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Human Saliva and the COVID-19 Infection

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Abstract

Human saliva is a complex mixture of various organic and inorganic compounds and host-derived molecules. Performs numerous functions. This comprehensive review will discuss the roles played by saliva in defence against the SARS-CoV-2 virus and the use of saliva as a diagnostic fluid in COVID-19 screening will be discussed along with a brief note on SARS-CoV-2 transmission through saliva. Saliva and SARS Cov 2: The antimicrobial and antiviral properties of saliva are conferred by the salivary peptides such as defensins, cathelicidins, and LL 37. Antiviral activity against the herpes virus, hepatitis C virus, ebola virus and to an extent HIV has been documented. Since the COVID-19 pandemic has now occurred as a new global threat, it is being investigated if saliva has certain properties that could defend against this infection. Studies have found the regular presence of the SARS-CoV-2 virus, the aetiological agent of the COVID-19 disease in saliva, hence saliva could be used as a diagnostic tool. Some interesting findings have highlighted the presence of the virus in salivary samples but documented its absence in throat swabs which is intriguing. Despite having multifaceted roles, the drawback of saliva also lies in its contribution to the transmission of the SARS-CoV-2 virus. Studies have shown that viable viruses can be transmitted through saliva from person to person through coughing and sneezing. Hence saliva could be regarded as a double-edged sword in the COVID-19 pandemic.

Keywords: *Antiviral, COVID-19, Diagnostic Tool, Saliva, SARS Cov 2.*

Introduction

A critical and pivotal dynamic fluid of the oral environment is saliva, which is comprised of inorganic and organic substances. Saliva plays a vital role in major physiologic functions such as mastication, deglutition and speech. During mastication, the ingested food contents are dissolved in saliva and transported to the taste receptors present in the tongue for taste perception. The process of digestion begins in the oral cavity and is

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initiated by salivary amylase responsible for starch and glycogen breakdown, and lipase secreted by lingual salivary glands (Von Ebner's glands) plays for the digestion of fat. The functions of saliva include the protection of oral tissues by lubrication, maintenance of pH, speech, taste perception, mastication, deglutition and enzymatic digestion. Decreased secretion of saliva has been shown to increase the risk of oral diseases such as dental caries, and oral candidiasis [1]. Recent studies have uncovered additional functions of salivary glands and saliva. Salivary glands contain and possibly secrete several vital physiologic substances such as growth factors, vasoactive peptides, and regulatory peptides. Thus, in addition to the alimentary function, the salivary glands may a role in other functions [2, 3].

Saliva is a mucoserous exocrine secretion. Whole saliva is a combination of secretions from major salivary glands such as parotid, submandibular and sublingual glands and minor salivary glands found in the lower lip, tongue, palate, cheeks, and pharynx., gingival crevicular fluid containing oral bacteria and food debris [4, 5]. The terms major and minor salivary glands refer to the glands' anatomic size and quantity of salivary secretion. However, minor salivary glands secrete more protective components [6].

Salivary flow rate per day varies between 1 and 1.5 L in a healthy individual. During an unstimulated salivary flow, 20% of saliva is contributed by the parotid gland 65% from submandibular, 7% to 8% from the sublingual, and less than 10% from numerous minor glands. During stimulated salivary flow rates parotid gland contributes to the majority of the total salivary secretion of 50% [6].

It has been recognized that other than digestion, lubrication and aid in mastication, saliva is a storehouse of many protective molecules that make it a defence fluid. Saliva protects against a spectrum of bacterial, viral and fungal infections. Saliva has also been explored as a diagnostic and prognostic tool by several researchers [7, 8, 9, 10]. Further sections of this article will highlight the antiviral properties of saliva and the relevance of saliva in the COVID-19 pandemic.

Antiviral Properties of Saliva

Like other mucosal surfaces, the oral cavity is continuously exposed to several microbes and toxic chemical substances through ingestion. The oral cavity is an open system to facilitates several important biological functions such as speech, mastication and assistance for respiration. However, it is bathed by the flow of copious amounts of saliva from the salivary glands that maintain the dynamicity and bring in a variety of protective substances to defend against infections and assaults. The salivary defence system is predominantly innate. In other words, it is nonspecific and quick and is an important first-line defence against infections caused by bacteria and viruses. Salivary flow aids in rigorous cleaning of the oral cavity, a continuous process [11]. The focus of this section is to elucidate the antiviral properties of saliva. The salivary constituents that possess both antibacterial and antiviral properties include Cathelcidin (LL-37), Lactoferrin, Lysozyme, Mucins, Peroxidase, Salivary agglutinin (gp340, DMBT1), sIgA, SLPI, α, β Defensins [12].

