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RESEARCH ARTICLE

SALIVARY BIOMARKERS OF DENTAL CARIES - A REVIEW ARTICLE

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Abstract

Saliva is the complex mixture of fluids that surrounds the oral tissues, secreted by the major and minor salivary glands[1]. Human saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosal surfaces in different ways. Dental caries is recognized as a multi-factorial infectious disease caused by complex interactions among acid-producing bacteria, fermentable carbohydrates and many host factors including saliva. Saliva is easy to collect, to store, and can be obtained at low cost in sufficient quantities for analysis by a non-invasive method. Thus it has the potential to be used in the early detection and diagnosis of various oral diseases due to the abundant biomarkers present in saliva. Saliva 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. This article aims to providea brief overview of salivary biomarkers present which can be used for the early detection nd prevention of dental caries in humans.

Introduction:-

Saliva is considered to be the “mirror of our body” as it is an indicator of general health not just the oral cavity. Saliva is the complex mixture of fluids that surrounds the oral tissues, secreted by the major and minor salivary glands[1]. Human saliva not only lubricates the oral tissues, making oral functions such as speaking, eating, and swallowing possible, but also protects teeth and oral mucosal surfaces in different ways. The resting saliva maintains the lubricating and antimicrobial functions of saliva. Stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents[2].

Saliva as a biological fluid serves as the diagnostic tool in health and disease for more than 2000 years[3]. It can be used as a diagnostic tool in various systemic diseases like hereditary disorders, autoimmune diseases, infectious diseases, as well as in the assessment of therapeutic levels of drugs and monitoring of illicit drug use, and also for forensic evidence and others.

Most importantly saliva plays a great role in diagnosis of oral diseases like dental caries, periodontal disease and oral cancer. In the last few decades, there has been a focus on the utilization of saliva for microbiological tests that give an indication of dental caries.

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Dental caries is recognized as a multi-factorial infectious disease caused by complex interactions among acid-producing bacteria, fermentable carbohydrates and many host factors including saliva[4]. Dental Caries is the most prevalent oral disease in Asian and Latin American countries, while it appears to be less common and less severe in most African countries[5]. It remains a major health issue in the United States and worldwide with a prevalence of more than 40 percent in young children and about 90 percent in the adult population[5].

Saliva 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[6]. The salivary flow is important in the prevention of caries, and there is a high risk of caries in individuals with a low unstimulated salivary flow. All salivary functions, such as buffering and clearance are dependent on the salivary flow rate.

There are certain epidemiological studies which have shown that children who experience colonization by mutans streptococci early in life are at greater risk of developing dental caries than those who are colonized later[7,8]. A study of oral Candida species suggest their cariogenic potential since they exhibit acidogenic hetero fermentative properties, especially in the presence of carbohydrates, and co aggregation with other bacteria in biofilms[4].

These studies help us to understand the role of salivary biomarkers in prediction of dental diseases. The saliva is recognized as one of the important factor which helps in preservation and maintenance of oral and systemic health irrespective of its quantity whether large or small[9]. Hence this article aims to to provide an overview of the current understanding of the salivary biomarkers which are used for the prediction, diagnosis, prognosis and management of dental diseases.

History

From the Australopithecines (over a million years ago) to the Neolithic (since 10,000 years ago), carious lesions have been found in almost every population studied. Caries, however, was very uncommon amongst fossil hominids into the Paleolithic and Mesolithic. A Sumerian text from 5000 BC describes a **tooth worm** as a cause of caries[10]. The EbersPapyrus ,an Egyptian text from 1550, mentions diseases of teeth. The rate of caries remained low through the Bronze age and Iron age, but sharply increased during the Middle ages. During the Roman occupation of Europe, wider consumption of cooked foods led to a small increase in caries prevalence[11].

The prevalence of caries increased dramatically in the 19th century, as the Industrial revolution made certain items, such as refined sugar and flour, readily available. In the 1890s W.D.Miller gave chemo parasitic caries theory. This lead to need of proper diagnosis and treatment of dental caries[12].

Some decades ago, visual examination (light and mirror) and probing, supplemented by bitewing radiographs were the only tools available for clinical diagnosis of caries. However a variety of innovative technologies have been developed and introduced in the last few years to aid clinicians not only in early diagnosis of caries but make a firm diagnosis and to treat cases conservatively. Salivary diagnostic is one of these recent advancements.

