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Salivary Enzymes – A Diagnostic Marker for Periodontal Disease

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ABSTRACT

The role of saliva in the oral cavity and its multifunctional capability is known universally. Besides it has got another facet as it can be used as a diagnostic fluid to find out the periodontal disease activity. This article reviews the salivary enzymes which could be used as diagnostic marker to predict the onset of periodontitis, its progression and evaluation after the treatment. Since saliva is readily available in a non-invasive method and it reflects the oral and systemic disease, it is wise to use the saliva as diagnostic tool in periodontitis. The recent point-of-care technology has made salivary analysis rapidly detectable and easier to use. Further studies with these detection methods will throw a new ray of hope to the field of salivary diagnostics.

KEYWORDS: Saliva, Diagnostic fluid, salivary enzymes.

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INTRODUCTION

Saliva is clinically informative; it contains multiple bio markers which makes salivary diagnostics, a dynamic field. Saliva a bio-fluid is easily accessible via a totally non-invasive method[1]. As it contains locally and systemically derived markers of periodontitis, it offers the basis for a patient specific diagnostic test for periodontitis. Response of an organism to periodontal infection includes production of several enzymes which are released from stromal, epithelial, inflammatory or bacterial cells[2]. Purpose of this review is to give an overview of salivary enzymes and focus the use of salivary enzymes for the diagnosis of systemic and oral disease.

SALIVA

It is the secretion of the salivary gland and constitutes one of the largest secretions of the human body. It maintains the integrity of both the soft and hard tissues of the mouth and it constitutes one of the main natural defence system of the oral cavity. It is composed of 98% of water and the remaining 2 % is made up of electrolytes, mucins, antiseptic substances and various enzymes.[ά amylase, lysozyme, lipoprotein lipase, aminopeptidases etc.] "Oral fluid" is composed of saliva, gingival crevicular fluid contained in the dento gingival sulcus, mucosal transudate, cell debris, bacteria and food remnants[3].

BIOMARKER

A biomarker can be defined as a biological molecule found in blood, other body fluids or tissue that is a sign of normal or abnormal process, or of a condition or disease. Biomarker may be used to know how well the body responds to treatment.

SALIVA IN PERIODONTAL DISEASE

In saliva, the proposed markers for disease include proteins of host origin.[i.e., enzymes, immunoglobulin's.][4] The correlation between salivary biomarker and clinical features of periodontal disease has been evaluated for three aspects of periodontitis – inflammation, collagen degradation and bone turnover.

Higher enzyme activities were found in the chronic periodontal patients as compared to the healthy controls[5]. Nieman *et al* in 1993 stated that the enzyme activity in the whole saliva appears to reflect the severity of periodontal disease and the salivary enzymes have the potential as adjunctive to assess periodontal inflammation and the response to periodontal treatment[6].

Enzymes in saliva originate from host cells and from bacteria. Kaufman *et al* in 2000 suggested that in comparison with healthy subjects, higher concentration of salivary enzymes exists in patients with periodontitis[7, 8]. This is mainly due to the presence of connective tissue destruction seen in association with periodontal disease. The concentration of host derived collagenase, elastase and gelatinase are increased in patients with periodontitis before treatment and decrease following therapy.

SALIVA –A DIAGNOSTIC MEDIUM

Salivary diagnostics is an emerging important field in dentistry. Using saliva as diagnostics was made in the second half of the 20th century[9]. Michael and Kirk in the 1900's conducted sialochemical studies on oral fluid and reported that saliva has a specific component that would be diagnostics for various systemic conditions, including gout and rheumatism [10].

There are different methods available for collection of saliva. Stimulated saliva shows differences in quality and p H of the saliva. In unstimulated saliva, the flow rate is mostly affected by degree of hydration.

The greatest challenge for salivary diagnostics is to identify the disease diagnostic marker. Robust platforms for salivary biomarker discovery have been developed to empower salivary diagnostics to become an approach for health surveillance.

Possible various salivary markers for diagnosis of periodontitis are:

• Enzymes   • Proteins

• Immunoglobulin's   • Platelet activating factor

• Hepatocytic growth factor • Hormones

• C-reactive protein   • Vascular endothelial growth factor

• Neopterin   • Urate

This manuscript reviews the current literature on host derived and microbe detrimental enzymes that can be used for prediction of periodontal disease and focuses on the saliva as a potential sampling fluid of use.

