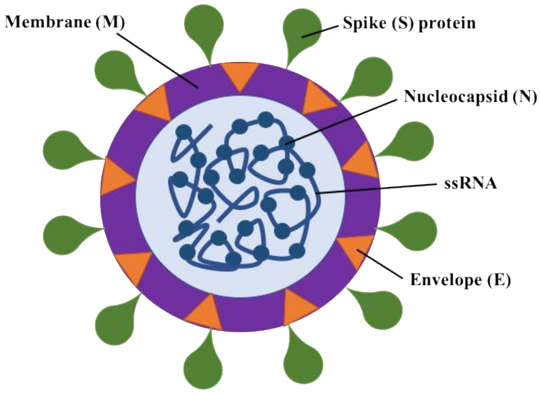

Figure 1 Structure of Covid-19. Main structure of SARS-CoV-2; Spike (S) protein (green), Envelope protein (orange), Membrane protein (purple), Nucelocapsid (blue dot), and single stranded RNA (blue). *Source:* Figure modified from [8].

3. PATHOGENESIS OF COVID-19

The virus life cycle is divided into 5 stages, namely, attachment, penetration, biosynthesis, maturation, and release [6]. The entry of the Covid-19 virus begins with the entry of the virus into the body of the host cell. The entry of the virus into the body can be directly through the inhalation of droplets or aerosols into the nasal mucosa or through direct contact with saliva, touched by hands, and enters the body through the oral mucosa, nose, or eyes. Moreover, it can be through indirect contact by touching objects that have been contaminated by humans confirmed positive for Covid-19 [9].

SARS-CoV-2 through the Spike (S) protein will first attach to the surface of the host cell membrane in the angiotensin-converting enzyme 2 (ACE-2) receptor, called attachment. After being attached to the plasma membrane, the virus will fusion with the plasma membrane or enter the cell through endocytosis, called penetration. Following attachment to the plasma membrane, the spike (S) protein can be activated through 2 step-sequential protease cleavages, produce S1 and S2 cleavage site, and generate S_1 and S_2 subunits, respectively. The S1 subunit is responsible for binding to the plasma membrane, while the S2 subunit has a role for the fusion of the viral and plasma membrane. After entering the cells, the viral RNA will translocate into the nucleus for replication. The mRNA virus is used to produce viral protein and continued with assembling, called biosynthesis. The new viral protein is produced and finally released out from cells through exocytosis (Figure 2)[6],[7].

4. POSSIBLE INVASION OF SARS-CoV-2 to ORAL TISSUE

It has been reported that ACE-2 plays a critical role in the entry of the virus to the host cells and causes infection. Angiotensin-converting enzyme 2 expressions were found in the intestine, stomach, bile duct, liver, lung, thyroid, esophagus, bladder, breast, uterus, prostate, and oral cavity. This receptor is evenly distributed in several locations of the oral cavity. Oral tongue showed the highest expression followed by the floor of the mouth, base of the tongue, and other sites such as buccal or gingival tissue [10]. It was also reported the high expression of ACE-2 in the salivary gland compared to the lung. This salivary gland can be a reservoir for this virus, especially in asymptomatic patient [11]. Since the attachment of SARS-CoV-2 to the host cell goes through the ACE-2 receptor, it makes the oral cavity very susceptible to this virus.

5. POTENTIAL TRANSMISSION OF COVID-19

SARS-COV-2 is transmitted from an infected person to a susceptible person through 3 modes of transmission, such as airborne, direct, and indirect modes.

5.1. Airborne

The airborne transmission is generated by respiratory droplets through breathing, speaking, singing, coughing, and sneezing from the nose or mouth from an infected person to a susceptible person. The particles produced from droplets is in the various size and carry the particle of the virus [12].