Active Viral Activity and Isolation of Viable Viruses from Saliva

The viruses that have been found in active forms in human saliva include HSV, HIV, VZV, EBV, HPV, hepatitis A, hepatitis C, Ebola, Norwalk virus, HHV 6 and 8, measles, rabies, adenoviruses, and prions [12]. Although numerous antiviral components are present in saliva, it is surprising that saliva is still inhabited by the above viruses. The predominant reason for this phenomenon could be that these antiviral proteins may be in a free form in solution or may be bound to the

hard and soft tissue components thereby being unavailable for action [12]. Also, the concentrations of the proteins could vary with the salivary flow rate complicating the concept of an ideal antiviral concentration.

There have been some focused studies on antiviral proteins such as salivary agglutinin also called gp340 or DMBT-1. It has been found that this protein has activity against HIV 1 and Influenza A [13, 14]. However, the same substance has limited activity or no significant activity against HSV, adenovirus, SIV and HIV 2. Studies have reported that salivary agglutinin could bind with gp120 on the surface of the virus and could inhibit HIV infectivity [15].

The antiviral proteins present in saliva possess very limited antiviral potency. Hence it can be understood that several infectious viruses are present in the oral cavity despite the presence of antiviral proteins in the oral cavity. For instance, the salivary protein gp340 interacts with bacteria and 2 viruses (HIV-1 and influenza). However, there is a variation in the concentration of this salivary protein in different individuals wherein some individuals possess increased levels of proteins with antibacterial effects while others possess proteins with antiviral activity.

The above section deals with the antiviral activity of saliva against various viruses. However today the globe is facing severe threats from the SARS-CoV-2 virus which has caused the COVID-19 pandemic. The future sections will deal with the relationship between saliva and the COVID-19 pandemic.

The Sars Cov 2 Entry into the Oral Cavity and Plausible Interactions with Saliva

Coronavirus disease 2019 (COVID-19) was first reported in the city of Wuhan, Hubei Province, Central China, and has then created havoc, spreading to many developing and developed nations of the world. COVID-19 is caused by a novel coronavirus also referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Considering, the mammoth destruction caused by the disease, the World Health Organization (WHO) has announced COVID-19 as a public health emergency of international concern (PHEIC). Currently, scores of humans are being afflicted by this disease which causes severe respiratory distress and multi-organ failure in a few leading to severe morbidity and mortality. Social distancing has been followed worldwide to prevent disease spread as this condition is predominantly spread by droplet transmission.

Coronaviruses (CoVs) belongs to the Ortho coronavirinae subfamily of the family Coronaviridae. The four genera of viruses in Ortho coronavirinae include Alphacoronavirus (α-CoV), Beta coronavirus (β-CoV), Gamma coronavirus (γ-CoV) and Delta coronavirus (δ-CoV). The alpha and beta subgenera affect humans and other mammals [16, 17]. SARS-CoV-2 is the 7th member of coronaviruses infects humans. Upper respiratory tract infections such as the common cold are caused by HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 and atypical pneumonia is caused by SARS-CoV and MERS-CoV. An insight into the virological aspects of the disease reveals that the size of the SARS-CoV-2 genome is 30 kb which encodes a large, non-structural polyprotein (ORF1a/b). Several proteins are formed as a result of proteolysis of this non-structural protein including proteins necessary for SARS-CoV-2 assembly and infection such as spike surface glycoprotein(S), the membrane protein (M), the envelope protein (E) and the nucleocapsid protein (N) and five accessory proteins such as ORF3a, ORF6, ORF7, ORF8 and ORF9 [18, 19, 20]. The spike surface glycoprotein is responsible for host cell attachment and is cleaved into the N-terminal S1 subunit and a membrane-bound C-terminal S2 region cleaved by proteases of the host [21]. The S1 subunit binds to the host receptor and causes

destabilization of the prefusion trimer. This causes the shedding of the S1 subunit and S2 subunit to transition into a highly stable post-fusion conformation [22].

The angiotensin-converting enzyme 2 (ACE2) present in the lower respiratory tract is the receptor for the surface spike (S) glycoprotein of SARS-CoV, which explains the site specificity of the infection. SARS-CoV-2 enters the respiratory epithelial cells and replicates rapidly. This in turn triggers a strong immune response, leading to cytokine storm syndromes and pulmonary tissue damage [23].