METHODS USED FOR COLLECTION OF SALIVA

The fluid collected for salivary diagnostic purpose is expectorated whole saliva. Resting saliva is usually collected in graduated tube or in proweighed vials, so that the flow rate per unit time can be measured. When volume measurement is not required, saliva can be collected by using cotton rolls, gauze or filter paper strips and then evaluated or centrifuged. Saliva can be aspirated directly from the floor of the mouth with plastic pipettes[11].

STIMULATED SALIVA

For an analytical purpose, if large volumes of saliva are required. It can be stimulated by a masticatory or gustatory stimulus. Softened paraffin wax or a wasted rubber band are the usual masticatory stimuli used for collection of stimulated saliva. 2% citric acid applied directly to the tongue is the standard gustatory stimulus[11].

SALIVARY ENZYMES IN PERIODONTAL DISEASE

Saliva has been used as a diagnostic fluid in medicine [Mandel 1990]. Enzymes in saliva can originate from cells in the salivary glands, micro organisms, epithelial cells, neutorphils and can be derived from gingival crevicular fluid. They play an important role in the destruction of periodontium.

The various enzymes are as follows:

• Aspartate amino transferase

• Alanine amino transferase

• Elastase

• Dipeptidyl peptidase

• β glucoronidase

• Lysozyme – antibacterial enzyme

• Chitinase.

• Lactoferrin

• Peroxidase.

• Alanine amino peptidase

• Alkaline phosphatase

• Amylase

• Arginase

• Myloperoxidase

• Matrix metallo proteinase 3, 8, 9 & 11

• Cathepsin – G

• Lactate Dehydorgenase.

• Cystatins etc.

The proteolytic and hydrolytic enzymes may serve as markers of the periodontal disease activity.

ASPARTATE AMINO TRANSFERASE

Relationship between AST levels & Periodontal diseases have focused on spectrophotometry and on commercial chair side tests such as pocket watch & perioguard. Concentration higher than 800U/ml of AST in the GCF is indicative of active periodontal disease. In pocket watch test an AST level of 1200 U/ml is the cut-off point.

Higher level of AST in saliva dictates the presence of periodontal pockets, gingival bleeding and suppuration[12].

Castro CF *et al* in 2011 found a very high degree of association between AST levels and peridontium and they have correlated with clinical assessment and other measures of disease both before and after therapy.

DIPEPTIDYL PEPTIDASE IV

It is a proteinase, which plays an important role in the destruction of periodontium. The mechanism of action is collagen degradation. Its level in periodontitis is increased [13].

ALANINE AMINO PEPTIDASE

AAP activity in whole saliva of periodontitis patients were found to be increased when compared to healthy controls[13]. It is a proteolytic enzyme that plays a role in peptide hydrolysis and involved in collagen degradation.

ENZYMES IN NEUTROPHILS

Neutrophil is an important cell type. In host defence against periodontic pathogens and its granules contain hydrolytic neutral enzymes & hydrolases. The hydrolytic neutral enzymes are elastase cathepsin G, Myeloperoxidases and lysozyme. Hyrolases include cathepsin B, cathepsin D & β Glucoronidase. Lactoferrin, Neutrophill collagenase, Matrix Metallo Proteinase – 8 & 9 are also stored in neutrophillic granules.

ALKALINE PHOSPHATASE

Gibert in 2003 predicted ALP as an indicator for future loss of periodontium. It may serve as marker in periodontal treatment planning and monitoring. Its level may also be useful as a potential bone turnover marker to establish the diagnosis and prognosis of periodontal disease. The ALP activity was greatest in mixed saliva and least in parotid saliva[14, 15]

ALANINE AMINO TRANSFERASE

Most studies found the correlation between the salivary enzyme level of ALT and chornic periodontitis. In 2010 Dr. Gopinath *et al* concluded that by studying the simple economical clinical parameters like salivary enzymes, we can assess the damage of periodontal tissues and may predict the future risk of atherosclerosis and periodontal patients[16].

LACTATE DEHYDROGENASE

Lactate Dehydrogenase activity is higher in patients with increased probing depth than the healthy patients[17]. Dr. Guzman *et al* in 2010, concluded the LDH activity is increased in saliva of smoking patients with chronic periodontitis[18]. LDH levels are higher in localized periodontitis than in generalized and chronic periodontitis. (Castro *et al* 2011)

LYSOZYME

It is also called as muraminidase. It is a hydrolytic enzyme that cleaves the linkage between structural components of the glycopeptide muramic acid, containing region of the cell wall of certain bacteria in vitro. It works on both gram negative and gram positive organisms.