5.1.1. Salivary droplets

The salivary droplets are comprised of bacteria, fungi, viral, exfoliated epithelial cells, food debris, serum element, white blood cells, gingival crevicular fluid [5]. The particles of droplets have a diameter of >10 μm and fall immediately to the ground after being exhaled from the mouth. The size particle of droplets is less than splatter. Spatter is defined as large particles of aerosol, which is > 50 µm. These particles cannot remain longer in the air and reach the area < 1m. Splatter can evaporate, leaving small particles called droplets nuclei, containing microorganisms and transmitting various diseases, including SARS-CoV-2. Large droplets produced by speaking activity takes a much longer time to sediment affected by evaporation and that it can increase viral air load. A person who speaks frequently can produce a steady-state air load more than 10^4 virions at a given time without being covered by mask. Droplets generated from speech and visualized under green laser light has approximately 1 mm in thickness and 150 mm in length. When the word "stay healthy" is pronounced, the size of the droplets ranging from 20 to 150 µm is generated. Similarly, if the mouth is covered with the cloth, the speaking-generated droplets are diminished [13]. It was reported that single strong cough-generating droplets can move to the air and reach less than 1.5 m in distance. The droplets exponentially increase with its size and ambient relative humidity (above 37%). Low relative humidity increases the evaporation and results in the remaining large droplets suspended in the air [14]. This finding was considered as the potency of the droplets-carrying virus in an indoor area with setting operation of humidity like hospitals, schools, department stores, as preventing the spreading of Covid-19.

5.1.2. Salivary Aerosol

The small particle size is suspended in a gas and can be respired generated by breathing and talking called aerosol or droplets nuclei [13]. The diameter of the aerosol is reported < 5 µm. Other research reported the heterogeneity of aerosol ranging from 0.001 to 100 µm. The smallest size of these particles can be remained in the air and traveled to other areas based on the air turbulence before it is dropped by gravity [15]. The simulation of coughing-generating aerosol spreading inside the examination room can be achieved by 5 minutes, take 9 minutes to reach the ground, and the clearance depends on the air circulating changes per hour. The people inside that room are in high potential in getting exposed to hazardous aerosols due to the jet of aerosol particles produced by coughing. Meanwhile, sneezing can travel up to 4.5 m in the distance. Dentists and dental clinicians are in high potential risk to Covid-19 due to aerosol-generating procedures, such as tooth preparation using high speed, ultrasonic scaler, and three-way syringe. The study simulation using an ultrasonic scaler with phantom jaws on a dental chair showed a surprising result. Immediately after scaling, the contamination was detected in various areas in the body, including the head, chest, and inner surface of the face mask of the operator and the assistant. The aerosol remained in the air 30 minutes after the scaling process [16]. Droplets and aerosol have different deposition sites in recipients when they are inhaled. The droplets can be deposited in the upper region of the respiratory tract, while the aerosol can be deeply penetrated in the alveoli of the lung. The exposure to the risk of the hazardous aerosol tends to occur to people without a mask, such as a surgical mask and N95 filtering-facepiece respirator (N95 FFR), which have filtered 95% of 0.1-0.3 µm aerosol particles·[17].

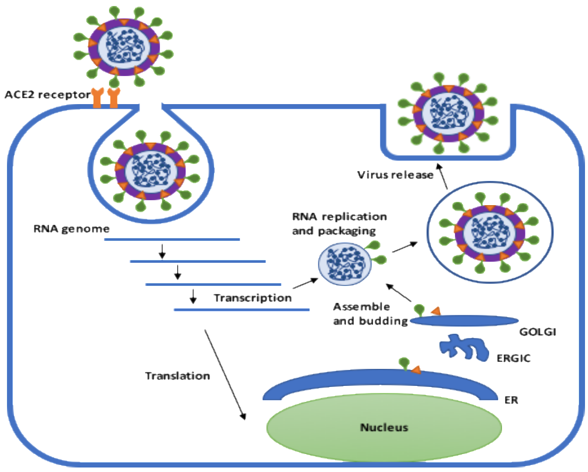

Figure 2 Pathogenesis of Covid-19. Five life cycle of virus; attachment, penetration, biosynthesis, maturation, and release. SARS-CoV-2 through the Spike (S) protein will first attach to the surface of the ACE-2 receptor (orange) followed by fusion with the plasma membrane through endocytosis (penetration). Viral RNA will translocate into the nucleus for replication continued with assembling, (biosynthesis). The new viral protein is produced and released out from cells through exocytosis (mature and release). *Source:* Figure modified from [18].