Saliva contains the serum constituents that can be used as a diagnostic tool in various diseases like hereditary disorders, autoimmune diseases, infectious diseases, as well as in the assessment of therapeutic levels of drugs and monitoring of illicit drug use, and also for forensic evidence and others. Moreover saliva also plays a great role in diagnosis of oral diseases like dental caries, periodontal diseases and oral cancer. Bacteriological tests of saliva plays the most important role in prediction of dental caries which is recognized as a multifactorial infectious disease caused by complex interactions among acid producing bacteria ,fermentable carbohydrates and many host factors including saliva .

Salivary biomarkers such as salivary flow, salivary pH salivary proteins, micro organismsetc, are used for prediction, diagnosis, prognosis and management of dental caries, as well as for evaluating the outcome of therapeutic regimens.

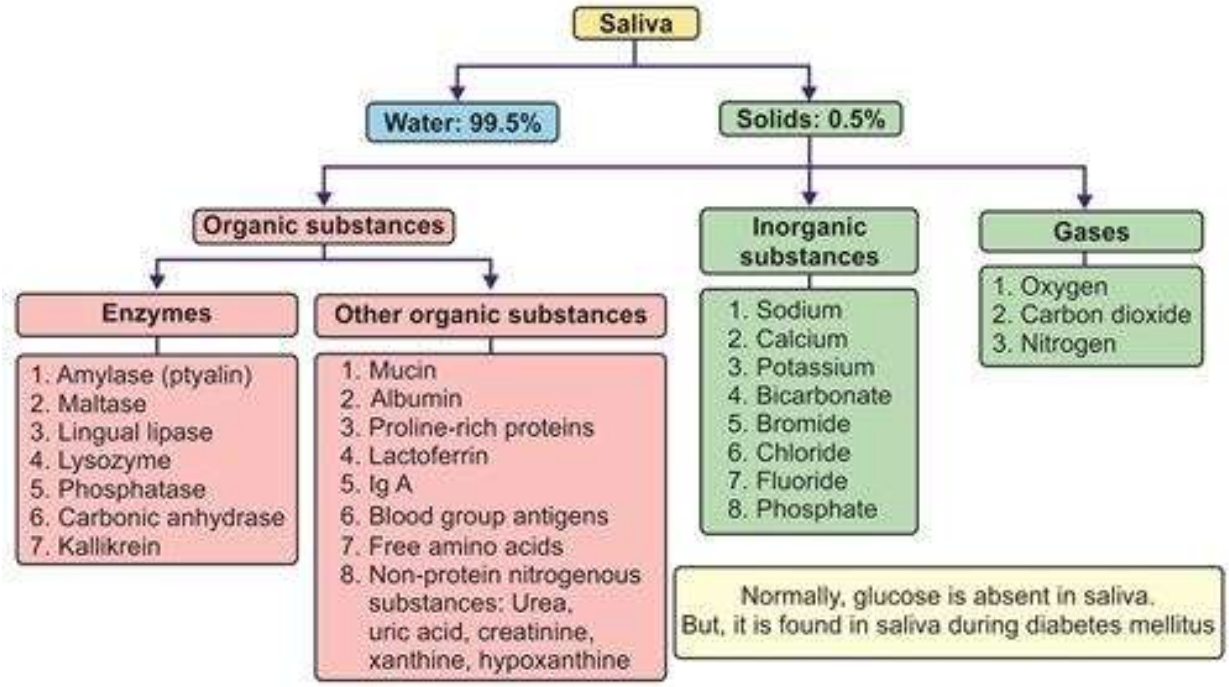
Discussion:-

Saliva is an exocrine secretion of the salivary glands mainly composed of water 99%, but it also contains electrolytes proteins, lipids and enzymes, Contaminants such as bacteria, epithelial cells, and gingival crevicular fluid and food debris are also detectable in saliva. These all can be used for diagnostic as well therapeutic purpose

for many diseases. All physical and chemical properties of saliva can also be used for screening, diagnosing and prognosing of multiple local and systemic health problems. The use of saliva as an alternative diagnostic tool is advisable since its collection is non invasive and possibly stress-free. Furthermore a large amount of saliva can be easily collected and stored with non invasive and cost saving procedures[13].Development of sensitive and precise salivary diagnostic tools and the formulation of defined guidelines following meticulous testing will allow salivary diagnostics to be utilized as chair side tests for various oral and systemic diseases in the near future.

Brief Overview of Saliva

Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates[11]. Immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia are also found in saliva.



Flowchart 1:- Composition of saliva.

Stimulated Vs Unstimulated Saliv[14]

Saliva can be collected from specific salivary glands or sampling whole saliva. In both cases, the samples have the same chemical composition, although the concentration of analytes can vary.