Gram negative organisms are generally more reisistant than gram positive organisms, because of the outer lipopolysacharide layer. The lysozyme also repel transient bacterial invaders of the mouth. Patients with low level of lysozymes in saliva are more susceptible to plaque accumulation. This may be considered as a risk factor for periodontal disease[19].

PEROXIDASE

It is produced by acinar cells in the salivary glands. Patients with periodontal disease have demonstrated high level of this enzyme in saliva. This enzyme removes the toxic H_2O_2, produced by oral micro-organisms and reduces acid production in the dental biofilm. Patients with periodontitis have demonstrated high levels of this enyme[20].

L PEROXIDASE

The thiocynate system in saliva is bactericidal in action. The mechanism of action is by preventing of the accumulation of lysine and glutamic acid, which are essential factors for bacterial growth.

MYELOPEROXIDASE

It is released by leukocytes and is bactericidal for Actinomycetes. The action is by inhibiting the attachment of actinomycetes strains to hydroxyappatite.

CYSTATINS

They are the physiological inhibitors of cysteine proteases and are ubiquitous in many body fluids. These enzymes originate from pathogenic bacteria, inflammatory cells and fibroblasts. They are considered to be protective against unwanted proteolysis like bacterial proteases and lysed leukocytes. It may play a role to inhibit proteases in periodontal tissues and also have an effect on calcium phosphate precipitation. It may function by modulating enzymes acting in periodontium. They also have collagenolytic effect that may cause tissue destruction[7]. Its level is more in saliva collected in sub mandibular and sub lingual salivary gland.

MATRIX METALLO PROTEINASE

They are the host proteinases responsible for both tissue degradation and remodeling. Host cells derived interstitial collagenases breaks the gingival and periodontal collagen and cause progressive periodontal tissue break down. Enzymes such as MMP 8, MMP 9 and MMP 13 are produced by leukocytes that cause connective tissue destruction of collagen and alveolar bone.

MMP 8 – Collagenase -2: It is a most prevalent MMP found in diseased periodontal tissue. Its level is highly elevated in saliva from patients with acute periodontal disease[21]

MMP 9 – Gelatinase: It is a produced by Neutrophills and degrade collagen inter cellular components. Mean MMP 9 levels are increased by two fold in patients with progressive attachment loss. Future use of MMP in oral fluid diagnostic may serve as a guide in periodontal treatment monitoring[22].

MMP 13: This is also referred as Collagenase -3. it is an another collagenolytic MMP. It may be useful for diagnosing and monitoring the course of periodontal disease and helpful in tracking the efficiency of periodontal therapy[23]. Tissue damaging activity of MMP's are controlled by the four members of the tissue inhibitors of metallo – proteinase family (TIMP).

β GLUCORONIDASE

Analysis of β Glucoronidase in saliva is a good diagnostic marker of periodontal disease. It was found useful in predicting the presence of periodontal disease. A significant association exists

between periodontal clinical   parameters and salivary β Glucoronidase activity. Increased concentration of β Glucoronidase in saliva may provide measurement of risk for the patients with periodontal disease[24].

Analysis of salivary levels of β Glucoronidase was evaluated in relationship to the periodontal parameters and provided evidence that increased salivary level of β Glucoronidase representative of GCF marker and it has been linked to an increased risk of peridontitis. It could be used as a diagnostic marker / screening test of periodontitis.

CATHEPSIN B

Eley *et al* in 1996 evaluated its use as a predictor of attachment loss. It may also be useful in distinguishing periodontitis from gingivitis and to plan and to monitor the treatment outcome[25].

ARGINASE

It is an arginine depleting enzyme present in the saliva. Its levels are increased in periodontitis patients. The increased salivary arginase activity in periodontitis causes a decrease in anit-bacterial property of saliva & cause periodontal tissue to become susceptible to periodontal pathogens.

CHITINASE

It plays a role in the defense against chitin containing oral pathogens. It is higher in the whole saliva of periodontitis patients. There is an evidence that chitinase activity gets 3-4 fold deviation after the treatment. To a lesser extent, the enzyme activity of β N acetyl hexosaminidase activity decreased as a result of periodontal therapy. This enzyme hydrolysis the glycosidase linkage.

SALIVARY LEUKOCYTE PROTEASE INHIBITOR IN PERIODONTAL DISEASE PROGRESSION

Periodontal disease is the most prevalent oral disease, affecting almost 90% of the Population. The true elastase activity as well as that of other proteases is regulated by specific inhibitors that are either produced locally or circulate in the plasma.