5.2. Direct Transmission

COVID-19 not only can be transmitted through the inhalation of droplets and aerosol, but also it can be spread by direct or indirect contact with the oral mucosa, nasal cavity, and eye mucous. Direct transmission can be defined as touching directly the saliva, patient's excrement, and other pollutants of confirmed Covid-19 patients. Dentists are a high-risk profession to Covid-19 due to the direct contact of the dental procedure with the oral cavity containing saliva. This saliva can directly be landed on the eye mucosal or nasal cavity if the dentist or health worker does not wear eye protection or mouth-nose cover mask [19].

5.3. Indirect Transmission

The indirect transmission of SARS-CoV-2 means that this virus can persist in the environment. The virus can remain in the fomites after the droplets/aerosol/splatter fall. The virus can contaminate the environment of a public area or private area, and viruses can survive on the surface of objects for some time. Investigation in a home environment in Guangzhou, China reported that SARS-CoV-2 was detected in the door handle at the home of a positive patient, and one person from a different family was infected after touching the button of the elevator on the same day with patient confirmed with Covid-19 [20]. Another study also reported the contamination of the environment due to the SARS-CoV-2 of an infected patient. The stability of SARS-CoV-2 in a different surface is described in Table 1. and stability in a different temperature described in Table 2. SARS-CoV-2 showed sensitive to heat [21].

Table 1. Stability of SARS-CoV-2 in different surfaces [21]

Surface	Time
Paper	3 hour
Tissue paper	3 hour
Wood	2 day
Cloth	2 day
Glass	4 day
Stainless steel	7 day
Plastic	7 day
Inner mask	7 day
Outer mask	> 7 day

Table 2. Stability of SARS-CoV-2 in different temperature [21]

Temperature	Time
4	14 day
22	7 day
37	1 day
56	10 minutes
70	1 minute

6. SALIVA AS POTENTIAL DIAGNOSTIC SPECIMEN

Saliva is a hypotonic fluid that contains mostly water, electrolytes, and other biomolecules. Saliva consists of a mixture of secretions between the major and minor glands presented in the oral cavity. Within a day, the saliva secretion varies from individual to individual, but the normal rate ranges from 500-1500 ml, while for the unstimulated salivary flow rate, each individual has around 0.3-0.5 ml / minute, and the stimulated salivary flow rate reaches 10 ml / minute [22].

Saliva has the function to maintain the health of oral tissue with protective properties of mucosa and teeth, including lubrication and hydration, protection from tooth erosion, or demineralization. Other functions possessed by saliva are such as regulating water content, removing viruses and metabolic products of organisms; therefore, saliva has the potential function as a diagnostic fluid for diseases, including viruses [23]. The potential of saliva to be utilized for SARS-CoV-2 detection can be based on several reasons. Oro/nasopharyngeal swab from the upper respiratory tract is the golden standard for Covid-19 detection so far. However, the collection procedure to take a specimen from this location is uncomfortable for the patient. It can even cause bleeding in thrombocytopenic patients. The close contact between health workers and patients during the specimen collection can be a high risk for transmission of this virus. Specimens taken from oro/nasopharyngeal swab cannot be used to quantify viral load. It is difficult to monitor the development of this virus by this method. In the other hand, the collection of saliva specimens is accessible. The patient can spit out the saliva by themselves and place it in a tube. This procedure does not need invasive action, which created easiness between both the patient and the health worker. Moreover, saliva specimen has the conformity of > 90% with specimen taken from oro/nasopharyngeal for viral detection. The last reason is that SARS-CoV-2 can be detected in high titer in saliva; thus, it can be used for monitoring this viral development [24].

SARS-CoV-2 is detected in saliva in a four-day median after the disease onset. A large amount of viral RNA is detected at about 7.08×10^3 to 6.38×10^8 copies/mL in saliva. The high viral RNA load is detected in saliva before the development of lung lesions. It was suggested that saliva can be used for early diagnostic for Covid-19 [25]. SARS-CoV-2 entering saliva in the oral cavity is at least mediated through 3 routes; 1) through upper and lower respiratory tract reaching the oral cavity, 2) blood serum present in a gingival crevicular fluid may contain a virus, 3) infection of the minor, and major salivary gland may enter the virus through ductus of salivary gland [5].

