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BIOMARKERS OF SALIVA: A REVIEW OF ITS ROLE IN PREDICTING DENTAL CARIES

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ABSTRACT

Dental caries is a major public health problem globally and is the most widespread non communicable disease (NCD). Severe dental caries affects general health and often causes pain and infection, which may result in tooth extraction. The costs of treating dental caries cause heavy financial burdens on individuals, families and society as a whole. But if one can predict this multifactorial disease in its early stages, it can significantly lessen the risk of patient discomfort, pain, help in prognosis, retention rates, and reduce recurrence. One of the intra oral host factors that affect caries development is saliva. As teeth are constantly bathed in saliva, the constituents and properties of this oral fluid play an essential role in the occurrence and progression of dental caries. Saliva consists of some important naturally occurring properties or functions which act as biomarkers and help in the prediction of dental caries. This paper is aimed to compose a review about the potential salivary biomarkers of dental caries.

KEYWORDS: Caries Susceptibility, Microorganisms, Proteins, Salivary Biomarkers.

INTRODUCTION

Saliva is said to be a "mirror of the body" because it is an indicator of health not just in the oral cavity but throughout the body. The oral tissues are surrounded by this complex mixture of fluid.[1] The lubricating and antimicrobial functions of saliva are maintained mainly by resting saliva. Stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents.[2] For more than 2000 years, saliva serves as the diagnostic tool in health and disease. It can be used to diagnose any pathology from the specific major salivary gland. Most importantly in the diagnosis of oral diseases like dental caries, periodontal disease and oral cancer, saliva plays an enormous and great role.[3] Dental caries which is recognized as a multi-factorial infectious disease is caused by complex interactions among acid-producing bacteria, fermentable carbohydrates and many host factors including saliva.[4] Saliva consists of some important naturally occurring properties or functions which act as biomarkers and help in the prediction of dental caries. Salivary biomarkers such as salivary flow, salivary pH, salivary proteins, microorganisms etc., are used for the prediction, diagnosis, prognosis and management of dental caries, as well as for evaluating the outcome of therapeutic regimens.[5]

The anticaries effect of saliva

Dental caries is a multifactorial disease and one of the major contributing factors is saliva. Salivary components like viscosity, flow, buffering capacity, etc., play a major role in the initiation, progression and prevention of the disease. It helps in the prevention of the caries by its antibacterial effect. Secretory IgA is the main immunoglobulin in saliva. It is the first line of defence of the host against pathogens which invade mucosal surfaces.[6]

One of the main aim in caries prevention is promoting the natural protective mechanisms of saliva. The pH of dental plaque is a key factor in the balance between acid demineralisation of the teeth and the remineralisation of the initial caries lesion. Plaque pH falls each time acid accumulates in the plaque due to bacterial acid production following the consumption of fermentable carbohydrates – mainly sugars – in foods and drinks. Conversely, plaque pH rises when the acids are washed away or neutralised by saliva, which contains the important buffer, bicarbonate. In healthy teeth, the loss of minerals is balanced by the reparative mechanisms of saliva.[7] This equilibrium can be depicted chemically by the equation overleaf – see Figure 1.[8]

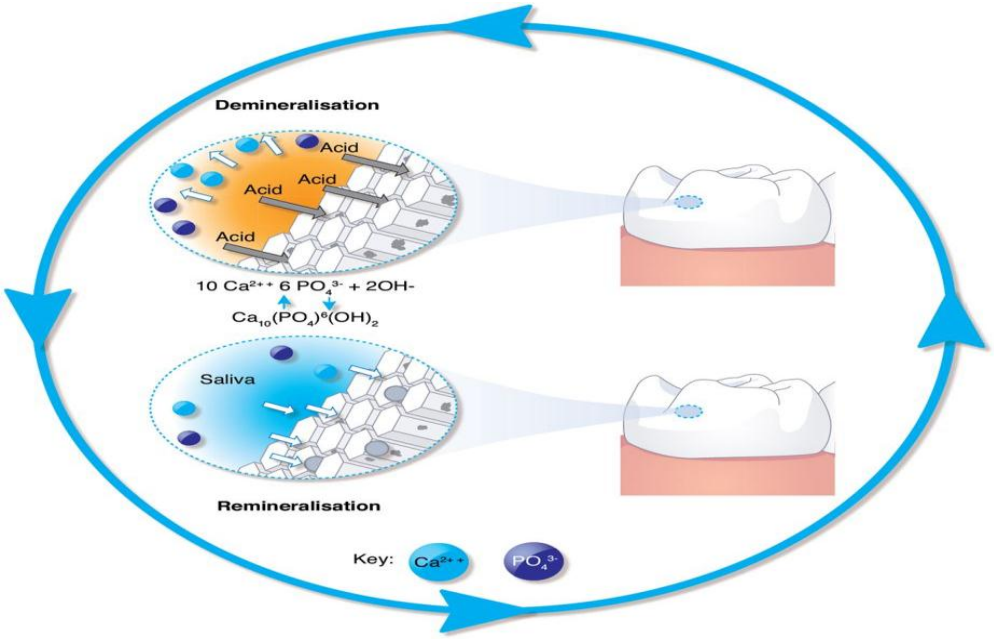

Fig. 1: The process of tooth remineralisation.