Unstimulated whole saliva-

It is the mixture of secretions that enters the mouth in the absence of exogeneous stimuli and depends on the daily basal salivary flow rate in the oral cavity[49].The sampling of unstimulated saliva is often preferred because it minimizes the dilution of analytes.

Stimulated saliva-

It is physiologically secreted in response to either masticatory or gustatory stimulations during food intake. Its composition depends on the gland size, food intake, smoking, gag reflex and type of stimulation given. Various stimulants such as paraffin wax, unflavored chewing gum base, cotton puff and rubber bands can be used to sample saliva by masticatory stimulation, whereas gustatory stimulation can be obtained using citric acid and sour candy drop

Biomarkers can be identified in both stimulated and unstimulated saliva. The sampling of unstimulated saliva is often preferred because it minimizes the dilution of analytes.

Methods of Saliva Collection[14]:-

There are many simple and non invasive methods to collect saliva to study biomarkers.

1. Passive drooling and draining method- Passive drooling is usually performed by asking the subject to "deburr" (let the saliva drop) into plastic tubes (e.g. polypropylene) with the head tilted forward.

2. Spitting - Spitting is the accumulation of saliva in the floor of the mouth followed by spitting it into a preweighed or graduated container, e.g. a funnel connected to a tube/container.

3. Swab-based sample - Whole saliva can be sampled by placing swabs or other absorbent materials in the mouth. The choice of the material should depend on the subject's tolerability (dimensions, taste and allergy).

Advantages Of Saliva As A Diagnostic Fluid[15]

1. Noninvasive diagnosis of disease and monitoring of general health.
2. Painless, patient suffers no discomfort and little anxiety in the collection process.
3. Simple in collection with a modest trained assistant and applicable in remote areas.
4. Relatively cheap technology as compared to other tests.
5. Cost effective applicability for screening large population.
6. Can be used to study special population where blood sampling is a problem e.g. children, anxious /handicap/ elderly patients.
7. Convenient for multisampling.
8. Safer for health professionals than blood tests.
9. Compared to blood and urine, saliva is also cheaper to store and ship.
10. In addition saliva does not clot and can be manipulated more easily than blood.

Limitations Of Saliva As A Diagnostic Fluid [15]

1. **Disparity between salivary and serum biomarkers.-**Biomarkers in the blood are commonly measured in micrograms which is one –millionths of a Gram. Biomarkers in saliva are commonly measurable in pictograms, one trillionth of a Gram ,ornanogram and so have to be very meticulously identified and assessed.
2. **Technique sensitive-**Absorbent devices may collect localized saliva rather than whole saliva which may affect results for some analytes. Head position also can affect pooling of saliva.
3. **Effect of change in salivary flow rate-**Most hormones display a diurnal rhythm of expression, and this can be true for certain salivary proteins as well.
4. **Variability among individual-** The biologic variation (BV) can be intraindividual variability which can be due to cyclical variations (circadian, monthly, or seasonal) or inter individual variations over the lifespan of the individuals (e.g., age-related hormonal changes).
5. **Contamination with serum-**Many serum markers can reach whole saliva in an unpredictable way (*i.e.* gingival crevicular fluid flow and through oral wounds) and can affect the diagnostic usefulness of many salivary constituents.
6. **Effect of systemic disorder-**The quantity and composition of saliva get affected by systemic disorders (Sjogren syndrome, viral infections, uncontrolled diabetes, hypertension & depression),numerous medications and radiation.

Saliva and Caries Risk[16]

Caries is a complex phenomenon involving internal defense factors, such as saliva, tooth surface morphology, general health and nutritional and hormonal status, and a number of external factors-for example, diet, the microbial flora colonizing the teeth, oral hygiene, and fluoride availability.[2]

Normal salivary output, as quantified by flow rate, is an extremely important intrinsic host factor providing protection against caries, and that pathologically diminished flow rate is a significant risk factor for caries development. Salivary low buffering capacity, low calcium and phosphate, and low specific sIgA show a less pronounced link to increased caries. There is no association with caries risk for salivary pH, other electrolytes or small molecules, total sIgA, IgG, IgM, and other salivary proteins, including those of the innate host defense system. This lack of correlation is due to the multiple levels of structure-function redundancies found in saliva[16] (Table 1).

