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RESEARCH ARTICLE

SALIVARY DIAGNOSTICS A NON-INVASIVE TOOL IN CHILDREN: A REVIEW

[1]Dr. Umapathy Thimmegowda and [2,*]Dr. Sanchitha Venkatahanumaiah

[1]MDS, Professor, Department of Pedodontics and Preventive Dentistry, Rajarajeswari Dental College and Hospital, No.14, Ramohalli Cross, Mysore Road, Kumbalgodu, Bangalore – 560074, Karnataka, India
[2]Post Graduate Student, Department of Pedodontics and Preventive Dentistry, Rajarajeswari Dental College and Hospital, No.14, Ramohalli Cross, Mysore Road, Kumbalgodu, Bangalore – 560074, Karnataka, India

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Corresponding author:
Dr. Sanchitha Venkatahanumaiah

ABSTRACT

Babies drool, old men dribble, and some individuals spit when they talk; the rest of us would rather not be reminded that saliva exists; practicing dentists consider it a nuisance that must be sponged, evacuated, or dammed. Saliva has shown remarkable potential as a diagnostic fluid over the years, as numerous illness biomarkers may also be identified in entire saliva, in addition to blood and other bodily fluids. Saliva has been examined intensively as a potential diagnostic tool over the last decade due to its easy and non-invasive accessibility, as well as its richness of indicators such as genetic material and proteins. In this review, we'll look at some of the new ways saliva can be used as a diagnostic tool in paediatric dentistry, spanning the "omic" spectrum.

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INTRODUCTION

Saliva has long been acknowledged as a diagnostic tool. Because of its noninvasive collection, easy handling, and preservation of samples, saliva is an ideal diagnostic medium for vulnerable groups such as newborns and children. Its distinct biomarker profiles aid in the diagnosis of a wide range of illnesses and disorders. Saliva genomics, proteomics, transcriptomics, metabolomics, and microbiome-based discoveries have all contributed to the development of complementary and powerful diagnostic information. Saliva is used to diagnose caries and aggressive periodontitis in infants and newborns, as well as a variety of other systemic disorders, metabolic diseases, cognitive functions, stress assessment, and evaluation of immunological and inflammatory reactions to immunisation.[1] Without extra laboratory testing, high-morbidity and high-mortality conditions, such as cancer and cardiovascular, metabolic, and neurological diseases, are difficult to detect.

The earlier a disease is recognised and diagnosed, the more probable it is that the patient will receive appropriate therapy, decreasing the severity of the disease. As a result, early identification is critical for the implementation of appropriate clinical treatment.[2] Salivary diagnostic assays have emerged in recent years as a result of breakthroughs in biotechnology paired with a clinical desire for more user-friendly and non-invasive platforms to better monitor disease, infection, and development. Perhaps no other patient group will benefit more from these advancements than children.[3] Using thorough "omic" analysis of saliva to get insight into development, infection, and disease could dramatically improve care and health access. Even so, a single drop of human saliva can provide a multitude of information. Saliva is offering paediatric caregivers and researchers with a fascinating new tool for inquiry, from forecasting physiological development and biological activities to microbiological and metabolic analysis.[3] As a result, this review sheds light on the diagnostic use of saliva in the paediatric population.

EMBRYOLOGY AND DEVELOPMENT

The salivary glands all have the same embryogenesis, in which they develop from oral epithelial growths into the underlying mesenchyme. The parotid anlagen first form in humans as firm epithelial placodes in the developing cheeks between the fourth and sixth embryonic weeks. The placodes for the submandibular glands emerge in the medial paralingual sulcus later in the sixth embryonic week. The sublingual gland anlagen emerge from several epithelial placodes during the seventh and eighth embryonic weeks, lateral to the submandibular glands, and the minor salivary glands mature late in the 12th fetal week.[4]

Fig 1. A summary of the key events in salivary embryogenesis:[4]