When the saliva pH or the plaque pH is below a 'critical value' of about 5.5, the saliva or plaque becomes unsaturated with respect to tooth mineral. As a result, tooth enamel can begin to dissolve. However, when the pH is above this value, the saliva and plaque are supersaturated with respect to tooth mineral. The calcium and phosphate ions in saliva then start to repair any damaged mineral crystals in the enamel – the process of remineralisation. Stimulation of saliva flow results in an increase in the washing out of acids (and sugars), and also an increase in the amount and concentration of bicarbonate buffer and of remineralising ions.[7]

Biomarkers and The multifactorial disease "Dental caries"

Saliva has the potential to be used in the early detection and diagnosis of caries. This is due to the abundant biomarkers present in saliva. They are:

I. Functional properties of saliva as biomarkers of caries

1. Salivary flow rate
2. Saliva pH and buffering capacity

II. Microorganisms

III. Salivary electrolytes

IV. Salivary Proteins (Proteomes)

1. Immunoglobulins
2. Acidic proline-rich proteins
3. Mucins 1 e 2(Mucous glycoproteins)
4. Agglutinins
5. Lactoferrin and lysozyme
6. Cystatin S and Statherin
7. Defensin
8. CD14
9. Glucosyltransferases
10. Amylase

I. Functional properties of saliva as biomarkers of caries

Individual salivation ranges from 0.3 to 0.7 ml of saliva/min on an average, producing about 1 to 1.5 litres daily. Human saliva is a clear, slightly acidic (pH 6.0 to 7.0) heterogeneous biofluid composed of 98% water and 2% other compounds, such as electrolytes, mucus, antibacterial compounds, and various enzymes.[9] As the caries process involves internal and external causal factors, salivary secretions can dramatically affect the rate of caries development.[10] The functional properties of saliva which act as Biomarkers Of Caries include the following: (1)Salivary Flow Rate and (2)Salivary pH and buffering capacity.[9]

(1) Salivary flow rate: Saliva plays many important roles but the most significant ones include building and maintenance of oral health. When salivary flow is decreased, oral health problems such as dental caries and oral infections can increase.[7] Severe reduction of salivary secretion not only results in a rapid deterioration in oral health but also has a harmful impact on the quality of life for the people who suffers. Patients suffering from dry mouth can experience difficulty with eating, swallowing, speech, ulceration of the oral mucosa, taste alteration, poor oral hygiene, a burning sensation of the mucosa, oral infections like Candida and rapidly advancing dental caries.[11]

Unstimulated whole saliva is the mixture of secretions found in the mouth in the absence of exogenous stimuli such as tastants or chewing. Stimulated saliva is secreted in response to masticatory or gustatory stimulation, or to other less common stimuli such as certain drugs (e.g. pilocarpine) or to activation of the vomiting centre.[11] At rest, without exogenous or pharmacological stimulation, there is a small, continuous salivary flow, an unstimulated

secretion, present in the form of a film that covers, moisturizes, and lubricates the oral tissues. This flow of saliva at rest is in the region of 0.4 0.5mL/minute in healthy subjects.[7] During sleep, the maximum flow will fall to less than 0.1 ml/minute and during eating the average stimulated flow rate is about 4 ml/minute.[11]

All other salivary functions, such as **buffering and clearance**, depend on the salivary flow rate. Cavities are most prevalent in patients with a lower salivary flow due to a decrease in the antibacterial, buffering and cleansing functions. The salivary flow dilutes the substances, cleans the oral cavity off carbohydrates, non-adherent bacteria, desquamated epithelial cells and food debris. This phenomenon is essential for decreasing the availability of sugars for the biofilm. The salivary viscosity reduces the hydration capacity of saliva, and consequently raises the caries risk.[9]