Strong Association with Caries Risk	Weak-to-Moderate Association with Caries Risk	No Association with Caries Risk

Flow Rate	Buffering Capacity; Calcium/Phosphate;	pH (static measurement); Glucose Clearance
	Specific sIgAImmunoglobulin	Rate/Concentration; Other Electrolytes & Small Organic Molecules; Total sIgA; IgG, IgM, Innate
		Immunity Factors

Some important characteristics of saliva which can be used as salivary biomarkers for dental caries are as follows:

1. Functional bio markers of saliva
2. Microbial bio markers
3. Electrolytes
4. Salivary Proteins

Functional Biomarkers of Saliva for Dental Caries:[17]

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

Salivary Flow Rate:

Saliva plays a significant role in maintaining oral health, helping to build and maintain the health of soft and hard tissues. When saliva flow is reduced, oral health problems such as dental caries and oral infections can develop[18].

Unstimulated Saliva Flow Rate:

At rest, without exogenous or pharmacological stimulation, there is a small, continuous salivary flow, aunstimulated 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 [18, 19]

MAJORFACTORS	MINORFACTORS
Degree of hydration	Gender
Body position	Age(above15years)
Exposure to light	Body weight
Previous stimulation	Gland size
Circadian rhythms	Psychic effects-thought/sight of food
Circannual rhythms	Functional stimulation

Stimulated Saliva Flow Rate:

Stimulated saliva is produced in response to a mechanical, gustatory, olfactory, or pharmacological stimulus, contributing to around40-50% of daily salivary production.

Nature of stimulus	Glandsize
Mechanical	Unilateral stimulation
Gustatory	Vomiting
Pharmacological	Olfaction
Food intake	Smoking
	Gag reflex

Clinical Significance of Salivary Flow Rate:

Practically 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 of carbohydrates, non-adherent bacteria[18], desquamated epithelial cells and food debris. This phenomenon is essential for decreasing the availability of sugars for the bio film. The salivary viscosity reduces the hydration capacity of saliva, and consequently raises the caries risk. People with low salivary flow are often, but not always, related to caries susceptibility and activity.

Saliva pH and Buffering Capacity:
The pH of dental plaque is a key factor in the balance between acid demineralization of the teeth and the demineralization 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 neutralized by saliva, which contains the important buffer, bicarbonate.[18]

Saliva has a pH normal range of 6.2-7.6 with 6.7 being the average pH. Resting pH of mouth does not fall below 6.3.

Saliva has two main buffersystems:[17]
1st is the carbonic acid-bicarbonate system is the most efficient in stimulated saliva
2nd is phosphate buffer system which is efficient in unstimulated saliva.

Bicarbonate in saliva serves as the main buffer against acid, working in conjunction with the phosphate and the protein buffersystems.[17]

Microbialbiomarkers of Saliva for Dental Caries[17]
Cariogenic bacteria are usually present in relatively small quantities in healthy saliva and plaque. However, with biological and environmental changes such as the increased frequency of fermentable carbohydrate consumption, conditions of low pH will favor the proliferation of bacteria.

The '**specific plaque hypothesis**' proposes a few specific species of bacteria as being responsible for caries, the **'ecological plaque hypothesis'** considers caries as an outcome of the overall activity of a heterogeneous mixture of microorganisms and a cariogenic shift of the plaque micro biomes. The acidic metabolites by microorganisms cause a local pH fall below a critical value (pH 5.5) resulting in the demineralization of the tooth tissue.[17]

Mutansstreptococciandlactobacilli
The main responsible bacteria for this disease are the endogenous strains **Streptococcus mutans, Streptococcus sobrinus**and **Lactobacillus species**, present in the biofilm. Among various species, *S. mutans*has been identified as a strong pathogen for caries; however, other species, such as S. sobrinus, may also play a minor role.[8] Early acquisition of *S.mutans*is associated with early childhood caries and future caries.[17]

1. Streptococcus mutans:*S. mutans*is able to metabolize a number of sugars and glycosides such as glucose, fructose, sucrose, lactose, galactose, mannose, cellobiose, glucosides, trehalose, maltose and a previously unrecognised, group of sugar-alcohols. In the presence of extra cellular glucose and sucrose, *S.mutans*synthesizes intracellular glycogen-like polysaccharides[21]
2. Lactobacilli: Lactobacilli are isolated from deep caries lesions but rarely just before the development of dental caries and in the early tooth decay. It is believed that they are pioneering microorganisms in the caries progress, especially in dentin.[21]

As a late colonizer, lactobacilli may not be a requisite for caries initiation. However, they may potently contribute to caries progression once lesions are established. As the level of lactobacilli in saliva also appears to reflect the acidogenic conditions associated with the consumption of abundant simple carbohydrates, it could serve as a useful indicator for a cariogenic diet.[17]

Salivary Electrolytes As Biomarkers For Dental Caries
Among the main salivary electrolytes, **sodium, fluoride, chloride, calcium, phosphate and bicarbonate** are considered of particular importance for protecting teeth from caries. The presence of fluoride in saliva is very important because it reduces acid production in the biofilm. Fluoride, calcium and phosphate ions keep saliva supersaturated with respect to hydroxyapatite and offer a reparative and protective environment for maintaining the integrity of dental tissues.