The oral cavity is one of the portals of entry of SARS Cov 2 as there is an abundance of ACE 2 expressing cells in the buccal mucosa, gingiva, palate and tongue [24]. After entry into the oral cavity, there is a definite plausible role played by saliva mediated by its antiviral properties to fight the SARS-CoV-2 entry and further infection. This has been elucidated by researchers who have hypothesized that hyposalivation could lead to acute respiratory infection. Hyposalivation may impair the protective mechanisms of the oral and respiratory mucosa as a physical barrier thereby facilitating viral adhesion and colonization. Also, hyposalivation may be associated with a decreased secretion of antimicrobial proteins [25].

As previously mentioned, saliva possesses several antiviral proteins and peptides that include Cathelcidin (LL-37), lysozyme, lactoferrin, mucins, salivary agglutinin (gp340, DMBT1), peroxidase, sIgA SLPI, α, β defensins, and cystatins, cystatin type II [26].

Salivary cystatins interfere with viral replication and thereby could play an important role in the host's defence against virus infections. Studies have reported the anti-viral activity of cystatin C against herpes and coronavirus is documented. Moreover, at a physiologic concentration cystatin D has been reported to inhibit the replication of coronavirus [26]. Thus, the presence of cystatin D in saliva may play a protective anti-viral role [27].

The salivary microvesicles contain several microRNAs (miRNAs) that prevent viral replication [28, 29]. The antiviral activity of miRNAs in saliva has been well established and saliva has been used for the management of ophthalmic herpes zoster. Also, unstimulated saliva from the submandibular gland at several-fold dilutions has been reported to inhibit the HIV-1 virus [28].

Considering the presence of many proteins with established anti-viral properties in saliva it could be hypothesized that saliva plays a pivotal role in innate defence against the SARS-CoV-2 virus. However, due to certain limitations like the presence of underlying systemic disease, the diurnal variation in salivary flow and variation in concentrations of antiviral proteins from person to person, there is always an absence of total protection from SARS-CoV-2.

Transmission of SARS Cov 2 Through Saliva

Salivary droplets are produced during speech, respiration, coughing and sneezing and they combine with moisture and droplet nuclei of microorganisms. However, the infectious and transmission intensity of the same pathogen differs with every individual. It could be attributed to the variations in quantity, distance, and size of saliva droplets in individuals. Around 3000 saliva droplets nuclei are produced during a cough which is comparable to the quantity generated during 5 minutes of speech. Approximately 40,000 salivary droplets are produced during a sneeze and cover several meters of air. During exhalation saliva droplets are produced more than one meter in the air. Considering the size of salivary droplets, smaller droplets travel for longer distances and larger droplets with higher mass fall to the ground [30].

The above-mentioned dynamics of coughing and sneezing have a very important

bearing on person-to-person transmission of SARS-CoV-2. SARS-CoV-2 infections are transmitted through contact or droplets [31]. SARS-CoV-2 could become airborne and infect individuals near an infected individual during coughing and sneezing. Hence social distancing has been strongly recommended to minimize community spread of the disease. Touching the surface of SARS-CoV-2 droplets on inanimate objects near the infected individuals is also an important mode route of transmission [31]. Also, the presence of SARS-CoV-2 in the saliva samples of the infected patients has been demonstrated by several studies [32]. Hence it could be inferred that SARS-CoV-2 could be transmitted from person to person through saliva.

Saliva as a Diagnostic Fluid in Covid 19

Currently, the Gold standard for diagnosis of SARS-CoV-2 infection is Real-time reverse transcription Polymerase Chain Reaction (rRT-PCR) obtained from nasopharyngeal and respiratory specimens [33]. The biggest disadvantage of nasopharyngeal swab sampling is the risk of virus transmission to the nurses and physicians who perform sample collection due to the close contact [34]. Also, the collection of samples poses discomfort to the patients. Hence non-invasive fluids like saliva are being investigated for SARS Cov 2 presence to confirm the disease. The advantages of saliva as a diagnostic sample include ease of sample collection, and decreased discomfort to the patient [35]. A few studies have reported the presence of the SARS-CoV-2 virus in saliva.