(2) Saliva pH and Buffering capacity: The pH of dental plaque is the most important component which helps to keep the balance between acid demineralisation of the teeth and the remineralisation of the initial caries lesion. Whenever acid accumulates in the plaque after the consumption of fermentable carbohydrates like sugars, the plaque pH falls. Whereas, plaque pH rises when the acids are washed away or neutralised by saliva.[7] This quantitative assessment of resistance to pH changes is referred to as **buffer capacity**. Saliva buffering capacity works by preventing the decrease in pH and protects the tooth from dental caries. Saliva has two main buffer systems: **1st** is the carbonic acid-bicarbonate system and **2nd** is phosphate buffer system. Individuals with a high salivary buffer capacity are often caries-resistant.[9]

When the saliva pH or the plaque pH is below a **'**critical value' of about 5.5 which is known as **"critical pH"**, the saliva or plaque becomes unsaturated with respect to tooth mineral. As a result, tooth enamel can begin to dissolve. However, when the pH is above this value, the saliva and plaque are supersaturated with respect to tooth mineral. The calcium and phosphate ions in saliva then start to repair any damaged mineral crystals in the enamel by the process of remineralization.[7] The buffering capacity of saliva increases with increasing flow rate as the bicarbonate ion concentration increases. The carbonic acid / bicarbonate system is the major buffer in stimulated saliva.[11]

II. Microorganisms

About 700 different bacteria species have been identified from the human oral microbiome. But cariogenic bacteria are usually present in relatively small quantities in healthy saliva and plaque because with biological and environmental changes such as the increased frequency of fermentable carbohydrate consumption, low pH will favour the proliferation of bacteria. The acidic metabolites by microorganisms cause a fall in the pH below a critical value (pH 5.5) resulting in the demineralization of the tooth tissue and dissolution of the hydroxyapatite crystals, the major component of enamel and dentin.[9]

An important role is played by the cariogenic bacteria, i.e. oral **streptococci**, especially of group **mutans** and lactic acid bacteria **(*Lactobacillus* spp**.) in the pathogenesis of dental caries.[9] Caries can also be caused by other bacteria, including members of the mitis, anginosus and salivarius groups of streptococci, *Propionibacterium*, *Enterococcus faecalis* and *Scardovia.* Bacteria of the species *Streptococcus mutans* is the main factor that initiates caries and very important factor of enamel decay.[12] In the initiation of caries, the main role of mutans streptococci is the formation of extracellular polysaccharides from sucrose which promotes their firm attachment to teeth, rapid fermentation of carbohydrates to acids and tolerance to low pH.[9] Mutans streptococci include bacteria of seven species, namely Streptococcus mutans, Streptococcus sobrinus, Streptococcus cricetus, Streptococcus rattus, Streptococcus ferus, Streptococcus macacae and Streptococcus downei.[9] Among these species, S. mutans has been identified as a strong pathogen for caries. Early acquisition of S. mutans is associated with early childhood caries and future caries. Mutans streptococci and lactobacilli are characterized by the ability to grow in an acidic environment and the property of rapid metabolism of sugars supplied in the diet to organic acids.[12]

The bacteria of the genus *Lactobacillus* are important in further caries development, especially in the dentin and they may potently contribute to caries progression once lesions are established. The level of lactobacilli in saliva also reflects the acidogenic conditions associated with the consumption of abundant simple carbohydrates.[9]

Synthesis of carbohydrate polymers: Most of the sucrose metabolized by S. mutans is utilized for its energy requirement and results in the production of lactic acid. However the sucrose which does not enter the cell may be used for the extracellular synthesis of carbohydrate polymers. S. mutans can polymerize the glucose and the fructose moieties of sucrose to synthesize two types of extracellular polymers, glucans and fructans respectively.

Of the two classes of polysaccharides, the glucans are of more importance in promoting S. mutans accumulation on teeth. Fructans serves as a reservoir of fermentable sugars for oral bacteria.[10]

Conventional bacterial test kits are almost exclusively culture-based, using dip-slide or selective culture broth. Some novel test kits, using immunoassay technology, have recently been developed and are expected to provide quick and highly specific numeration of cariogenic bacteria.[9]

Other microorganisms: The range of bacteria potentially involved in caries also include some non mutans streptococci, such as **Streptococcus sanguinis** and **Streptococcus salivarius**, and **Actinomyces** species; the latter contributes to the onset of root surface caries.[9]

Streptococcus sanguis: This α-hemolytic Streptococcus species being so ubiquitious, is consistently present in plaque obtained from both carious and non-carious sites. However, its proportions of the plaque microflora relative to *S.mutans* usually show a high association with sound rather than carious sites.[10]