It was reported that the initial viral load can be predicted as the severity of the diseases. The oral viral load has reportedly influenced the severity of Covid-19.

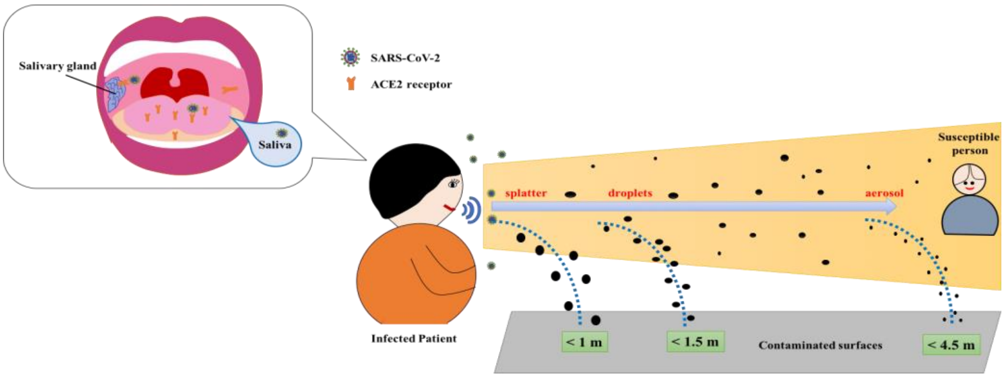

Figure 3. Saliva as transmission source and potential diagnostic specimens. Salivary splatter/ droplets/ aerosol can be spread through the air and dropped to the ground and possibly contaminated surface of fomites. The particles sizes affect the ability those salivary droplets to reach to others places. Oral cavity expressed ACE-2 receptors in tongue, salivary gland, epithelial mucosa which can be entry gate for SARS-CoV-2 to the host cells. Saliva also contain high titer of SARS-CoV-2. Combining all of these factors make oral cavity as high medium transmission of covid-19. *Source:* Figure modified from [26].

The screening using a saliva specimen has been widely studied. Saliva specimens consist of deep throat saliva (DTS), also known as posterior oropharyngeal saliva, and lower-respiratory tract specimens (LRT). Both DTS and LTR can be used for SARS-CoV-2 detection [27]. A study using DTS was reported. The patient had already confirmed to get infected by Covid-19 through nasopharyngeal or sputum specimens. First, the patient asked to cough up the saliva into a sterile bottle. The result showed that 11 out of 12 patients confirmed with COVID-19 were detected with SARS-CoV-2 in their saliva. Viral culture could demonstrate the live viruses of 3 patients. The serial saliva specimen showed there was the highest viral salivary load in the earliest taken specimens, and it gradually decreased. This result suggested that saliva could transmit the live virus and could be used for monitoring the development of the virus [28].

Another study compared the effectiveness of DTS and Nasopharyngeal swabs (NPS). The result showed that DTS was comparable to the NPS samples. The detection rate in DTS was higher compared to NPS, although it was not statistically significant. This study suggested that the performance of DTS was equivalent to NPS to be used for the detection of Covid-19 using RT-PCR [29]. However, the study conducted by Lai et al. showed a different result. Viral RNA concentration of DTS was lower and less sensitive compared to that in other respiratory tract specimens, such as NPS, sputum, and throat specimen [30],[31]. Sputum specimens, as example of LTR, show higher viral load compared to throat swab samples [32].

7. CONCLUSIONS

Early RNA viral detection and high titer viral load are the advantages of using saliva as a diagnostic medium to detect SARS-CoV-2. Saliva from the deep throat is considered as the best diagnostic tendency compared to other sources of saliva specimen. Saliva is the highest risk medium for the transmission of COVID-19. The large size of salivary droplets cannot remain in the air, and it immediately falls to the ground. In the other hand, aerosol or droplets nuclei can travel in the air and can be inhaled, resulting in the susceptibility of the person to be infected. The surface of the contaminated object is also potentially infectious. Oral tissue also directly possible infectious with high expression of ACE-2 receptors in tongue, salivary gland, epithelial mucosa. Combining all of these factors make oral cavity as high medium transmission of covid-19 (Figure 3).

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