Calcium
Clinical Consideration[22] –

1. Increase in salivary calcium levels has a beneficial effect by remineralising the tooth enamel but detrimental effect by remineralising the dental plaque that could favor the progression of periodontal diseases by formation of calculus.
2. Decrease in salivary calcium level makes the plaque more acideogenic which may result in demineralization of the enamel and more prone to dental caries.

Phosphate
Clinical Consideration[23] –

1. If the phosphate levels are too high it can remove calcium from the bones which make them brittle
2. The low levels of phosphate concentration in saliva impacts protection and rebuilding of tooth enamel. Teeth can chip easily .

Mechanism of action of Calcium and phosphate:
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 anderosion[24].

The equilibrium between calcium and phosphate ions in saliva and hydroxyapatite of the tooth is important to maintain the tooth structure. Under acidic conditions free hydrogen ions reacts with hydroxyl and phosphate ions forming water and mono hydrogen phosphate respectively, this affects the equilibrium between the solid and ionic phases and as a consequence calcium ions from apatite are released. The hydroxyapatite crystals will continue to dissolve until saturation is achieved or the pH rises.

Fluoride
Clinical Consideration[24] –

1. If the fluoride levels are more than 1.5 ppm it can cause dental fluorosis which can cause changes in the appearance of tooth enamel
2. Whereas <0.3 ppm of fluoride concentration in saliva impacts the activity of remineralisation.

SAL**IVARY PROTEINS (PROTEOME) AS BIOMARKER FOR DENTAL CARIES**
The salivary proteome is composed mainly of salivary proteins and salivary peptides[21].

Antimicrobial Salivary Protiens
Major SalivaryProteins:
Major salivary glycoproteins such as mucin, immunoglobulin etcare involved in the first line of defense of oral cavity[25].

Minor Salivary Proteins:
Besides the major proteins, which account for approximately 50% of its total protein, saliva contains a number of antimicrobial proteins that are present in lower concentrations. A number of these are enzymes which even in low concentration can exert significant biological activity. Examples of antimicrobial proteins with enzymic activity are lactoperoxidase and lysozyme etc.[25]

Protective Properties of Salivary Proteins
Saliva is composed by various proteins with antimicrobial activity.Changes in salivary proteome can provide changes in microbial flora, and consequently lead to caries progression.[17]

SALIVARYPROTEIN	PROPERTIES
Cystatins / VEGh	Protease inhibitor
Immunoglobulins	Inactivation and aggregation of bacteria
Lactoferrin	Growth inhibition
Lactoperoxidase	Growth inhibition
Lysozyme	Killing
Proline- rich proteins(aPRPs)	Adherence

Proline- rich proteins(bPRPs)	Unknown: membrane disturbing
Statherin	Adherence

Protective action of salivary proteins against dental caries

Saliva comprises different components. The parotid gland secretes lysine and arginine. Cadaverine and ammonia produced from lysine and arginine, respectively, neutralize the acidic environment generated by sugar processing and lower dental caries' risk. In addition, saliva contains some enzymes such as amylase, other proteins like histamines, some cystatins, and antibodies that act as antibacterial agents and inhibit bacterial growth and reduce the risk of dental caries[24](Fig 1)

