ECRONICON OPEN ACCESS

EC DENTAL SCIENCE
Review Article

Saliva Caries Biomarker

Mohammed Hany Ahmad Fouad[1]*, Hussain Abdulrahman Musayri[2], Rana Adel Reda[2], Abdulaziz Ali Mohamed Alshahrani[2], Omar Mohammed Alotaibi[2], Wael Adnan Mohammed Alayoubi[2], Nafea Awnallah Alsulami[3], Nader Obaid Alotaibi[3], Nouf Adnan Abuzenada[3], Mohammed Ayidh Alrehaili[4], Falah Saeed Alshahrani[4], Ahmed Abdullah Alaskari[5], Awad Saeed Alshahrani[6], Faisal Abdullah ALHegbani[7] and Bushra Ali Alqahtani[8]

[1]*Cairo University, Egypt*

[2]*Ministry of Health, Saudi Arabia*

[3]*King Abdulaziz University, Saudi Arabia*

[4]*Ministry of Interior, Saudi Arabia*

[5]*King Khaled University, Saudi Arabia*

[6]*Vision Colleges, Saudi Arabia*

[7]*Ministry of interior, Saudi Arabia*

[8]*Armed Forces Hospital, Saudi Arabia*

***Corresponding Author**: Mohammed Hany Ahmad Fouad, Professor of Restorative Dentistry, Registered in Saudi Commission for Health Specialties as Consultant and Cairo University, Egypt. Number: 05JD0323. **E-mail:** abdelghanymohamedhany@gmail.com

Received: October 09, 2021; **Published:** October 28, 2021

Abstract

Introduction: Saliva, the biological fluid secreted by the major salivary glands like the parotid, sublingual and submandibular glands, has a complex and dynamic biological property and is mostly present in buccal mucosa and the cervical area of teeth. Saliva has a varied composition with a lot of biochemical properties, which makes it an important biological fluid and hence it can be used for detecting various diseases related to the oral cavity like caries, periodontitis, and also many systemic diseases like cancer, infectious diseases, autoimmune disorders, and neurological disorders. Assessment of caries risk will help us identify caries at early stages and the overall incidence of caries in the patient's mouth. It will also help us to identify the new developing caries and the rate at which caries progression is taking place.

Aim of Work: This review aims at highlighting an overview of saliva as a caries biomarker that can be used for the early cessation of carious lesions, thereby decreasing the progressing caries rate.

Methodology: This review is a comprehensive research of PUBMED and Google Scholar from the years 2000 to 2020.

Conclusion: The complex composition of saliva makes it a very crucial biological fluid. Salivary fluid represents a wide range of biomarkers that help to identify many oral and systemic diseases. The ease of collection, reduction in time and money, and increased patient comfort make saliva a better fluid for biomarker representation when compared to blood or urine. Salivary Biomarkers help us to diagnose the disease at an early age and provide a more conservative management technique.

Keywords: *Saliva; Biomarker; Early Childhood Caries; Caries Risk Assessment; Enamel Pellicle*

Introduction

A biomarker can be identified as a characteristic indicator of any normal biological process, a pharmacological response to drug intervention, or a pathological process. The presence of biomarkers helps us identify and quantify the nature of a particular disease, its genetic presentation, progress, and response to therapeutic and environmental intervention. The changes observed in the biomarkers over time help to identify the systemic changes that have been occurring in the organism; thus, the main function of biomarkers boils down to prevent, diagnose and assess the prognosis and sequential progression of the disease. Saliva, which is the biological fluid secreted by the

major salivary glands like the parotid, sublingual and submandibular glands, has a complex and dynamic biological property and is mostly present in buccal mucosa and the cervical area of teeth [1].

The main functions of saliva are lubrication of the tissues around the teeth, helps in mastication, tasting of food, clearing the oral cavity of microorganisms and debris. It also keeps the tooth safe from demineralization. The biofilm present in the mouth is generally acidic in nature, and because of its buffering ability, saliva helps to neutralize the acid formed by it and the acid consumed by us from our diet. The enamel pellicle formed on the surface of the teeth as a protecting layer also comprises saliva as the main constituent. Because of its highly mineralized composition, it helps in remineralization and protects from demineralization of the tooth [2].

The secretion of saliva mainly depends on the sympathetic and parasympathetic nerve supply of the human body, and the flow and viscosity of saliva, in turn, decides its physiological function and properties. The salivary also varies according to the sex, diet, age, fluid intake, and the circadian rhythm of the patient. The main constituent of saliva is water which makes almost 99% of the volume, with the remaining one percent comprising of inorganic substance like magnesium, phosphate, fluoride, sodium, chloride, etc., and salivary protein, which influences the viscoelastic nature of saliva, thereby controlling the defense mechanism of saliva and main functions like aid in digestion, taste, and lubrication. The various proteins present in saliva are listed in table 1 [3].