Week	Event
4–6	Parotid anlagen arise from the ectoderm in the oral cavity
Late 6	Submandibular anlagen arise from the ectoderm in the oral cavity
7–8	Sublingual gland anlagen arise from the ectoderm in the oral cavity
Late 12	Minor salivary gland anlagen arise from the ectoderm and endoderm
10–13	Cavitation of future ducts occurs in the submandibular glands
12	Submandibular gland acini differentiate
12–13	Encapsulation of the submandibular and sublingual glands occurs
12–14	Lymphatic sacs anastomose to form ducts and plexi in the neck
14–16	Parotid gland encapsulation occurs after the parotid lymph nodes appear, and cavitation of the future ducts occurs in the sublingual glands
16–18	Cavitation of the parotid ducts occurs
16	Parotid terminal buds and acini appear
16	Submandibular gland serous activity starts
18	Parotid gland secretions start
24	Myoepithelial cells reach their peak density in the submandibular glands
28	Submandibular gland serous secretions decrease
35	Myoepithelial cells reach their peak density in the parotid glands

HISTOLOGY: The acini, or terminal secretory unit, are the basic functional unit of a salivary gland. Epithelial secretory cells, such as serous and mucous cells, make up the terminal secretory unit, regardless of size or location. The serous, mucous, and myoepithelial cells are organised in an acinus or acini (plural) with a central lumen and a broadly spherical or tubular shape.[5] Because of the extension of lumen between the cells called intercellular canaliculi, the central lumen of each acini may have a star-shaped appearance. The acini's primary lumen continues through a fine network of ducts that constantly merge and get larger, finally merging into the major excretory duct. The ductal system is made up of these ducts. The lumen of mucous acini is bigger than that of serous acini (end piece). In serous acini, the secretory terminal unit is made up of 8–12 serous acini that surround a central lumen (Fig. 2). Unlike the serous acini, mucous cells are connected by a variety of intracellular connections. Only acini with demilunes are thought to have intercellular canaliculi.

Mucous acini sometimes have a bonnet or crescent-shaped covering made of serous cells. Demilunes are what they're called.[5] (Fig. 2).

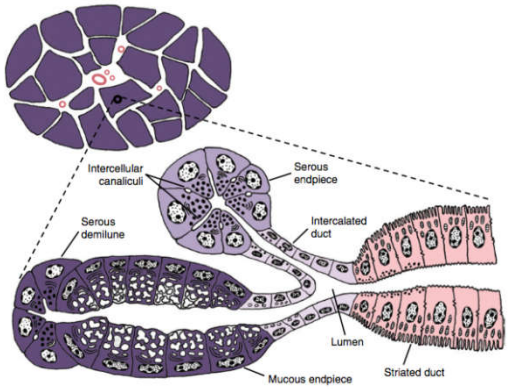

Figure 2. Diagram of serous and mucous end pieces, intercalated and striated duct

FORMATION, SECRETION AND REGULATION: Saliva is made up of two distinct stages. The secretory end pieces and intercalated ducts create primary saliva, an isotonic fluid containing the majority of the organic components and all of the water released by the salivary glands, in the first stage. The primary saliva is modified in the second stage as it goes through the striated and excretory ducts, mostly by the reabsorption and release of electrolytes. The hypotonic saliva that reaches the oral cavity is the ultimate product.[5] Salivary secretion may be defined as "A unidirectional movement of fluid, electrolytes and macromolecules into saliva in response to appropriate stimulation". This simple statement encapsulates most aspects of the secretory process.[6] The process of secretion is made up of three steps: Fluid filtration from blood plasma to the acinar lumen, exocytosis of cytoplasmic granuli-containing proteins into the acinar lumen, and mechanical contraction of the secretory end-pieces controlled by specialised myoepithelial cells are all initiated by nerve cholinergic activation.[7] The Autonomic Nervous System is primarily responsible for saliva secretion. Unmyelinated fibres from the parasympathetic and sympathetic nervous systems innervate the salivary glands, which are dispersed in tiny bundles to the endpieces and ducts. The most powerful stimulation for saliva release is the flavour of food ingredients and chemicals. The nucleus of the solitary tract (NST) in the medulla oblongata receives taste sensations via cranial nerves VII, IX, and X.[8]