SLPI is a major leukocyte protease inhibitor, and stable, highly basic non glycosylated protein associated with glandular secretion including saliva. The proposed marker for diagnosing the disease status include protein of host origin naming enzymes, immunoglobulin and SLPI. The accumulation of SLPI into local environment may represent on intrinsic feed back mechanism to

prevent harmful effects of inflammation. Because of its anti-Proteolytic, anti-microbial and anti-inflammatory properties SLPI probably plays a protective role by maintaining a balance between proteases and anti-proteases.

Analysis of SLPI levels aid in survey of patient with terminal disease. SLPI levels were found to be reduced in the terminal stage of periodontitis. This is merely due to degradation of SLPI by cathepsin L bacterial cysteine protease and termination of complex with elastase. SLPI levels to be raised in initial stages of periodontitis, SLPI accumulate in the local environment probably to inhibit the action of increased elastase activity.

LACTOFERRIN

It is a major iron binding protein in saliva. Its level is elevated in Saliva of Periodontitis patients and its reduced capacity of lactoferrin to bind iron in the saliva of Aggressive periodontal patients. During gingival inflammation it is strongly upregulated and it is detected at a higher concentration in saliva of patients with periodontal diseases as compared to the healthy patients[26].

TESTING FOR HIV

For screening and accurately diagnose HIV infection, rapid point-of care HIV tests have been developed recently. Interstitial transudate rich in IgG antibodies are used for diagnosing HIV infection. Oral fluid tests results are considered preliminary and require confirmatory tests such as Elisa or Western blot[27].

EMERGING SALIVARY DIAGNOSTIC TOOLS

Salivary proteotome and salivary transcriptome are used as tool boxes for early detection, disease progression and therapeutic monitoring. Salivary protein and RNA'S can be used to detect oral cancer.

SALIVARY PROTEOTOME

Proteotome is the protein complement of the genome. Proteomics is the analysis of the portion of the genome. By using two- dimensional gel electrophoresis mass spectrometry 'short gun" proteomic approach 309 distinct proteins in the saliva have been identified. Global analysis of the human salivary proteomes can provide a comprehensive spectrum of oral and general health. Accordingly to Denny *et al*, 1166 salivary proteins have been identified[28].

SALIVARY TRASCRIPTOME

It is an emerging concept; RNA molecules elevated in oral cancer tissue are also elevated in saliva thus giving the chance to probe the scope and diagnostic uses of saliva. The RNA molecules discovered are usually stable in saliva. Researcher's in forensic science are focusing on multiplex m RNA profiling for new identification of body fluids including saliva[29].

MEDIATORS IN GINGIVAL CREVICULAR FLUID

They are detected in saliva. GCF derived constituents in saliva have been analysed as an approach to the development of diagnostic test for periodontal diseases[30]. Simple patient based salivary analysis may provide a feasible cost effective approach for large scale screening of patients. An example shall be provided, if an inflammatory marker identified in GCF which has been studied in saliva.

DISCUSSION

Saliva, a mirror of oral & systemic health is a valuable source for clinically relevant information because it contains biomarkers specific for the periodontal disease. Since saliva is non-invasive and repeatedly sampled, it holds considerable promise as a biologic fluid and is can be analysed for diagnosis of periodontal disease.

Clinical & radiographic parameters provide a measure of past destruction and are critical to early diagnosis[31]. A goal of periodontal diagnostic measure should provide useful information to clinician in treatment planning. Conventional Diagnostics tests – lack the capacity to identify highly susceptible patients who are at risk for future periodontal breakdown. Advanced diagnostic techniques are mostly seen in the research setting & seldom in clinical practice. Optimal innovative approach would correctly determine the presence of increased disease activity and predict the sites that are vulnerable for future breakdown.

CONCLUSION

In the field of Periodontics, the diagnosis relies only on clinical and radiographic parameter, providing only limited information about patients and amount of risk for further periodontal breakdown. Numerous markers in saliva have been used as diagnostic tests for periodontal disease with high specificity & sensitivity. Because of the simple and non-invasive method of collection, salivary diagnostics test appear to hold promise for future.

By linking diagnostic and therapeutic approach together, especially in the host modulatory treatment for periodontal disease, the discovery of salivary biomarker is highlighted. Novel techniques such as lab-on-a chip microfluidic devices have the potential to provide determination of patients periodontal disease risk profile, predict disease activity & response to therapeutic intervention. Although challenges remain, the use of saliva as diagnostic fluid appears promising for future application to diagnose periodontal disease. Studies in large populations are necessary to confirm the importance & usefulness of specific diagnostic marker.

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