Salivary Protein	Function
1. Proline rich protein	Helps in plaque formation
2. Mucins	Helps in pellicle formation
3. Histatin	Antimicrobial and antifungal properties
4. Cystatins	Inhibition of cysteine proteases
5. Statherins	Inhibits precipitation of calcium phosphate salts
6. Salivary Immunoglobulins	The main defense system of saliva
7. Lactoferrin	Defense against bacterial injuries
8. Staterin	Controls calcium homeostasis

Table 1: *Salivary proteins and their function [3].*

Apart from all the above-mentioned proteins and inorganic substances, saliva also contains many other markers like growth factors, hormones, various genetic DNA, and RNA. Any changes in the pathophysiological condition of the body change the flow, consistency, and composition of the saliva, hence making saliva a parameter to assess changes in the body [4].

Dental caries is one of the most prevalent diseases in the world and affects more than 5 billion people throughout the world. The treatment of dental caries is only restorative techniques which can be either conservative or surgical, but in the end, the patient ends up losing little or a huge amount of the tooth structure. Assessment of caries risk will help us identify caries at early stages and the overall incidence of caries in the patient's mouth. It will also help us to identify the new developing caries and the rate at which caries progression is happening. This will, in turn, help in the classification of patients at higher risk of developing caries in which various preventive measures can be taken [5].

Importance of saliva as a biomarker

Saliva has a varied composition with a lot of biochemical properties, which makes it an important biological fluid and hence it can be used for detecting various diseases related to the oral cavity like caries, periodontitis, and also many systemic diseases like cancer, infectious diseases, autoimmune disorders, and neurological disorders. Saliva helps in the formation of enamel pellicle, which again can contribute largely in providing biomarkers for detection of gastrointestinal diseases and acts as a source of saliva as the protein constituent of enamel pellicle represents salivary composition [6].

The distinct advantage of using saliva as the biomarker biological fluid is its compositional similarity with blood as it has more than three thousand proteins, in which half of the composition is also seen in blood, making it an effective biomarker for systemic diseases. In a study conducted by Punyadeera., *et al.* they concluded that protein found in saliva has a huge correlation with serum both in humans and animals [7]. RNA protein for oral cancer is found in a higher number in saliva compared to serum and has a higher receiver operating characteristics value (ROC). In cases where the particular biomarker is present both in saliva and serum, the concentration of the biomarker is more in saliva, and because of the ease of sampling, saliva becomes a more preferred source of sample collection [8].

1.	Less discomfort and manipulation of the patient during sample collection as compared to blood or urine.
2.	The process of collection is non-invasive and painless
3.	Lower biological risk is observed
4.	Patient compliance is higher in all age groups, including geriatric and pediatric patients
5.	Cost is lowered as no special training or equipment is required.

***Table 2**: Advantages of using Saliva as a biomarker [9].*

Sample collection of saliva for use as a biomarker

The collection of saliva for any investigation can be divided into 4 ways:

1. Total collection of saliva
2. Collection from an individual gland
3. Stimulated saliva - by chewing, or any drug-induced stimulation
4. Unstimulated Saliva- Collected at rest.

In cases of biomarker analysis, total saliva collection is the best practice. The Source of saliva can be the sputum, spit, or suction. Cases, where saliva has to be collected from a particular gland require specific armamentarium, like for collection from the parotid glands use of Lashley cups or a modified version like the Carlson Crettendon cup (Figure 1) and from submandibular and sublingual glands use of a Wolff saliva collector are prevalent (Figure 2). One of the main factors that have to be kept in mind is the time when the saliva is collected as it varies according to the circadian rhythm of various individuals, and a thorough study of the circadian cycle of the individual must be done before collection is planned. Once the collection of saliva is completed, the main challenge that arises is storage, as salivary proteins are at a higher risk for degradation as compared to serum proteins [10].