COMPOSITION AND FUNCTION OF SALIVA

On average, a normal person produces 1-1.5L of saliva every day. The Salivary Flow index, as shown in table 1, is used to measure the level of salivation by both resting and stimulated production.[9]

Table 1. Saliva flow range for stimulated and unstimulated conditions.[9]

SF (stimulated)	Range
Normal	1-3mL/min
Low	0.7-1 mL/min
Hyposalivation	>0.7 mL/min
SF (unstimulated)	Range
Normal	0.25-0.35 mL/min
Low	0.1-0.25 mL/min
Hyposalivation	>0.1 mL/min

Table 2. Composition of whole saliva[9]

Parameter	Characteristics
Volume	600–1,000 mL/day
Flow rate (resting)	0.2–0.5 mL/minute
pH	6.7–7.4
Osmolality	~50–75 mOsm/kg
Electrolytes	Na^+, K^+, Cl^-, HCO^-, Ca^{2+}, Mg^{2+}, HPO^{2-}, SCN^-, F^-
Protein concentration	~0.5–1.5 mg/mL
Major proteins	Amylase, proline-rich proteins, mucins, histatins, cystatins, peroxidase, lysozyme, lactoferrin, immunoglobulin A, defensins, cathelicidin-LL37
Small molecules	Glucose, amino acids, urea, uric acid, lipids
Other components	Growth factors, insulin, cyclic AMP-binding proteins, serum albumin

FUNCTIONS OF SALIVA[10]

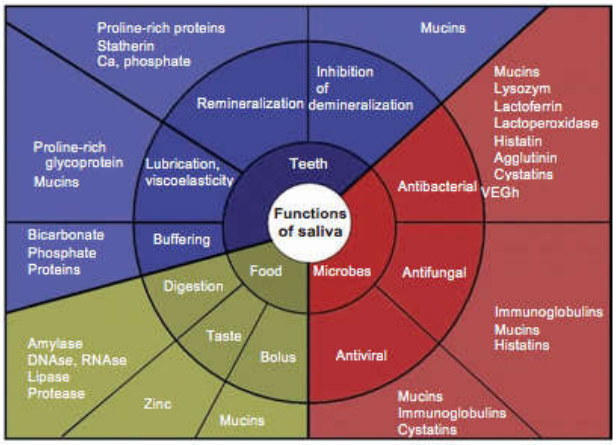

Figure 3 - A look at the connections between saliva's many functions and the salivary elements involved. Several salivary proteins are involved in multiple functions

SALIVARY BIOMARKERS: Any measurable and quantifiable biological object that can serve as an indicator for health-related assessments is referred to be a biomarker. Salivary diagnostics is a dynamic and evolving discipline that incorporates the notion of molecular diagnostics and uses salivary biomarkers to aid in the diagnosis of a variety of oral and systemic disorders. Saliva is a generally available and easy to get specimen that can be obtained using non-invasive procedures and contains a variety of hormones and antibodies that have proven to be invaluable in the screening and diagnosis of diseases.[11] Biomarkers generated from serum, gingival crevicular fluid, and mucosal transudate is found in saliva. Markers develop in saliva as a result of systemic and oral illnesses. Saliva that has not been stimulated includes a higher concentration of diagnostic indicators than saliva that has been stimulated and is widely utilised for diagnostic purposes. Because of the foreign compounds (citric acid) utilised to increase salivary secretion, stimulated saliva has low concentration proteins and a pH change.[12]

Salivary biomarkers can be used in the diagnosis of various systemic disorders are mentioned below:[12]

- Oncology
- Cardiovascular diseases
- Viral diseases
- Bacterial infections
- Fungal infections
- Endocrinology
- Psychiatry
- Drug monitoring
- Biomarkers are also used in the diagnosis of oral diseases:
- Periodontal disease
- Sjogren's syndrome
- Oral cancer
- Dental caries
- Orofacial pain