In two recent studies saliva samples were collected when the patients were instructed to cough out saliva and transported with a viral transport medium and the presence of SARS CoV-2 was assessed by real-time reverse transcription-quantitative polymerase chain reaction (rRT-PCR). [36, 37]. The samples collected using this technique contain secretions from the salivary gland, tracheobronchial tree and nasopharynx. SARS CoV- 2 was detected in the saliva of 20 out of the 23 infected individuals. Negative salivary SARS CoV 2 was reported in 33 individuals who were previously reported negative from nasopharyngeal or sputum specimens Thus the overall diagnostic specificity was reported to be 87%.

In another study collection of saliva was done through passive drool. Salivary samples possibly containing respiratory secretions were collected intraorally using a pipette during endotracheal intubation and mechanical ventilation and SARS CoV 2 viral load was assessed using rRT-PCR that detected. Quantification was not performed. Salivary SARS CoV 2 was detected in 25 patients previously diagnosed with COVID-19 from pharyngeal or bronchoalveolar swabs. Another important finding in the study was the presence of SARS CoV 2 in the saliva of 2 patients on the same days that their pharyngeal or bronchoalveolar swabs were reported negative for the virus. Thus, there could be a possibility that individuals could be contagious through their saliva even when tested negative for the virus from pharyngeal swabs [38].

Han et al. [39] reported a salivary viral load of 105 copies/mL that was similar to that of pharyngeal swabs and lesser than from bronchoalveolar swabs in a 27-day-old COVID-positive neonate

Thus, saliva could be a diagnostic tool for COVID-19. The variations and alterations in serum during COVID-19 infection could reflect in the saliva. During severe infection, elevated levels of acute phase proteins (APPs) like C-reactive protein (CRP) and ferritin in serum have been reported. [40, 41]. These markers are elevated earlier to the appearance of clinical signs and hence could be early biomarkers [42, 43]. Several interleukins IL-6 and IL-10, the mediators of acute phase protein response, are elevated in COVID-19 infection. Studies have demonstrated the correlation between serum and salivary levels

of CRP in human and animal models [44, 45]. Also, ferritin, haptoglobin, serum amyloid A, different interleukins, and adenosine deaminase (ADA) can be measured in salivary samples [44]. Thus, these inflammatory markers could potentially serve as salivary biomarkers for the assessment of the severity and prognosis of the disease.

Also, there have been a few studies that have assessed the diagnostic use of saliva in COVID-19 infection. They are presented in Table 1 as shown below.

Table 1. Studies that have Demonstrated Presence of SARS Cov 2 in Saliva

S No	Author Name	Year	Sample Size	Source of Saliva	Method of Detection	Result
1.	Azzi et al [38]	2020	25	Drooling saliva	R T PCR	25/25 positive
2.	To et al [32]	2020	12	self-collection by coughing	Real time reverse transcriptase PCR	11/12 positive
3.	Chen et al [46]	2020	31	Oropharyngeal swab and saliva	RT PCR	13/31 positive
4.	To et al [37]	2020	23	Oropharyngeal saliva	reverse transcriptase quantitative PCR (RT-qPCR)	20/23
5.	Williams et al [47]	2020	39	Saliva unstimulated pooled and collected	Reverse transcriptase PCR (RT-PCR)	33/39

Conclusion

The usefulness of saliva as an innate host defence mechanism has been highlighted in this review. Saliva has many protective molecules with anti-viral activity against a spectrum of viruses. In the same fashion, it could defend the host from the deadly COVID-19 infection also. However, many factors contribute to a potent and robust anti-viral activity including salivary flow, the concentration of anti-viral peptides and so on. Saliva in addition to its protective role could also serve as a diagnostic tool in COVID-19 screening. It is one of the most non-invasive fluids to collect and use for screening bacteria and viruses. Finally, it could also be worthwhile mentioning that saliva is a double-edged sword in the COVID-19 scenario as it is also one of the methods of disease transmission. Saliva has also been explored as a diagnostic tool for various diseases [48.49]. In the same way, saliva also could be explored as a diagnostic tool for COVID-19. Hence, it lies in the hands of every health professional and patient whether to exploit the usefulness of saliva or to fall prey to its ill effects in transmitting disease. Hence, awareness of the role of Saliva and COVID is vital for dental professionals [50].