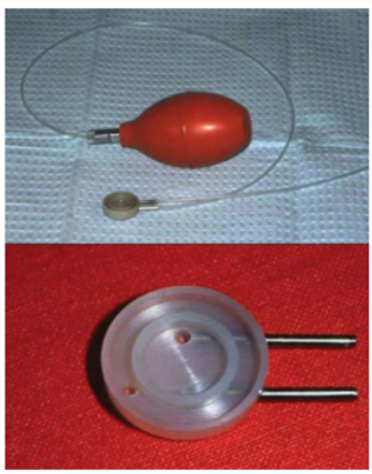

***Figure 1:** Carlson-Crettendon cup for collection of saliva from parotid gland [10].*

Figure 2: *Custom made Wolff cup for collection from submandibular and sublingual gland [10].*

Caries risk assessment using saliva

Saliva is a source of many microorganisms that are present in a small quantity in healthy individuals. The main physiology of caries formation is the interaction of dietary carbohydrates that produce acid by interacting with cariogenic bacteria present in saliva. *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* are often seen associated with a high rate of caries formation, with *S. mutans* being the primary agent for caries progression. The main characteristics of *Streptococci* that lead to a higher caries formation rate are its ability to survive at a very low pH, while it causes a very high rate of carbohydrate fermentation and has the ability to firmly attach to the surfaces of teeth. *S. mutans* are seen in infants who are in their predental stage, which is largely attributed to transmission from their mother [11]. In a study conducted by Corby., *et al.* [12] where they profiled the 16S rDNA of dental caries, they concluded that *S. mutans* was found in high numbers in children with caries and also in geriatric patients with root caries. In order to reduce the caries risk of a child, it's important that the *S. mutans* colonization is arrested in the mother, which decreases the chances of early childhood caries [12]. *Candida* species have also been associated with caries due to their ability to colonize with other bacterial species and increased fermentation of carbohydrates. Many studies have revealed that an increased rate of *Candida* species is seen in patients with a higher caries rate [13].

Early childhood caries (ECC) is one of the most detrimental oral diseases found in patients with age 6 or less. Biomarkers found in saliva can help in the diagnosis and early prevention of the disease. One of the most important markers found in saliva is *S. mutans* which is shown to have an increased correlation with ECC [14]. *Candida albicans* showed a five times higher risk association of caries when found in saliva [15].

The composition of saliva varies in patients with a higher caries rate as compared to a normal patient's. The main difference seen was higher levels of proline-rich peptide in the caries-free group, which showed antimicrobial properties. Various differences in the salivary proteins compared between both groups are mentioned in table 3 [16-18].

Caries free individuals	High carious rate individuals
1. Higher Calcium concentration	1. High total protein value
2. High bicarbonate concentration	2. Higher antioxidant level
3. Higher Urease activity	3. Higher α-amylase level
4. Higher concentration of salivary proteins like histatin, statherin, proteinase, etc.	4. A higher quantity of MUC1 and MUC5B

Table 3: *Difference in salivary composition between carious and non-carious individuals [16-18].*

Other factors related to saliva helping in caries risk assessment

1. Salivary flow rate: A reduced rate of salivary flow facilitates a longer exposure time of the teeth to cariogenic bacteria, thus increasing the chances of caries. The salivary flow rate can be reduced due to various reasons, including diseases like Sjogren's syndrome, drug-induced or due to dietary incompetency [19].
2. Acidity and buffering capacity of saliva: Buffering capacity refers to the resistance shown by saliva to changes in the acidic condition. In cases where the buffering capacity of saliva is lowered due to any reason, chances of caries development increase [20].

Use of saliva as biomarker for other diseases

Periodontitis: Patients with chronic periodontitis showed inflammatory biomarkers and elevated levels of salivary proteins like heavy chain immunoglobulins, serum albumin, and α-amylase and a lowered value of cystatin protein [21].

Oral cancer: Oral cancer has the highest prevalence amongst all the other head and neck cancers, with squamous cell carcinoma being the most common form of oral cancer seen. A study conducted by Nagler., *et al*. concluded that patients with squamous cell carcinoma had increased levels of cytokeratin 19, CA 125, and tissue polypeptide antigen [22]. In another study conducted by He., *et al.* they concluded increased levels of an antigen associated with oral cancer like CA50 were found in the saliva of patients with oral cancer [23].

Gastric cancer: Target molecules used for salivary biomarkers in the case of gastric cancer are amino acids. D-Amino acid was found to be at an increased level in the saliva of patients with Gastric cancer. Other proteins like Cystatin B and glycoproteins were also found in increased quantities in patients with gastric cancer [24].

Pancreatic cancer: Few salivary microbiotas were found to be associated with patients with pancreatic cancer like *Streptococcus mitis, N. elongata* and *Porphyromonas*. These microbes were found to be in a higher number in cancer patients when compared to the healthier individuals and could be used as an early and non-invasive diagnostic tool for the detection of pancreatic cancer [25].