Biomarkers in Pediatric Dentistry

1) Early childhood caries (ECC) - Salivary microorganisms can be used as biomarkers to assess ECC risk: The species most frequently related with tooth decay in youngsters include a group of cariogenic bacteria, including mutant streptococci, S. mutans, and Streptococcus sobrinus.[13] The use of salivary proteins as biomarkers to predict ECC susceptibility and outcomes: The mucin family in human saliva is divided into two types: high-molecular-weight mucins (MG1), which have molecular weights greater than 1000 kDa, and low-molecular-weight mucins (MG2), which have molecular weights between 150 and 200 kDa. The acquired enamel pellicle is predominantly made up of MG1, which acts as a bacterial attachment site, a permeable barrier to organic acid challenge, and may enhance bacterial colonisation on oral surfaces.[13]

2) Children's Obstructive Sleep Apnea Syndrome - Children's salivary cortisol production increases significantly without a change in salivary a-amylase concentrations. In addition, the hypothalamus-pituitary-adrenal axis is more active in the morning and evening, which is particularly noticeable. Salivary a-amylase levels in OSA children fluctuate physiologically throughout the day, with a higher level in the morning compared to the evening.[14]

3) Dental anxiety and stress - salivary cortisol can be used as a stress and depression biomarker. Stress can damage the immune system and prevent immunoglobulin synthesis. 15 Salivary alpha-amylase has also been identified as a sensitive marker to stimuli that activate the sympathetic nervous system (e.g., adrenaline). The content of alphaamylase in saliva increases dramatically in response to stress, making it a significant salivary biomarker of stress.[15]

SALIVA COLLECTION, PROCESSING AND STORAGE

Saliva can be collected in different forms

- Resting or unstimulated whole saliva
- Stimulated whole saliva
- Glandular saliva (mainly parotid) - with or without stimulation, sub-mandibular/ sub-lingual saliva
- Palatine saliva.[16]

Methods for collection of saliva per se: Whole saliva - Draining, spitting, suction, and swabbing are some of the ways for collecting entire saliva currently available.[17]

Glandular Saliva / Gland Specific Saliva

Parotid saliva is obtained with the use of a cannula, a Lashley cup, or a modified Carlson Crittenden device. It's worth noting

that parotid saliva contains twice as much protein and organic material than submandibular and sublingual saliva.[17]

Submandibular/sublingual saliva

- Suction method: The Stensen's duct is blocked with a cotton roll or a Lashley cup. The saliva that has gathered in the mouth's floor is then aspirated with a syringe, micropipette, or mild suction.[17]
- Cannulation: The Wharton's duct can be cannulated using tapered polyethylene tubing. The most significant downside of this approach is the thin conduit, which is prone to rupture.[17]
- Segregator method - An apparatus capable of collecting submandibular and sublingual saliva while subjected to masticatory and gustatory stimuli has been developed and published.[17]

Saliva from minor salivary glands: Palatine saliva, buccal saliva, and labial saliva are all examples of saliva from minor salivary glands. The periopaper / sialopaper absorbent method can be used to collect labial and buccal saliva. The periotron can be used to determine the amount of saliva present.[18] Recent techniques- Most newer techniques are variations on expectoration methods. Oragene, Saligene, Oracol, and Verofy are only a handful of them.[17] Sample storage - It is advised that saliva samples be frozen at or below -20 oC as soon as possible following collection. If a freezer is not accessible, specimens can be kept at 4 degrees Celsius to avoid bacterial growth and further degradation of salivary components (no longer than 6 h). Specimens can also be preserved for several years at -80 oC with little to no deterioration. Snap-freezing in liquid nitrogen and the use of enzyme inhibitors are two other storage and specimen processing methods.[19]

BIOENGINEERING CELL MODELS AND ARTIFICIAL SALIVARY GLAND: Complex biological processes like development, tissue function, immune response, and wound healing are coordinated through precise and dynamic regulation of cell behaviour, which is mostly accomplished through active conversation between cells and their environment in bioengineering cell models.[20] Hyposalivation is a significant clinical concern because decreased saliva production leads to dental caries, periodontitis, microbial infections, and difficulties with basic oral functions (e.g., speaking, mastication, and swallowing), all of which significantly reduce afflicted patients' quality of life.[21]