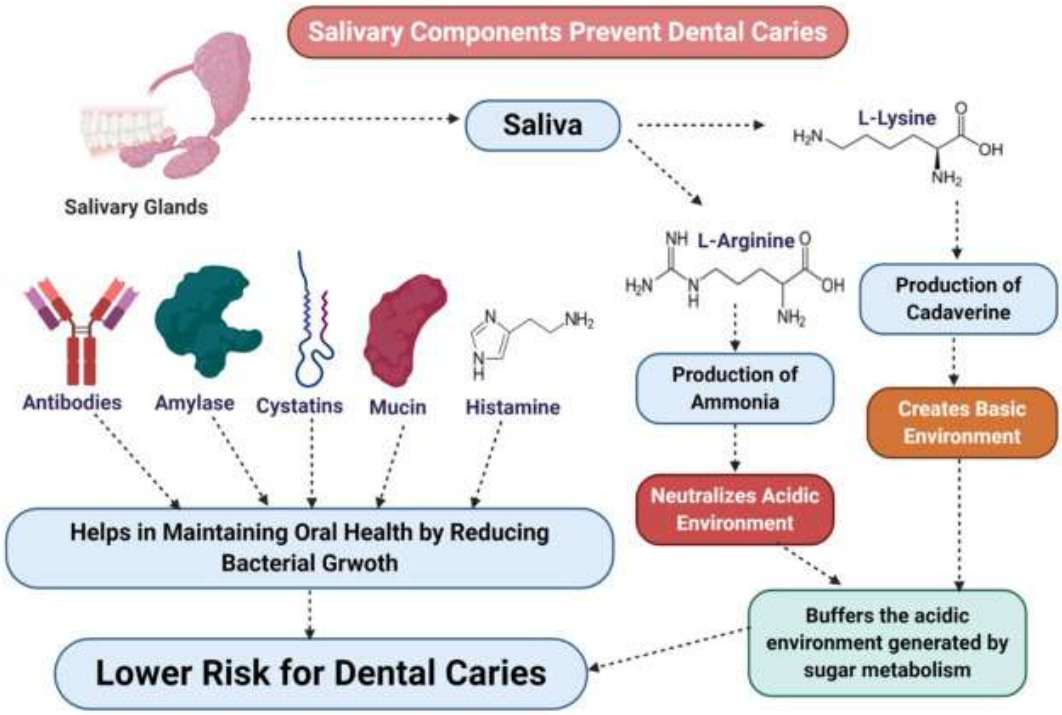


Fig 1:- Protective action of salivary proteins against dental caries.

Antimicrobial Salivary Peptides

In saliva at least three types of antimicrobial peptides can be distinguished: histatins, defensins and hCAP18/LL37, a human cathelicidin. Of these antimicrobial salivary peptides, the histatins are of utmost importance.

Protective Properties of Salivary Peptides

These antimicrobial peptides have a broad antimicrobial activity not only against bacteria, but also against yeasts. Such peptides can be used as templates to develop a new generation of antibiotics, because they work very rapidly and efficiently, while they are negligibly cytotoxic and do not evoke resistance [19](Table 2).

Salivary Peptides	**Properties**
CathelidinLL3	Antimicrobial activity
Histatins	Antifungal and antibacterial
DefensinvHNP1-3	Anti viral activity
Beta-defensins hBD1 hBD2 hBD3	Microbial activity and anti viral properties
Statherin	Remineralization of enamel

The Various Salivary Proteins:

1.Immunoglobulin's

Immunoglobulin or antibodies are glycol proteins produced by plasma cells. There are 5 types of Immunoglobulin in human[25]The immunoglobulin in saliva primarily belong to the IgA subclass (>85%).

Anti Caries Properties of Immunoglobulin[25]

1. They play an antibacterial role by inhibiting bacterial metabolism, neutralizing bacterial toxins and enzymes and agglutination of bacteria.
2. It promotes the inhibition of bacterial adherence, by the reduction of the hydrophobicity of bacteria.
3. It acts synergistically with other defense mechanisms, such as the lactoferrins, peroxidases, agglutinins and mucins.

Proline-rich proteins[17]

Proline-rich proteins are a class of intrinsically unstructured proteins that contain several repeats of a short proline-rich sequence. Proline-rich proteins can be divided into acidic and basic families.

1. The acidic proline-rich proteins adheres strongly to recently cleaned tooth surfaces. Acidic proline-rich proteins account for 25-30% of all proteins in saliva and play a role in the formation of dental pellicle and influence initial microbial colonization on tooth surfaces.
2. Basic Proline-rich proteins,bind to hydroxyapatite of enamel crystals and inhibit the precipitation of calcium and phosphate and maintain mineralization and demineralization of tooth surface by maintaining the calcium and phosphorus level.BasicProline-rich proteins also play an important role in binding the cell wall of microbes such as streptococci, thereby protecting the dental enamel from the microbial adherence and neutralizing the acids produced by microbes.[17]

Mucins 1e2 (Mucousglycoproteins)

Mucous glycol proteins (mucins) constitute a family with two members,-molecular-weight mucins (MG1)and low-molecular-weight mucins (MG2).There is inverse relationship between Mucins 1 and 2 levels and the prevalence of dental caries.