Conclusion

The complex composition of saliva makes it a very crucial biological fluid. Salivary fluid represents a wide range of biomarkers that help to identify many oral and systemic diseases. The ease of collection, reduction in time and money, and increased patient comfort make saliva a better fluid for biomarker representation when compared to blood or urine. Salivary Biomarkers help us to diagnose the disease at an early age and provide a more conservative management technique.

Bibliography

1. Selwitz R H., *et al*. "Dental caries". *The Lancet* 369.9555 (2007): 51-59.
2. Berg J H. "Early dental caries detection as a part of oral health maintenance in young children". *Compendium of Continuing Education in Dentistry* 26.5-1 (1995): 24-29.
3. Chiappin S., *et al*. "Saliva specimen: a new laboratory tool for diagnostic and basic investigation". *Clinica Chimica Acta* 383.1-2 (2007): 30-40.
4. Kaczor-Urbanowicz K E., *et al*. "Saliva diagnostics–Current views and directions". *Experimental Biology and Medicine* 242.5 (2017): 459-472.
5. Fontana M and Zero D T. "Assessing patients' caries risk". *The Journal of the American Dental Association* 137.9 (2006): 1231-1239.
6. Martini T., *et al*. "Proteomics of acquired pellicle in gastroesophageal reflux disease patients with or without erosive tooth wear". *Journal of Dentistry* 81 (2019): 64-69.
7. Punyadeera C., *et al*. "One-step homogeneous C-reactive protein assay for saliva". *Journal of Immunological Methods* 373.1-2 (2011): 19-25.
8. Li Y., *et al*. "Salivary transcriptome diagnostics for oral cancer detection". *Clinical Cancer Research* 10.24 (2004): 8442-8450.

9. Dawes C and Wong D T W. "Role of saliva and salivary diagnostics in the advancement of oral health". *Journal of Dental Research* 98.2 (2019): 133-141.

10. Navazesh M and Kumar S K. "Measuring salivary flow: challenges and opportunities". *The Journal of the American Dental Association* 139 (2008): 35S-40S.

11. Berkowitz R J. "Acquisition and transmission of mutans streptococci". *CDA* 31.2 (2003): 135-138.

12. Corby P M., *et al*. "Microbial risk indicators of early childhood caries". *Journal of Clinical Microbiology* 43.11 (2005): 5753-5759.

13. Raja M., *et al*. "Association of oral candidal carriage with dental caries in children". *Caries Research* 44.3 (2010): 272-276.

14. Kirthiga M., *et al*. "Risk factors for early childhood caries: a systematic review and meta-analysis of case control and cohort studies". *Pediatric Dentistry* 41.2 (2019): 95-112.

15. Xiao J., *et al*. "Candida albicans and early childhood caries: a systematic review and meta-analysis". *Caries Research* 52.1-2 (2018): 102-112.

16. Monica M., *et al*. "Analysis of salivary level of alpha-amylase as a risk factor for dental caries". *Acta Medica Transilvanica* 23.1 (2018).

17. Yang T Y., *et al*. "Role of saliva proteinase 3 in dental caries". *International Journal of Oral Science* 7.3 (2015): 174-178.

18. Nascimento M M., *et al*. "Correlations of oral bacterial arginine and urea catabolism with caries experience". *Oral Microbiology and Immunology* 24.2 (2009): 89-95.

19. FenolI-Palomares C., *et al*. "Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers". *Revista Espanola de Enfermedades Digestivas* 96.11 (2004): 773-783.

20. Leone CW and Oppenheim FG. "Physical and chemical aspects of saliva as indicators of risk for dental caries in humans". *Journal of Dental Education* 65.10 (2001): 1054-1062.

21. Gonçalves LDR., *et al*. "Comparative proteomic analysis of whole saliva from chronic periodontitis patients". *Journal of Proteomics* 73.7 (2010): 1334-1341.

22. Nagler R., *et al*. "Concomitant analysis of salivary tumor markers—a new diagnostic tool for oral cancer". *Clinical Cancer Research* 12.13 (2006): 3979-3984.

23. He H., *et al*. "A joint detection of CEA and CA-50 levels in saliva and serum of patients with tumors in oral region and salivary gland". *Journal of Cancer Research and Clinical Oncology* 135.10 (2009): 1315-1321.

24. Zhang Z., *et al*. "Non-invasive detection of gastric cancer relevant d-amino acids with luminescent DNA/silver nanoclusters". *Nanoscale* 9.48 (2017): 19367-19373.

25. Asai Y., *et al*. "Elevated polyamines in saliva of pancreatic cancer". *Cancers* 10.2 (2018): 43.

Volume 20 Issue 11 November 2021