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Could saliva play a role in controlling the Covid-19 pandemic?

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SUMMARY

The pneumonia outbreak observed in Wuhan, China, in December 2019 evolved into a general multi-system infection. This is attributed to a virus, from coronaviruses, the SARS-CoV-2 or 2019-nCoV the increased transmissibility of which lead to a spread across the world and the subsequent declaration of a pandemic. Saliva and in particular saliva droplets of different density play a significant role in the Covid-19 infection spread and contamination of the general population, leading to the development of severe acute respiratory syndrome in affected patients.

Keywords: Salivary glands, saliva droplets, COVID-19 transmission, ACE2 receptor.

Human saliva is a unique body fluid secreted by the salivary glands. Because saliva can carry several viruses, including SARS-CoV-2, the possibility of transmitting viruses through saliva, especially those that cause respiratory infections, is unavoidable. SARS-CoV-2 can be detected through saliva diagnostic tests, which have many advantages for healthcare professionals and patients.Saliva is mainly composed of water (94-99%), with organic molecules accounting for approximately 0.5% and inorganic molecules for 0.2% (1). It has the functions of lubricating oral mucosa, digesting food, cleaning and protecting the oral cavity, and is one of the most important factors affecting homeostasis of the oral cavity (2). A normal adult secretes approximately 600 ml of saliva every day (1). In addition to salivary gland excreta, saliva also contains food residue, serum components, oral microorganisms, and their metabolites, exfoliated epithelial cells and white blood cells. Regardless of tissue type (teeth, alveolar mucosa, keratinized gingiva, or buccal mucosa), surface-associated bacterial communities vary along the oral cavity from the front to the back of the mouth, and that on exposed tooth surfaces, the gradient is pronounced on lingual compared to buccal surfaces (3). Outbreak of pneumonia announced in Wuhan, China, in December 2019 has become a rapidly-spread infectious disease across the world, which led to a mass epidemic that forced the WHO to announce on January 30, 2020 that this outbreak had constituted a public health emergency of international concern (4). Pneumonia confirmed and announced in Wuhan, China, is attributed to a virus belonging to the coronaviruses family named nCoV. The spread of 2019-nCoV infection started in December 2019 in Wuhan, Hubei Province, further rapidly spread to many provinces in China as well as to other countries.

Coronaviruses are enveloped in single-stranded RNA viruses (26-32 Kb) (5). The RNA genome of SARS-CoV-2 has 29,811 nucleotides, encoded in 29 proteins. Studying these different components of the SARS-CoV-2 virus, as well as how they interact with host cells, it is found that they utilize four structural proteins of coronaviruses including the spike surface glycoprotein (S), which is responsible for the virus binding to the host cell receptors and thus inserting into the cell (6). The remaining of the four structural proteins include the E and M proteins, from the viral envelope; the N protein (Fig.1), which binds to the virus's RNA genome. Many investigations were focused on the Spike protein S, which play the principal role as a key to enter host cells. SARS-CoV-2 has at least three separate routes to be present in saliva. SARS-CoV-2 in the lower and upper respiratory tract reaches the oral cavity along with the droplets; SARS-CoV-2 in the blood may enter the mouth through the gingival crevicular fluid; and major and minor infection of the salivary gland, with the ensuing release of particles into the saliva through salivary ducts. SARS-CoV-2 can be attached to Angiotensin-converting enzyme 2 (ACE2) receptors through the S Spike protein on the epithelium of salivary glands, fuse with them,

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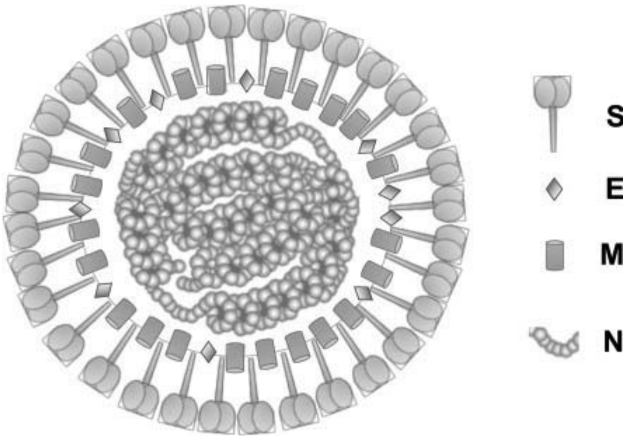

Fig. 1. Diagrammatic representation of coronavirus virion. S – spike protein; E – envelope protein; M – membrane protein; N – nucleocapsid protein encapsidating the RNA genome (image from source 30).