Streptococcus salivarius: This species is found predominantly on the tongue, soft tissue and in saliva but is not found in high numbers in plaque. It produces copious amounts of a very viscous extracellular polymer which is a levan (fructan). However, its low numbers in human plaque suggest that it is not of great significance in human caries.[10]

Filamentous bacteria: Several types of filamentous organisms will initiate root surface carious lesions in experimental animals. ***Actinomyces*** and ***Rothia*** species have been found in human dental plaque and dental calculus in significant numbers. *Actinomyces viscosus*, an acidogenic bacterium which also stores intracellular polysaccharides, is almost always among the predominant flora of plaque overlying root lesions.[10]

Streptococcus milleri: This fairly homogenous group is based on its morphological characteristics but serologically it is very heterogeneous. Although some strains induce fissure caries in experimental animals, its importance in dental caries in humans is not known at present.[10]

III. Salivary electrolytes

The most important salivary electrolytes for protecting teeth from caries are **sodium, fluoride, chloride, calcium, phosphate and bicarbonate.** There are other electrolytes that are present in low concentrations, less than 1 mM, include **sulphate, thiocyanate, iodide and magnesium.**[10] The process of remineralization and demineralization is regulated by saliva. These phenomena are controlled by the concentration of salivary calcium, phosphate, fluoride, and pH.[9]

Fluoride reduces acid production in the biofilm, therefore, its presence in saliva is very important. For maintaining the integrity of dental tissues, fluoride, calcium and phosphate ions keep saliva supersaturated with respect to hydroxyapatite and offer a reparative and protective environment.[9]

Calcium and trivalent phosphate (PO_4^{3-}) ions, along with hydroxyl ions, maintain the saturation of saliva with respect to tooth mineral, and are therefore important in calculus formation and in protecting against the development of both caries and erosion.[11]

A calcium and phosphate rich environment also facilitates remineralization of incipient carious lesions or demineralized zones of enamel. Accessibility of saliva is an important factor in permitting remineralization of incipient carious lesions.[10]

Thus we can find an inverse relationship between dental caries and levels of salivary calcium, and between dental caries and organic or inorganic phosphorous.[9]

IV. Salivary proteins

A large number of proteins are present in saliva which helps in the protection of the oral tissues. For example, lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinin and mucins. Besides, several peptides such as histatins, defensins and the only human cathelicidin, LL-37 has been found to have bacteria killing activity.[13] More than 1400 proteins are found to be present in salivary proteome which mainly consists of proteins. The association of salivary proteome with dental caries is an important step towards the identification of biomarkers to caries and thus helps in the prediction of caries susceptibility, letting the intervention in a presymptomatic state.[9]

Protective properties of the major salivary proteins[13]

- Agglutinin- aggregation of bacteria
- Cathelicidin (LL37)- broad-spectrum killing of bacteria
- Cystatins/VEGh- protease inhibitor
- Defensins- broad-spectrum killing of bacteria
- EP-GP- unknown
- Histatins- broad-spectrum killing of bacteria
- Immunoglobulins- inactivation and aggregation of bacteria
- Lactoferrin- growth inhibition
- Lactoperoxidase- growth inhibition
- Lysozyme- killing
- MUC5B (mucin MG1)- proton-diffusion barrier in pellicle
- MUC7 (mucin MG2)- aggregation
- Proline-rich glycoprotein- unknown: aggregation?
- Proline-rich proteins (aPRPs)- adherence
- Proline-rich proteins (bPRPs)- unknown: membrane disturbing
- Statherin- adherence

❖ **The various salivary proteins**

1. ***Immunoglobulins:*** The immunoglobulins in saliva mainly belong to the IgA subclass (>85%) and, to a lesser extent, to the IgG and IgM subclasses. The entire population of salivary immunoglobulins presents a broad-spectrum defense system by binding the majority of microorganisms present in saliva.[13] Of the total salivary proteins immunoglobulins make up 5–15%. Salivary antibodies play an antibacterial role by inhibiting bacterial metabolism, agglutination of bacteria and neutralizing bacterial toxins and enzymes. Also, it promotes the inhibition of bacterial adherence, by the reduction of the hydrophobicity of bacteria. The normal level of sIgA (Salivary IgA) in individuals without systemic or immunological diseases ranges from 4-30 mg/dL. It can change in case of salivary flow rate. A decreased level of sIgA is associated with an increase in root caries and candidiasis in elderly people.[9]

2. ***Acidic proline-rich proteins:*** Acidic proline-rich proteins account for 25-30% of all proteins in saliva. Proline-rich glycoproteins exerts buffering capacity, antibacterial

property and lubrication. These proteins inhibit the precipitation of calcium and phosphate by binding to hydroxyapatite of enamel crystals.[9]