Conflict of Interest

None

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None

References

[1]. Anderson, P., Hector, M. P., & Rampersad, M. A., 2001, Critical pH in Resting and Stimulated Whole Saliva in Groups of Children and Adults. *International Journal of Paediatric Dentistry*, 11(4), 266–273, https://doi.org/10.1046/j.1365-263x.2001.00293.x

[2]. Alamoudi, N., Farsi, N., Faris, J., Masoud, I., Merdad, K., and Meisha, D., 2004, Salivary Characteristics of Children and its Relation to Oral Microorganism and Lip Mucosa Dryness. *The Journal of Clinical Paediatric Dentistry*, 28(3), 239–248. https://doi.org/10.17796/jcpd.28.3.h2477450700l550

[3]. Baskar, S. N., 1997, Orban's Oral Histology and Embryology. (11th ed. St. Louis: Harcourt Asia PIE Ltd., Mosby).

[4]. Edgar, W. M.,1992, Saliva: Its Secretion, Composition and Functions. *British Dental Journal*, 172(8), 305–312, https://doi.org/10.1038/sj.bdj.4807861

[5]. Roth, G., Calmes, R., 1981, Salivary Glands and Saliva. Oral Biology, (CV Mosby, St Louis).

[6]. Edgar, W. M., 1990, Saliva and Dental Health. Clinical Implications of Saliva: Report of a Consensus Meeting. *British Dental Journal*, 169(3-4), 96–98, https://doi.org/10.1038/sj.bdj.4807284

[7]. K, H. S., R, G., Ramani, P., & Veeraraghavan, V. P., 2024, Longitudinal Study on Salivary IL-6 Trajectories in Postoperative OSCC Patients After Chemotherapy and Radiotherapy. *Journal of Stomatology, Oral and Maxillofacial Surgery*, 101909. Advance Online Publication. https://doi.org/10.1016/j.jormas.2024.101909

[8]. Alam, M. K., Zaman, M. U., Alqhtani, N. R., Alqahtani, A. S., Alqahtani, F., Cicciù, M., & Minervini, G., 2024, Salivary Biomarkers and Temporomandibular Disorders: A Systematic Review Conducted According to PRISMA Guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Journal of Oral Rehabilitation*, 51(2), 416–426. https://doi.org/10.1111/joor.13589

[9]. Thomas, J. T., Joseph, B., Varghese, S., Thomas, N. G., Kamalasanan Vijayakumary, B., Sorsa, T., Anil, S., & Waltimo, T., 2024, Association Between Metabolic Syndrome and Salivary MMP-8, Myeloperoxidase in Periodontitis. Oral Diseases, Advance Online Publication. https://doi.org/10.1111/odi.15014

[10]. Fathima, R., Ramamoorthi, R., Gopalakrishnan, S., Jayaseelan, V. P., & Muniapillai, S., 2024, Expression of Salivary Levels of S100A7 in Oral Submucous Fibrosis and Oral Leukoplakia. *Journal of Oral and Maxillofacial Pathology: JOMFP*, 28(1), 84–89. https://doi.org/10.4103/jomfp.jomfp_113_23

[11]. Dawes, C., 1998, Recent Research on Calculus. *The New Zealand Dental Journal*, 94(416), 60–62.

[12]. Malamud, D., Abrams, W. R., Barber, C. A., Weissman, D., Rehtanz, M., & Golub, E., 2011, Antiviral Activities in Human Saliva. Advances in Dental Research, 23(1), 34–37. https://doi.org/10.1177/0022034511399282

[13]. Wu, Z., Van, Ryk, D., Davis, C., Abrams, W. R., Chaiken, I., Magnani, J., & Malamud, D., 2003, Salivary Agglutinin Inhibits HIV Type 1 Infectivity Through Interaction with Viral Glycoprotein 120. AIDS Research and Human Retroviruses, 19(3), 201–209, https://doi.org/10.1089/088922203763315704

[14]. White, M. R., Crouch, E., Vesona, J., Tacken, P. J., Batenburg, J. J., Leth-Larsen, R., Holmskov, U., and Hartshorn, K. L., 2005, Respiratory Innate Immune Proteins Differentially Modulate the Neutrophil Respiratory Burst Response to Influenza a Virus. Lung Cellular and Molecular Physiology. *American Journal of Physiology*, 289(4), L606–L616, https://doi.org/10.1152/ajplung.00130.2005

[15]. Nagashunmugam, T., Malamud, D., Davis, C., Abrams, W. R., & Friedman, H. M.,1998, Human Submandibular Saliva Inhibits Human Immunodeficiency Virus Type 1 Infection by Displacing Envelope Glycoprotein gp120 from the Virus. *The Journal of Infectious Diseases*, 178(6), 1635–1641, https://doi.org/10.1086/314511