Anticaries property of Mucins

1. The mucins of saliva form a seromucosal cover that protects, lubricates, prevent the dehydration of oral cavity.
2. It also protects tissues against the proteolytic attacks of microorganisms.
3. It has antibacterial properties.
4. Mucins interact with several strains of streptococci and promote their agglutination, thereby accelerating the clearance of bacteria from the oral cavity.
5. The mucins are present in acquired pellicle from tooth surfaces and protect teeth surface from demineralization.[17]

Lactoferrin-

Lactoferrin (**LF**), also known as **lacto transferrin** (**LTF**), is a multifunctional protein. Lactoferrin has the ability to bind and kill bacteria via direct interactions through the strongly basic N-terminal region of the glycoprotein that consists of 47 amino acids

Anticaries property of Lactoferrin

1. It alter the permeability of the outer membrane of Gram-negative bacteria and releases bacterial lipo polysaccharides LPS.[31]
2. Lactoferrin competes with various microorganisms in binding to free iron; this competition mechanism has a bacteriostatic and bactericidal effect on various microorganisms that depend on this ion to survive.[17]

Lysozyme

Lysozyme is an antimicrobial enzyme produced by animals that forms part of the innate immune system.

Lysozyme is found in high amounts in body fluids such as saliva, serum, tears and amniotic fluid, as well as in low amounts in bile, urine and cerebrospinalfluid.

Anticaries property of Lysozymes

1. Lysozyme promotes bacterial clearance through aggregation and adherence.[26]
2. Lysozyme can activate bacterial autolysins and destroy the cell walls.
3. Lysozyme is an enzyme that destroys the bacterial cell wall of some bacteria.[17]

Lactoperoxidase[27]:

Lactoperoxidase is a peroxidase enzyme secreted from mammary, salivary and other mucosal glands including the lungs, bronchii and nose that functions as a natural and the first line of defense against bacteria and viruses.

Anticaries property of 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.

Cystatin S

Cystatins belongs to a heterogeneous family of proteins with a conserved consensus sequence in their active site. Cystatins are cysteine proteinase inhibitors, antimicrobial and immune modulatory and are present in all mucosal secretions.[26]

Anticaries property of Cystatin

1. Cystatins is responsible for the inhibition of cysteine proteases.
2. 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.
3. The removal of the phosphate groups of cystatin reduces the affinity of the protein to hydroxyapatite.[17]

Conclusion:-

Human saliva is a unique clear, slightly acidic (pH 6.0 to 7.0) biological fluid composed of 99% water and 1% other compounds, such as a variety of electrolytes including sodium, potassium, calcium, magnesium, bicarbonate and phosphates. Saliva also has immunoglobulins proteins, enzymes &mucins. On average, individual salivation can range from 0.3to0.7ml of saliva per minute, producing a range of 1to1.5litres daily. Saliva is easy to collect, to store, and can be obtained at low cost in sufficient quantities for analysis by a non-invasive method. Thus it has the potential to be used in the early detection and diagnosis of various oral diseases due to the abundant **biomarkers** present in saliva.

As a highly prevalent multi factorial disease, dental caries afflicts a large proportion of the world's population. 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. Various inorganic (water and electrolytes) and organic (proteins and peptides) components may protect teeth from dental caries. This occurs via several functions, such as clearance of food debris and sugar, aggregation and elimination of microorganisms, buffering actions to neutralize acid, maintaining super saturation with respect to tooth mineral, participation in formation of the acquired pellicle and antimicrobial defense. Despite some controversial findings, the main body of the literature supports an elevated caries prevalence and/or incidence among people with a pathologically low saliva flow rate, compromised buffering capacity and early colonization or high titer of mutans streptococci in saliva. [75].

These salivary biomarkers may be used for the prediction, diagnosis, prognosis and management of dental caries, as well as for evaluating the outcome of therapeutic regimens. This was an attempt to provide an overview of the current understanding of salivary biomarkers for dental caries. A few salivary tests, such as salivary flow rate, buffering capacity and bacterial tests (for *S. mutans*and lactobacilli), have entered dental clinical practice and can be used to assist the assessment of patients' caries risk. Various salivary parameters should be combined with socio demographic, behavioral and clinical factors for a better estimate of patients' caries risk. Thus, advances in salivary analysis technology and further salivary research may lead to breakthroughs (important discovery) in salivary biomarkers for dental caries.Although Saliva **" Lacks the drama of blood, the sincerity of sweat and emotional appeal of tears"** therefore saliva is still an overlooked opportunity to reduce the burden and cost of diagnosis of Dental caries[26].