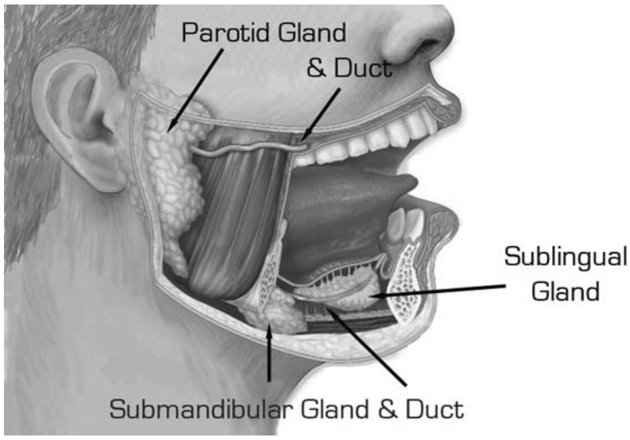

Fig. 2. The major salivary glands, three pairs in total, parotid, submandibular and sublingual glands (image from source 31).

replicate, and disrupt cells to trigger apparent signs and symptoms, such as discomfort, inflammation, and pain in major salivary glands. As a result, it spreads more easily and is more infectious while patients might appear as asymptomatic (7, 8). ACE2 is expressed especially strongly in human lungs with lung diseases. The new strain of coronavirus, that caused the pandemic outbreak, the 2019 nCoV (or SARS-CoV-2), is of strong virulence and caused severe acute respiratory infections (SARIs) and is spreading globally (9, 10) (Fig. 1). Saliva is produced by three pairs of major salivary glands: parotid glands produce a serous, watery secretion, submaxillary (mandibular) glands produce a mixed serous and mucous secretion, sublingual glands secrete a saliva that is predominantly mucous in character (11) (Fig. 2). Saliva is secreted 90% from major salivary glands and 10% from minor salivary glands with pH ranging from 6 to 7 (12, 13). It contains fluid, desquamated oral epithelial cells, microorganisms, blood, respiratory secretions, and gastric acid from reflux. It also contains a large number of proteins such as immunoglobulins, mucins, enzymes, metabolites, hormones. There is a relationship between saliva fluid properties and droplet airborne transmission paths and anthropogenic aerosol (14) (Fig.3). SARS-CoV-2 caused severe disease (COVID-19), and rapidly spread worldwide since the beginning of 2020. SARS-CoV-2 mainly spreads by speaking, singing, coughing, sneezing, droplet inhalation, and contact. SARS-CoV-2 has been detected in saliva samples, making saliva a potential transmission route for Covid-19 (15-18, 26). Using high-speed imaging, the researchers showed that when the mouth opens to produce speech sounds, a film of lubricating saliva initially spreads across the lips. As the lips part, the liquid film then breaks into filaments (16). Outward airflow from the lungs stretches and thins the filaments until they eventually rupture and disperse into the air as miniscule droplets — all within fractions of a second. Deep exhalation produces small and dense droplets that are easier to penetrate the respiratory system. Particles, solid or liquid, that are distributed in the air can remain suspended in the surrounding air for a longer period of time and increase the contamination probability among the population from SARS-CoV-2 infected individuals (24) (Fig. 4). This droplet-producing mechanism is especially pronounced for so-called stop-consonants or “plosives” like “p” and “b”, which require the lips to firmly press together when forming the vocalized sound. Other sounds known as denti-alveolar plosives, such as “t” and “d” which involve the tongue touching the upper teeth and the jaw ridge just behind the teeth, likewise produce droplets at a much greater rate than when forming vowel sounds. A deeper understanding of this droplet formation and dispersal process should lead to new and better mitigation strategies, helping to slow down the current coronavirus pandemic along with future outbreaks (16, 17). The composition of saliva allows the detection of pathogens and the quantification of biomarkers which provide clinical information about the immunological, inflammatory, endocrine and metabolic status of the individual (28). The measuring of salivary alpha-amylase (sAA, enzymatic vs. concentration) evaluates the influence of different ways of reporting in sAA interpretation. The type of saliva particles density of SARS-CoV-2 determines the infectious action of the droplets (20). Besides lungs, salivary glands and tongue are possibly another hosts of 2019-nCoV due to expression of ACE2. Due to its nature saliva is ideal medium