[16]. Banerjee, A., Kulcsar, K., Misra, V., Frieman, M., & Mossman, K., 2019, Bats and

Coronaviruses. Viruses, 11(1), 41, https://doi.org/10.3390/v11010041
[17]. Yang, D., & Leibowitz, J. L., 2015, The Structure and Functions of Coronavirus Genomic 3' and 5' Ends. Virus Research, 206, 120–133, https://doi.org/10.1016/j.virusres.2015.02.025
[18]. Ramaiah. A., Arumugaswami, V., 2020, Insights into Cross-Species Evolution of Novel Human Coronavirus 2019-nCoV and Defining Immune Determinants for Vaccine Development. bioRxiv https://doi:10.1101/2020.01.29.925867
[19]. Chan, J. F., Kok, K. H., Zhu, Z., Chu, H., To, K. K., Yuan, S., and Yuen, K. Y., 2020, Genomic Characterization of the 2019 Novel Human-Pathogenic Coronavirus Isolated from a Patient with Atypical Pneumonia After Visiting Wuhan. Emerging Microbes & Infections, 9(1), 221–236, https://doi.org/10.1080/22221751.2020.1719902
[20]. Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., Meng, J., Zhu, Z., Zhang, Z., Wang, J., Sheng, J., Quan, L., Xia, Z., Tan, W., Cheng, G., & Jiang, T., 2020, Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host & Microbe, 27(3), 325–328, https://doi.org/10.1016/j.chom.2020.02.001
[21]. Yuan, Y., Cao, D., Zhang, Y., Ma, J., Qi, J., Wang. Q., et al., 2017, Cryo-EM Structures of MERS-CoV and SARS-CoV Spike Glycoproteins Reveal the Dynamic Receptor Binding Domains. Nat Commun, 8:15092, https://doi:10.1038/ncomms15092
[22]. Walls, A. C., Xiong, X., Park, Y. J., Tortorici, M. A., Snijder, J., Quispe, J., Cameroni, E., Gopal, R., Dai, M., Lanzavecchia, A., Zambon, M., Rey, F. A., Corti, D., & Veesler, D., 2019, Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion. Cell, 176(5), 1026–1039.e15. https://doi.org/10.1016/j.cell.2018.12.028
[23]. Paules, C. I., Marston, H, D., & Fauci, A. S., 2020, Coronavirus Infections-More than Just the Common Cold. *JAMA*, 323(8), 707–708, https://doi.org/10.1001/jama.2020.0757
[24]. Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., & Chen, Q., 2020, High Expression of ACE2 Receptor of 2019-nCoV on the Epithelial Cells of Oral Mucosa. *International Journal of Oral Science*, 12(1), 8, https://doi.org/10.1038/s41368-020-0074-x
[25]. Iwabuchi, H., Fujibayashi, T., Yamane, G. Y., Imai, H., & Nakao, H., 2012, Relationship Between Hyposalivation and Acute Respiratory Infection in Dental Outpatients. Gerontology, 58(3), 205–211. https://doi.org/10.1159/000333147
[26]. Magister, S., & Kos, J., 2013, Cystatins in Immune System. *Journal of Cancer*, 4(1), 45–56. https://doi.org/10.7150/jca.5044
[27]. Collins, A. R., & Grubb, A., Cystatin, D., 1998, A Natural Salivary Cysteine Protease Inhibitor, Inhibits Coronavirus Replication at its Physiologic Concentration. Oral Microbiology and Immunology, 13(1), 59–61, https://doi.org/10.1111/j.1399-302x.1998.tb00753.x
[28]. Dawes, C., Pedersen, A. M., Villa, A., Ekström, J., Proctor, G. B., Vissink, A., Aframian, D., McGowan, R., Aliko, A., Narayana, N., Sia, Y. W., Joshi, R. K., Jensen, S. B., Kerr, A. R., & Wolff, A., 2015, The Functions of Human Saliva: A Review Sponsored by the World Workshop on Oral Medicine VI. Archives of Oral Biology, 60(6), 863–874, https://doi.org/10.1016/j.archoralbio.2015.03.004
[29]. Irmak, M. K., Erdem, U., & Kubar, A., 2012, Antiviral Activity of Salivary microRNAs for Ophthalmic Herpes Zoster. Theoretical Biology and Medical Modelling, 9(1), 21, https://doi.org/10.1186/1742-4682-9-21
[30]. Baghizadeh Fini, M., 2020, Oral Saliva and COVID-19. Oral Oncology, 108, 104821. https://doi.org/10.1016/j.oraloncology.2020.104821
[31]. Centers for Disease Control and Prevention Transmission of Coronavirus Disease 2019 (COVID-19). Accessed 18th Mar 2020, Available at: https://www.cdc.gov/coronavirus/2019-ncov/about/transmission.html
[32]. To, K. K., Tsang, O. T., Yip, C. C., Chan, K. H., Wu, T. C., Chan, J. M., Leung, W. S., Chik, T. S., Choi, C. Y., Kandamby, D. H., Lung, D. C., Tam, A. R., Poon, R. W., Fung, A. Y., Hung, I. F., Cheng, V. C., Chan, J. F., & Yuen, K. Y., 2020,