References:-

1. Cunha-Cruz J, Scott J, Rothen M, Mancl L, Lawhorn T, Brossel K, Berg J. Salivary characteristics and dental caries: evidence from general dental practices. J Am Dent Assoc. 2013 May;144(5):31-40.
2. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. Adv Dent Res. 2000 Dec;14:40-7.
3. V Swathi, Jha A, Mamtha. The diagnostic applications of saliva.IOSRJournalofDentalandMedicalSciences2016;15(9):96-101.
4. Guo L, Shi W. Salivary biomarkers for caries risk assessment. J Calif Dent Assoc. 2013 Feb;41(2):107-9, 112-8.
5. Petersen PE. The World Oral Health Report 2003; Continuousimprovement of oral health in the 21st century- the approach of theWHOGlobalOral HealthProgramme2003.
6. Gao X, Jiang S, Koh D, Hsu CY. Salivary biomarkers for dental caries. Periodontol 2000. 2016 Feb;70(1):128-41.
7. Alaluusua S, Renkonen OV. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res. 1983 Dec;91(6):453-7.
8. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J Dent Res. 1993 Jan;72(1):37-45.
9. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001 Feb;85(2):162-9.
10. Ruby JD, Cox CF, Akimoto N, Meada N, Momoi Y. The Caries Phenomenon: A Timeline from Witchcraft and Superstition to Opinions of the 1500s to Today's Science. Int J Dent. 2010;2010:432 -447.
11. Bertilsson C, Borg E, Sten S, Hessman E, Sjöblom H, Lingström P. Prevalence of Dental Caries in Past European Populations: A Systematic Review. Caries Res. 2022;56(1):15-28.
12. Herschfeld JJ. W.D. Miller and the "chemico-parasitic" theory of dental caries. Bull Hist Dent. 1978 Apr;26(1):11-20.
13. Ghosh S, Dhobley A, Avula KK, Joseph S, Gavali N, Sinha S. Role of Saliva as a Non-Invasive Diagnostic Method for Detection of COVID-19. Cureus. 2022 Jul 29;14(7): 274- 281.
14. Francesca G. Bellagambi, TommasoLomonaco, Pietro Salvo, Federico Vivaldi, Marie Hangouët, Silvia Ghimenti, Denise Biagini, Fabio Di Francesco, Roger Fuoco, AbdelhamidErrachid. Saliva sampling: Methods and devices. An overview. Trends in Analytical Chemistry.2020; Vol 124:115-121.
15. Madalli, Vijaylaxmi. (2013). Saliva-A Diagnostic Tool. IOSR Journal of Dental and Medical Sciences. 11. 96-99.
16. Leone CW, Oppenheim FG. Physical and chemical aspects of saliva as indicators of risk for dental caries in humans. J Dent Educ. 2001 Oct;65(10):1054-62.
17. Bansode PV, Pathak SD, Wavdhane MB, Birage PP. Salivarybiomarkers of dental caries. IOSR Journal of Dental and MedicalSciences 2018;17(3):12-18.
18. Dodds M, Roland S, Edgar M, ThornhillM. Saliva a review ofitsroleinmaintainingoralhealthandpreventingdentaldisease.BDJTeam2; 2015.p.1-11.
19. Edgar M, Dawes C, O'Mullane D. Saliva and oral health. 4th edition.Stephen HancocksLimited.2012.Pg 1-172.
20. Iorgulescu G. Saliva between normal and pathological. Important factors in determining systemic and oral health. J Med Life. 2009 Jul-Sep;2(3):303-7.
21. KarpinskiTM,SzkaradkiewiczAK.Microbiologyofdentalcaries.JBiolEarth Sci2013; 3(1): M21-M24.
22. Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. Int J ClinPediatr Dent. 2019 Mar-Apr;12(2):139-144.
23. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: a narrative review. F1000Res. 2020 Mar 9;9:171-185.
24. Triller M. Le fluor, Fluoride, a preventive agent of caries: mechanisms, sources, risks. Arch Pediatr. 1998 Oct;5(10):1149-52.
25. Van NieuwAmerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Res. 2004 May-Jun;38(3):247-53.
26. Hemadi AS, Huang R, Zhou Y, Zou J. Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment. Int J Oral Sci. 2017 Nov 10;9(11):42-45
27. Sharma S, Singh AK, Kaushik S, Sinha M, Singh RP, Sharma P, Sirohi H, Kaur P, Singh TP. Lactoperoxidase: structural insights into the function,ligand binding and inhibition. Int J BiochemMol Biol. 2013 Sep 13;4(3):108-28.
28. Davies Forsman, L., Kim, H.Y., Nguyen, T.A. *et al.* Salivary Therapeutic Drug Monitoring of Antimicrobial Therapy: Feasible or Futile?. *Clin Pharmacokinet.2024* Vol(**63)**: 269–278.