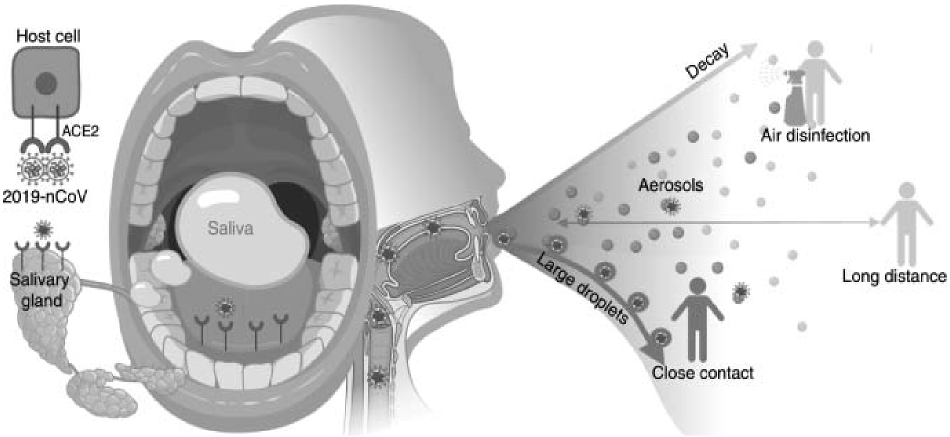

Fig. 3. Saliva: potential diagnostic value and transmission of 2019-nCoV (image from source 32).

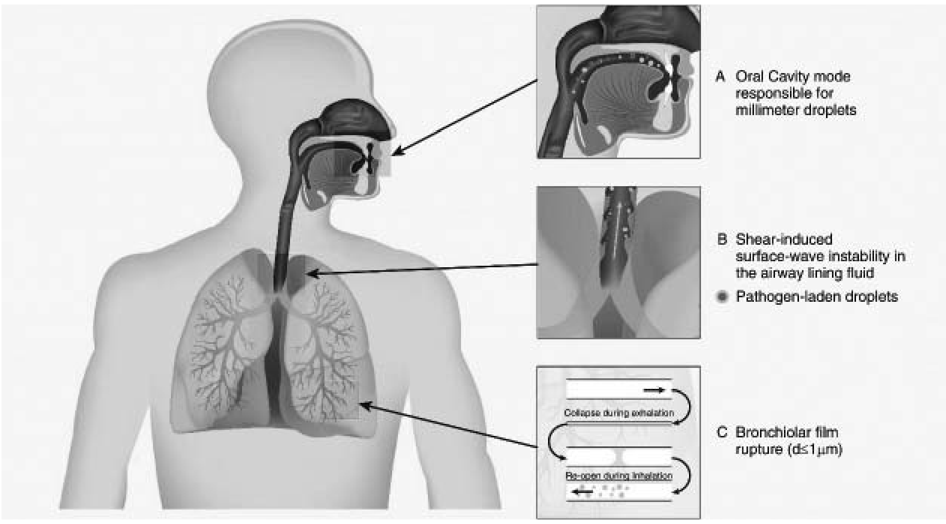

Fig. 4. Deep exhalation produces small and dense droplets. Schematic showing the site of origin and mechanisms of droplet generation from the respiratory tract (image from source 33)

for non-invasive analysis techniques and can be used for evaluating the patient's infection. However, its low concentration did not allow its usage for diagnosis, a limitation that has been overcome with the development of molecular methods and nanotechnology. In addition, studies had already described the detection of respiratory viruses by using molecular techniques, something which implies that SARS-CoV-2 might be detected in saliva, and analyzed for health assessment (18, 19). The velocity and length of time that droplets stay in the air are also dependent on a range of factors, including the volume at which the speaker is talking, their age, and how dry their mouth is. ACE2 receptor has been reported to be the main host cell receptor of 2019-nCoV and plays a crucial role in the virus entry into the lung cell leading to the host infection. Liu et al investigated the distribution of ACE2 epithelial cells (angiotensin converting enzyme 2-positive cells). The findings of this study are consistent with the study of Xu et al who analyzed bulk single cell RNA-seq profiles in order to investigate the susceptibility of the oral cavity to 2019-nCoV(29). The study demonstrated that the mucosa of the oral cavity could express the ACE2 receptor higher in tongue than buccal or gingival tissues. Furthermore epithelial cells were enriched by ACE2 positive cells something that provides evidence that the ACE2 expressing cells (ACE2 cells) in oral tissues could be possible routes of entry for the 2019-nCoV and so the oral cavity is likely a source of data of patient's saliva. Furthermore, the collection of saliva is appropriate for patients for whom the collection of nasopharyngeal or pharyngeal swab can cause discomfort or bleeding for instance in patients with