Current hyposalivation treatments include I patient education, diet, and lifestyle changes; (ii) prevention of dental and oral mucosal diseases; (iii) symptom management; (iv) sialogogues or salivary gland stimulants (e.g., the muscarinic receptor agonists pilocarpine and cevimeline), which induce saliva secretion from residual acinar cells; and (v) artificial saliva. However, because current treatments primarily address surface-level symptoms and provide only brief relief, it is critical to create an alternate treatment that has a longer-lasting effect.[21] The creation of a functional artificial salivary gland is a fresh alternative for many people suffering from hyposalivation. Autologous primary cells should ideally be used in clinical settings. A patient's healthy tissue might theoretically be harvested prior to radiation therapy. The cells might be grown on a scaffold and put back into the patient during treatment. This is not an option in circumstances where the salivary glands are badly injured or nonexistent. These people would have to rely on a donor-cell-grown artificial salivary gland.[21]

Current cell models utilized in the development of an artificial salivary gland:[21]

- Tumor-derived cell lines – HSY, HSG, SMIE and RSMT-A5.
- Immortalized cell lines - SMG-C6 and SMG-C10, Par-C10 and Par-C5.
- Primary cells
- Progenitor cells

SALIVARY APPLICATIONS IN PEDIATRIC DENTISTRY: When compared to blood, the main drawback of saliva as a diagnostic fluid is that several essential macromolecules are discovered to be in low concentration.[22]

SALIVARY PROTEOMICS: Diabetes research is one example of salivary proteomics applications. Diabetic patients with type 1 and type 2 diabetes have saliva proteome alterations. Statherin, proline-rich peptide PB, salivary acidic proline-rich phosphoprotein 1/2, and histatin 1 were shown to be much less prevalent in the saliva of children with type 1 diabetes, but isoforms of S100 calcium-binding protein A9 were found to be significantly more plentiful. 1 It was discovered that salivary insulin growth factor (IGF-1) is a promising indication of skeletal maturity.[23]

SALIVARY MICROBIOMICS: Salivary analysis in paediatric populations is used to detect immunological responses to mumps, measles, HIV, hepatitis B, and herpes simplex virus, as well as vaccine response, by measuring saliva Ig levels. 1 Measuring the level of salivary antibodies allows for the detection of morbillivirus infection, which causes measles (with 97 percent sensitivity and 100 percent specificity), Paramyxoviridae, which causes mumps (with 94 percent sensitivity and 94 percent specificity), and Togaviridae, which causes rubella (with 94 percent sensitivity and 94 percent specificity) (98 percent sensitivity and 98 percent specificity).[22] Children with ASD have also been found to have microbial changes in their mouths. Pathogens such Haemophilus in saliva and Streptococcus in plaques were found to be much more abundant in ASD patients, while commensals like Prevotella, Selenomonas, Actinomyces, Porphyromonas, and Fusobacterium were found to be significantly less abundant.[24]

Salivary biomarkers in early childhood caries:[25]

It is classified as:

I Biological constituents of saliva: Streptococcus, Lactobacillus, and Actinomyces are the most common microbial species linked to ECC. Atopobium genus, Aggregatibacter sp., Streptococcus oralis, S.mitis, S.infantis, S. parasanguinis, A. defectiva, G. haemolysans, Selemonas sp., and Porphyromonas sp. have all been reported to have a role in caries development.[25] II Physical properties of saliva - Reduced salivary flow rate in children with caries, as measured by unstimulated salivary flow rate of 0.290.08ml/min, can be used as a warning indication before caries develops in young children. 25 Caries susceptibility has a clear negative association with salivary buffer capacity, with buffer capacity dropping to 0.4 units in caries-prone children.[26]

Salivary viscosity measurement is critical since high salivary viscosity has been linked to an increased risk of oral illnesses, including dental caries and periodontal disease.[27]

Chemical constituents of saliva.[25]

Electrolytes in saliva[25]

Calcium and phosphate contents	Decreased in individuals with active carious lesions. Calcium and phosphorus measured colorimetrically have shown their values as less as 2 units in caries active children.
Fluoride	Ion specific electrode and spectrophotometric values of F ranges from 0-1ppm in children with active caries.
Concentration of NO	>50µM and values <40µM in caries active and caries free children are seen. The increased concentration of NO suggests its defense role towards caries.
Copper	Spectrophotometric measurements of Cu show values of 0.2- 0.3 units in children with caries. This inverse relationship between copper and dental caries gives its predictive role for ECC.