Consistent Detection of 2019 Novel Coronavirus in Saliva. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 71(15), 841–843. https://doi.org/10.1093/cid/ciaa149

[33]. Hong, K. H., Lee, S. W., Kim, T. S., Huh, H. J., Lee, J., Kim, S. Y., Park, J. S., Kim, G. J., Sung, H., Roh, K. H., Kim, J. S., Kim, H. S., Lee, S. T., Seong, M. W., Ryoo, N., Lee, H., Kwon, K. C., & Yoo, C. K., 2020, Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. Annals of Laboratory Medicine, 40(5), 351–360. https://doi.org/10.3343/alm.2020.40.5.351

[34]. Ng, K., Poon, B. H., Kiat Puar, T. H., Shan Quah, J. L., Loh, W. J., Wong, Y. J., Tan, T. Y., & Raghuram, J., 2020, COVID-19 and the Risk to Health Care Workers: A Case Report. Annals of Internal Medicine, 172(11), 766–767. https://doi.org/10.7326/L20-0175

[35]. Chojnowska, S., Baran, T., Wilińska, I., Sienicka, P., Cabaj-Wiater, I., & Knaś, M., 2018, Human Saliva as a Diagnostic Material. *Advances in Medical Sciences*, 63(1), 185–191, https://doi.org/10.1016/j.advms.2017.11.002

[36]. To, K. K., Tsang, O. T., Yip, C. C., Chan, K. H., Wu, T. C., Chan, J. M., Leung, W. S., Chik, T. S., Choi, C. Y., Kandamby, D. H., Lung, D. C., Tam, A. R., Poon, R. W., Fung, A. Y., Hung, I. F., Cheng, V. C., Chan, J. F., & Yuen, K. Y., 2020, Consistent Detection of 2019 Novel Coronavirus in Saliva. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America, 71(15), 841–843. https://doi.org/10.1093/cid/ciaa149

[37]. To, K. K., Tsang, O. T., Leung, W. S., Tam, A. R., Wu, T. C., Lung, D. C., Yip, C. C., Cai, J. P., Chan, J. M., Chik, T. S., Lau, D. P., Choi, C. Y., Chen, L. L., Chan, W. M., Chan, K. H., Ip, J. D., Ng, A. C., Poon, R. W., Luo, C. T., Cheng, V. C., Yuen, K. Y., 2020, Temporal Profiles of Viral Load in Posterior Oropharyngeal Saliva Samples and Serum Antibody Responses During Infection by SARS-CoV-2: An Observational Cohort Study. The Lancet. Infectious diseases, 20(5), 565–574, https://doi.org/10.1016/S1473-3099(20)30196-1

[38]. Azzi, L., Carcano, G., Gianfagna, F., Grossi, P., Gasperina, D. D., Genoni, A., Fasano, M., Sessa, F., Tettamanti, L., Carinci, F., Maurino, V., Rossi, A., Tagliabue, A., & Baj, A., 2020, Saliva is a Reliable Tool to Detect SARS-CoV-2. *The Journal of Infection*, 81(1), e45–e50, https://doi.org/10.1016/j.jinf.2020.04.005

[39]. Han, M. S., Seong, M. W., Heo, E. Y., Park, J. H., Kim, N., Shin, S., Cho, S. I., Park, S. S., & Choi, E. H., 2020, Sequential Analysis of Viral Load in a Neonate and Her Mother Infected With Severe Acute Respiratory Syndrome Coronavirus 2. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(16), 2236–2239, https://doi.org/10.1093/cid/ciaa447