3. ***Mucins 1 e 2 (Mucous glycoproteins):*** The mucins of saliva form a seromucosal cover that lubricates, protects, prevent the dehydration, protects tissues against the proteolytic attacks of microorganisms and maintain the viscoelasticity of saliva. It also has antibacterial properties. These type of proteins accelerate the clearance of bacteria from the oral cavity by interacting with several strains of streptococci, enhancing their agglutination and protect teeth surface from demineralization.[9]

4. ***Agglutinins:*** This mucin-like glycoprotein mediates the aggregation of many oral bacteria and interacts with unattached bacteria, resulting in clumping of bacteria into large aggregates, which are more easily swallowed or flushed away.[9]

5. ***Lactoferrin and Lysozyme:*** Latoferrin is an antibacterial enzyme which can bind and kill bacteria via direct interactions through the strongly basic N-terminal region of the glycoprotein. Lactoferrins are also capable of neutralizing the interaction between bacterial lipopolysaccharides (LPS) and host defense cells. This interaction can alter the permeability of the outer membrane of Gram-negative bacteria and release LPS. Because of its antimicrobial activity, salivary LF is thought to play a major role in caries susceptibility.[14] Lactoferrin has bacteriostatic, bacteriocidal, fungicidal, antiviral and anti-inflammatory activity. Lysozyme can activate bacterial autolysins and destroy the cell walls. Lysozyme promotes bacterial clearance through aggregation and adherence and destroys the bacterial cell wall of some bacteria.[9]

6. ***Lactoperoxidase:*** A factor exists in milk, tears and saliva which can inhibit the growth and acid formation of some bacteria. This substance (enzyme) is known as lactoperoxidase. It oxidizes thiocyanate (SCN^-) in the presence of hydrogen peroxide which is formed by many oral organisms. This antibacterial system is known to be inhibitory towards lactobacilli and some streptococci.[10]

7. ***Cystatin S and Statherin:*** Cystatins belongs to a heterogeneous family of proteins and are cysteine proteinase inhibitors, antimicrobial and immunomodulatory present in all mucosal secretions.[13] Cystatin S can be phosphorylated in five sites. The phosphorylated forms have an important function in the regulation of calcium levels and in the pellicle

formation. The removal of the phosphate groups of cystatin reduces the affinity of the protein to hydroxyapatite. The most important function of Statherin is inhibition of precipitation in supersaturated solutions of calcium, thus, acting as a primary regulator of mineralization in the oral cavity.[9]

These proteins have an inverse correlation with occlusal caries. Higher levels of statherin and cystatin S are observed in caries-free individuals.[9]

8. ***Defensin:*** These are small, cationic proteins with antimicrobial activity. The peptides can kill a variety of gram positive and gram-negative bacteria, fungi and enveloped viruses. They are of two types- α-defensins and β-defensins. Higher salivary a-defensins (HNP1, 2, 3) have been found in caries-free children.[9]

9. ***CD14:*** It is involved in innate immunity and mediates the activation of endothelial cells, polymorphonuclear leucocytes and epithelial cells. CD14 acts as an important anti-cariogenic factor as an inverse relationship is seen between the presence of sCD14 in saliva and caries lesions. It helps in the binding between the epithelial cells and bacteria and activates the production of cytokines for the recruitment of phagocytes.[9]

10. ***Glucosyltransferases:*** These proteins help in the synthesis of glucans which serve as a binding site for streptococci and other microorganisms. Glucans participate in oral colonization and formation of the oral biofilm. Glucosyltransferases (GTFs) from S. mutans are a candidate for the production of dental caries vaccine.[9]

11. ***Amylase:*** The α-amylase is referred to as a good indicator of the function of the salivary glands, and represents 40% to 50% of the protein content of saliva. It helps in the control of S.mutans colonization. Amylase bound to bacteria in plaque may facilitate hydrolysis of dietary starch to provide glucose for metabolism by plaque microorganisms in close proximity to the tooth surface. In addition, amylase may bind with high affinity to a selected group of oral streptococci and contribute to bacterial clearance.[9]

CONCLUSION

Salivary biomarkers have many potential roles in the diagnosis and management of dental caries and appears to be a potential source of biomarkers for dental caries. However, the association between many salivary factors and dental caries has yet to be established. Further studies are needed to define whether the individual has an increased risk of caries. No

salivary parameter identified thus far is able to select caries-susceptible patients with high sensitivity and specificity on a single test basis. Various salivary parameters should be combined with sociodemographic, behavioral and clinical factors for a better estimate of patients' caries risk.

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