Salivary proteins[25]

Salivary Antimicrobial peptides	Ig A & G have shown concentration of 196.14±100.07 mg/dl; 9.78±3.26 mg/dl respectively in caries active children which are significantly higher compared to caries free.
Defensins	alpha defensins in ECC could not be established. Beta defensins measured using ELISA have shown a significant increase in ECC.
Histatins	HST levels in saliva of children with severe ECC, as high as 50ng/ml.
Free amino acids	Proline is most frequently absent in caries free group whereas absence of glycine was observed in children experiencing caries.
Glucosyltransferase	S. mutans produces 3 types of Gtf: GtfB, GtfC, GtfD. The level of GtfB enzyme shows a significant increase with the increase of caries experience.
Salivary amylase	S.mutans and Lactobacillus, promote their removal from the oral cavity by the salivary clearance and lowering the risk of dental caries. However, the evidence is controvertible.

Salivary glycoproteins[25]

Mucins	Role of mucins in ECC still remains doubtful.
Proline-rich proteins	Mass spectrometric analysis has revealed an elevated PRP peaks [32], whereas gel electrophoresis have shown reduction in ECC.
Agglutinin	A correlation between increased levels of agglutinin in saliva and increased numbers of S. mutans in dental plaque and susceptibility to dental caries are seen.
Lactoferrin	Lower concentrations of salivary lactoferrin estimated using ELISA kits may be a risk factor for dental caries in children.
Lysozyme	Controversial evidence that supports the relationship between lysozyme and ECC obtained using western blotting and ELISA kits suggests its role in as a risk factor for ECC.
Soluble CD14	It seems that this inflammatory protein is developed in response to the bacterial stimulus of ECC.

Periodontal disease - Research on the salivary biomarker profile in children with regard to periodontal condition has found a link between the intensity of inflammation and IgA content. MIP-1a, in particular, is linked to a child's risk of developing localised aggressive periodontitis (LAP) and could be employed as an early salivary biomarker for the disease.[1,28]

CONCLUSION

Saliva can be used as a diagnostic blood substitute. Biomarkers in saliva can be very useful in the early diagnosis of a range of diseases and disorders in newborns and children. It is a complex and dynamic biological fluid that contains many different chemicals. Saliva alleviates patients' discomfort and increases compliance because it is easy, simple, painless, and noninvasive to collect, especially in newborns and toddlers, where frequent blood sample can be both painful and laborious. Saliva's functioning utility has long been assumed to outweigh its diagnostic potential.