[40]. Wan, S., Xiang, Y., Fang, W., Zheng, Y., Li, B., Hu, Y., Lang, C., Huang, D., Sun, Q., Xiong, Y., Huang, X., Lv, J., Luo, Y., Shen, L., Yang, H., Huang, G., & Yang, R., 2020, Clinical Features and Treatment of COVID-19 Patients in Northeast Chongqing. *Journal of Medical Virology*, 92(7), 797–806, https://doi.org/10.1002/jmv.25783

[41]. Peng, Y. D., Meng, K., Guan, H. Q., Leng, L., Zhu, R. R., Wang, B. Y., He, M. A., Cheng, L. X., Huang, K., & Zeng, Q. T., 2020, Zhonghua Xin Xue Guan Bing Za Zhi, 48(6), 450–455, https://doi.org/10.3760/cma.j.cn112148-20200220-00105

[42]. Cerón, J. J., Martinez-Subiela, S., Ohno, K., & Caldin, M., 2008, A Seven-Point Plan for Acute Phase Protein Interpretation in Companion Animals. *Veterinary Journal* (London, England: 1997), 177(1), 6–7, https://doi.org/10.1016/j.tvjl.2007.12.001

[43]. Wan, S., Yi, Q., Fan, S., Lv, J., Zhang, X., Guo, L., Lang, C., Xiao, Q., Xiao, K., Yi, Z., Qiang, M., Xiang, J., Zhang, B., Chen, Y., & Gao, C., 2020, Relationships Among Lymphocyte Subsets, Cytokines, and the Pulmonary Inflammation Index in Coronavirus (COVID-19) Infected Patients. *British Journal of Haematology*, 189(3), 428–437, https://doi.org/10.1111/bjh.16659

[44]. Tvarijonaviciute, A., Martinez-Lozano, N., Rios, R., Marcilla de Teruel, M. C., Garaulet, M., and Cerón, J. J., 2020, Saliva as a Non-Invasive

Tool for Assessment of Metabolic and Inflammatory Biomarkers in Children. Clinical Nutrition (Edinburgh, Scotland), 39(8), 2471–2478, https://doi.org/10.1016/j.clnu.2019.10.034

[45]. Parra, M. D., Tecles, F., Martínez-Subiela, S., & Cerón, J. J., 2005, C-Reactive Protein Measurement in Canine Saliva. Official Publication of the American Association of Veterinary Laboratory Diagnosticians. *Journal of Veterinary Diagnostic Investigation Inc*, 17(2), 139–144. https://doi.org/10.1177/104063870501700207

[46]. Chen, L., Zhao, J., Peng, J., Li, X., Deng, X., Geng, Z., Shen, Z., Guo, F., Zhang, Q., Jin, Y., Wang, L., & Wang, S., 2020, Detection of SARS-CoV-2 in Saliva and Characterization of Oral Symptoms in COVID-19 patients. Cell proliferation, 53(12), e12923. https://doi.org/10.1111/cpr.12923

[47]. Williams, E., Bond, K., Zhang, B., Putland, M., & Williamson, D. A., 2020, Saliva as a Noninvasive Specimen for Detection of SARS-CoV-2. *Journal of Clinical Microbiology*, 58(8), e00776-20. https://doi.org/10.1128/JCM.00776-20

[48]. Sagar, S., Ramani, P., Moses, S., Gheena, S., & Selvaraj, J., 2024, Correlation of Salivary Cytokine IL-17A and 1,25 Dihydroxycholecalciferol in Patients Undergoing Orthodontic Treatment. *Odontology*, 112(3), 966–975. https://doi.org/10.1007/s10266-023-00890-1

[49]. Alam, M. K., Zaman, M. U., Alqhtani, N. R., Alqahtani, A. S., Alqahtani, F., Cicciù, M., & Minervini, G., 2024, Salivary Biomarkers and Temporomandibular Disorders: A Systematic Review conducted according to PRISMA guidelines and the Cochrane Handbook for Systematic Reviews of Interventions. *Journal of oral rehabilitation*, 51(2), 416–426. https://doi.org/10.1111/joor.13589

[50]. Kritika, S., Mahalaxmi, S., Srinivasan, N., & Krithikadatta, J., 2023, Deciphering the Role of Saliva in COVID 19: A Global Cross-Sectional Study on the Knowledge, Awareness and Perception Among Dentists. *BMC Oral Health*,23(1), 424. https://doi.org/10.1186/s12903-023-03152-2