Table. Possible medical procedures generating aerosols

Medical procedures	Mechanism of aerosol generation
Bronchoscopy	Induced cough, respiratory tract
Cardiopulmonary resuscitation	Induced cough, respiratory tract
Noninvasive ventilation	Possible mechanical dispersal of aerosols, respiratory tract
Tracheal intubation	Induced cough, respiratory tract
Manual ventilation	Possible mechanical dispersal of aerosols, respiratory tract
Surgery	Cutting bone and tendon, and irrigation aerosolize blood
Sputum induction	Induced cough, respiratory tract
Nebulizer Treatment	Possible mechanical dispersal of aerosols, respiratory tract
Suctioning	Possible mechanical dispersal of aerosols, respiratory tract
Laser Plume	Mechanical dispersal of aerosols

thrombocytopenia (20). The rapid spread of the new coronavirus responsible for pneumonia infection, suggesting a human to human transmission, implies a role for the droplets. Droplets which spread the respiratory infections, within a short or a long distance, and by lying on surfaces and so infect them and the people who touch them, these are the most described paths of how the coronavirus can infect many people in a short period of time. As it has been referred by To *et al.* the new virus contained in saliva gives positive viral particles (18). Cough and speech can be a source of infection. Saliva virus transmission can be observed in asymptomatic patients and not only in symptomatic with COVID-19. (21). It is suspected that asymptomatic persons are potential sources of spreading the SARS-CoV-2. It is probable that the infected saliva is present and may be transmitted during the incubation period, where the illness is brief and nonspecific. The detection of SARS-CoV-2 and a high sputum viral load in a convalescent patient arouses concern about prolonged shedding of SARS-CoV-2 after recovery. Asymptomatic patients and patients during incubations period (5-6 days on average, but lately has been shown that this period exceeds to 14 days) are also carriers of SARS-CoV-2 and remains to be proved whether patients in their recovering phase are a potential source of transmission. Moreover, watery droplets are more dangerous for SARS-CoV-2 transmission and the asymptomatic patients can also be a serious risk (11, 21, 22). Other important modes of transmission are direct contact and through fomite generated by medical procedures that produce aerosols (Table) as it is referred by Rabaan AA *et al.* (24). Rapid increase of a SARS-CoV-2 variant with multiple Spike protein mutations observed in various areas present a continuous danger for the pandemic spread, increased transmissibility or the ability to evade the host immune response. (22). In April 2021, 13 vaccines were authorized by at least one national regulatory authority for public use: two RNA vaccines (the Pfizer–BioNTech vaccine and the Moderna vaccine), five conventional inactivated vaccines (BBIBP-CorV, CoronaVac, Covaxin, WIBP-CorV and CoviVac), four viral vector vaccines (Sputnik V, the Oxford–AstraZeneca vaccine, Convidecia, and the Johnson & Johnson vaccine), and two protein subunit vaccines (EpiVac-Corona and RBD-Dimer). In total, the vaccine development pipeline according to the Vaccine Centre, London School of Hygiene and Tropical Medicine as of March 2021, 308 vaccine candidates were in various stages of development, with 73 in clinical research, including 24 in Phase I trials, 33 in Phase I–II trials, and 16 in Phase III development. The vaccines may protect population from Covid-19 epidemic spread through droplets. Early evidence though suggests infections in fully vaccinated persons caused by the SARS-CoV-2 or even Delta variant of SARS-CoV-2 may be transmissible to others (27). Moreover, apart the vaccines, face masks are an effective medium to prevent interhuman transmission, and together with social distancing and quarantine are the rational measures to manage the COVID-19 pandemic.

It may be concluded that saliva through droplets is responsible for the spreading of SARS-CoV-2 in the general population. The control of the pandemic of COVID-19 relies significantly on the restriction of saliva infected droplets.

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