REFERENCES

1. Pappa, E., Kousvelari, E. and Vastardis, H., 2019. Saliva in the "Omics" era: A promising tool in paediatrics. *Oral diseases*, *25*(1), pp.16-25.
2. Zhang Y, Sun J, Lin CC, Abemayor E, Wang MB, Wong DT. The emerging landscape of salivary diagnostics. Periodontology 2000. 2016 Feb;70(1):38-52.
3. Hassaneen M, Maron JL. Salivary diagnostics in pediatrics: applicability, translatability, and limitations. Frontiers in Public Health. 2017 Apr 20;5:83.
4. Oral histology – Tencates – 8th edition.
5. Oral histology and embryology – Orbans – 14th edition.
6. Gupta S, Ahuja N. Salivary Glands. Histology. 2019 Jan 10:63.
7. Porcheri C, Mitsiadis TA. Physiology, pathology and regeneration of salivary glands. Cells. 2019 Sep;8(9):976.
8. ARTHUR
9. da Silva Marques DN, da Mata ADSP, Patto JMV, Barcelos FAD, de Almeida Rato Amaral JP, de Oliveira MCM, Ferreira CGC. Effects of gustatory stimulants of salivary secretion on salivary pH and flow in patients with Sjögren's syndrome: a randomized controlled trial. Journal of oral pathology & medicine, 2011; 40(10): 785-792
10. Salivary diagnostics – David T Wong; first edition.
11. Malhotra T, Sachdeva A, Bhateja S, Arora G. Salivary biomarkers as a diagnostic tool. IP Journal of Surgery and Allied Sciences. 2019 Apr 15;1(1):1-4.
12. Babu NA, Masthan KM, Balachander N, Gopalakrishnan T, Elumalai M. Biomarkers in saliva. Biomedical and Pharmacology Journal. 2015 Apr 26;5(2):367-70.
13. Jasim, H. A. Carlsson, B. Hedenberg-Magnusson, B. Ghafouri, and M. Ernberg, "Saliva as a medium to detect and measure biomarkers related to pain," Scientific Reports, vol. 8, no. 1, pp. 3220–3229, 2018
14. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. International journal of oral science. 2017 Nov;9(11):e1.
15. Furlan NF, Gavião MB, Barbosa TS, Nicolau J, Castelo PM. Salivary cortisol, alpha-amylase and heart rate variation in response to dental treatment in children.

Journal of Clinical Pediatric Dentistry. 2012 Sep 1;37(1):83-7.
16. Zimmermann BG, Park NJ, Wong DT. Genomic targets in saliva. Annals of the New York Academy of Sciences. 2007 Mar 1;1098(1):184-91.
17. Priya KY, Prathibha KM. Methods of collection of saliva-a review. International Journal of Oral Health Dentistry. 2017;3(3):149-53.
18. Navazesh M. Methods for collecting saliva. Annals of the New York Academy of Sciences. 1993 Sep 1;694(1):72-7
19. Bhattarai KR, Kim HR, Chae HJ. Compliance with saliva collection protocol in healthy volunteers: strategies for managing risk and errors. International journal of medical sciences. 2018;15(8):823.
20. Asthagiri AR, Lauffenburger DA. Bioengineering models of cell signaling. Annual review of biomedical engineering. 2000 Aug;2(1):31-53.
21. Nelson J, Manzella K, Baker OJ. Current cell models for bioengineering a salivary gland: a mini-review of emerging technologies. Oral diseases. 2013 Apr;19(3):236-44.
22. Kaczor-Urbanowicz, K. E., Martin Carreras-Presas, C., Aro, K., Tu, M., Garcia-Godoy, F., & Wong, D. T. (2017). Saliva diagnostics - Current views and directions. *Experimental Biology and Medicine (Maywood)*, 242(5), 459–472.
23. Wetie, N., Armand, G., Wormwood, K. L., Russell, S., Ryan, J. P., Darie, C. C., & Woods, A. G. (2015). A pilot proteomic analysis of salivary biomarkers in autism spectrum disorder. *Autism Research*, 8(3), 338–350.
24. Qiao, Y., Wu, M., Feng, Y., Zhou, Z., Chen, L., & Chen, F. (2018). Alterations of oral microbiota distinguish children with autism spectrum disorders from healthy controls. *Scientific Reports*, 8(1), 1597.
25. Sruthi KS, Yashoda R, Manjunath PP. Diagnostic potential of saliva as a biomarker in early childhood caries: A review. children. 2019;11(12):14.
26. Kuriakose S, Sundaresan C, Mathai V, Khosla E, Gaffoor FM. A comparative study of salivary buffering capacity,flow rate, resting pH, and salivary Immunoglobulin A in children with rampant caries and caries-resistant children. J Indian Soc Pedod Prev Dent. 2013; 31:69-73.
27. Radhi NJ, Yas BA. Salivary viscosity in relation to oral health status among a group of 20-22 years old dental students. Iraqi Journal of Community Medicine. 2013; 26(3):219-24.
28. Bhat M, Bhat D. Salivary diagnostics in oral diseases. InSaliva and Salivary Diagnostics 2019 May 28. IntechOpen

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