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Salivary Biomarkers for Periodontal Disease – A Review

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ABSTRACT
Periodontitis is a chronic inflammation of the periodontium caused by persistent bacterial infection that leads to the breakdown of connective tissue and bone. The identification of susceptible individuals or sites at risk from disease, and the diagnosis of active phases of periodontal disease, represent a challenge for both clinicians and oral health researchers. Generally, clinical parameters are used in dental practice for periodontal disease yet several drawbacks exist with the clinical standards for addressing the needs of the public at large in determining the current status/progression of the disease. saliva a likely source for identifying unique biomarkers that reflect oral and systemic health changes. Many inflammatory biomarkers associated with oral diseases have been detected, such as interleukins, tumour necrosis factor-alpha (TNF-a) and matrix metalloproteinases (MMPs) Our findings suggest that several biomarkers are associated with distinct biological stages of these diseases and demonstrate promise as practical biomarkers in identifying and managing periodontal disease, showing that biomarkers of the molecules with cytokine-like activity like interleukin-1-beta and hepatocyte growth factor are the most robust salivary biomarkers for periodontal disease The majority of these studies have progressed through biomarker discovery, with the identified molecules requiring more robust clinical studies to enable substantive validation for disease diagnosis.

Keywords: *Biomarkers, periodontal disease, interleukins, microorganisms, host*

I. INTRODUCTION

Periodontitis and gingivitis are the most common forms of periodontal disease, among which periodontitisis one of the most prevalent oral diseases and a major cause of tooth loss in adults[1]. India suffers lot of disparities in terms of oral health care and 95% of the Indian population suffers from periodontal disease[2]. Peridontitis is an inflammatory disease that affects the tooth-supporting structures due to interactions between oral bacteria and the host's immune response.

It is widely accepted that the initiation and the progression of periodontitis are dependent on the presence of virulent microorganisms capable of causing disease. Although the bacteria are initiating agents in periodontitis, the host response to the pathogenic infection is critical to disease progression. The initial lesion in the development of periodontitis is the inflammation of the gingiva in response to a bacterial challenge. Changes involved in the transition from normal gingiva sulcus to the pathologic periodontal pocket are associated with different proportions of bacterial cells in dental plaque.

Host immunoinflammatory response to initial and continuous bacterial attack lead to collagen and bone destruction. This occurs by release of cytokines some produced normally and some by cells involved in inflammatory process such as polymorphonuclear leukocytes (PMNs), monocytes, and other cells. Triggering humoral immunity leads to activation of inflammation, and inflammatory cells and then their release of tissue destructive enzymes. Cellular immunity-activated T cells activate antigen-presenting cells and macrophages, which releases a number of cytokines: IL-1β, TNF-α and IL-6; which lead to cytotoxic responses to periodontal tissue.

Clinical parameters such as probing depth, attachment level, bleeding on probing, plaque index, and radiographic assessment of alveolar bone loss provide information on the severity of periodontitis but they do not measure disease activity. Microbiological tests, analysis of host response, and genetic analyses have been proposed in an effort to monitor and identify patients at increased risk for periodontitis.

Human saliva is composed of 98% water and 2% of other compounds, such as electrolytes, mucus, antibacterial compounds and various enzymes. Multiple functions of saliva include rinsing, solubilization of food substances, food and bacterial clearance, lubrication of soft tissues, bolus formation, dilution of detritus, swallowing, speech and facilitation of mastication, all of which are related to its fluid characteristics and specific components[4]. Whole saliva is an important physiologic fluid that contains a high complex mixture of substances. Salivary gland secretions contain locally produced proteins, as well as other molecules from the systemic circulation. Variable amounts of blood, serum, serum products, gingival crevicular fluid (GCF), electrolytes, epithelial and immune cells, microorganisms, bronchial products and other foreign substances also are present in whole saliva. It is this rich mixture of substances that makes saliva a likely source for identifying unique biomarkers that reflect oral and systemic health changes[5].So, saliva is an optimal biologic fluid to serve as a near patient diagnostic tool for periodontitis[6].

A biomarker is a substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention[7]. A biomarker must be indicative of physiological health, pathological process and response to a therapy. It should be discriminatory and validated in clinical studies[8]. The analysis of salivary biomarkers offers some advantages. One of these is that whole saliva represents a pooled sample from all periodontal sites, thereby giving an overall assessment of disease status. A second advantage is that levels of salivary analytes reflect current disease activity as well as severity. Third, salivary analytes offers a way of assessing subject-level risk or status. Finally, collection of whole saliva is easy, noninvasive and rapid and requires no special equipment or expertise[5]. This saliva is not only advantageous due to its ease of collection but it also contains certain elements that reflect the activity of all periodontal sites and therefore provides an indication of disease status in the mouth as a whole rather than at individual sites. Studies on oral health in the literature have focused on biomarkers that have association with inflammatory, connective tissue destruction and bone remodeling phases of periodontal disease[9].

Many inflammatory biomarkers associated with oral diseases have been detected, such as interleukins-1b, -6, -8 (IL-6, IL-1, IL-8), tumour necrosis factor-alpha (TNF-a) and matrix metalloproteinases (MMP)-8 and -9. Apart from diagnosing periodontal disease using saliva, it has also been increasingly evaluated as a diagnostic fluid for detecting caries risk, oral cancer , breast cancer , salivary gland diseases and systemic disorders such as hepatitis and the presence of human immunodeficiency virus (HIV) or hepatitis C virus[4]. A number of potential biomarkers associated with periodontitis have been reported and further optimal biomarkers should be carefully selected based on the pathogenesis of periodontitis. It is important to evaluate the potential of select salivary biomarkers of periodontalhealth or disease within the context of their natural daily variation in an orally and systemically healthy cohort of subjects to determine their use in clinical decision-making[9].

II. OVERVIEW

It has long been established that a simple and non-invasive diagnostic tool that allows rapid screening, provides accurate predictive information and enables reliable evaluation of periodontal disease status would be of great value to both dentists and patients. Saliva is a secretion of the salivary and mucous glands and is of major importance in the maintenance of oral health. The biochemical analysis of saliva is particularly important in dentistry. Estimation of the risk of disease onset and severity, monitoring of disease progression and evaluation of therapeutic efficacy for infectious diseases of the oral cavity like periodontal disease can be performed by analyzing an array of constituents within saliva. Salivary constituents that have been studied as potential diagnostic biomarkers for periodontal disease mainly include locally produced proteins of host and bacterial origin (enzymes, immunoglobulins and cytokines) bacteria and bacterial products. Specific salivary proteomic biomarkers have been identified for three key features of the pathogenic processes in periodontal disease – inflammation, collagen degradation and bone turnover[10].

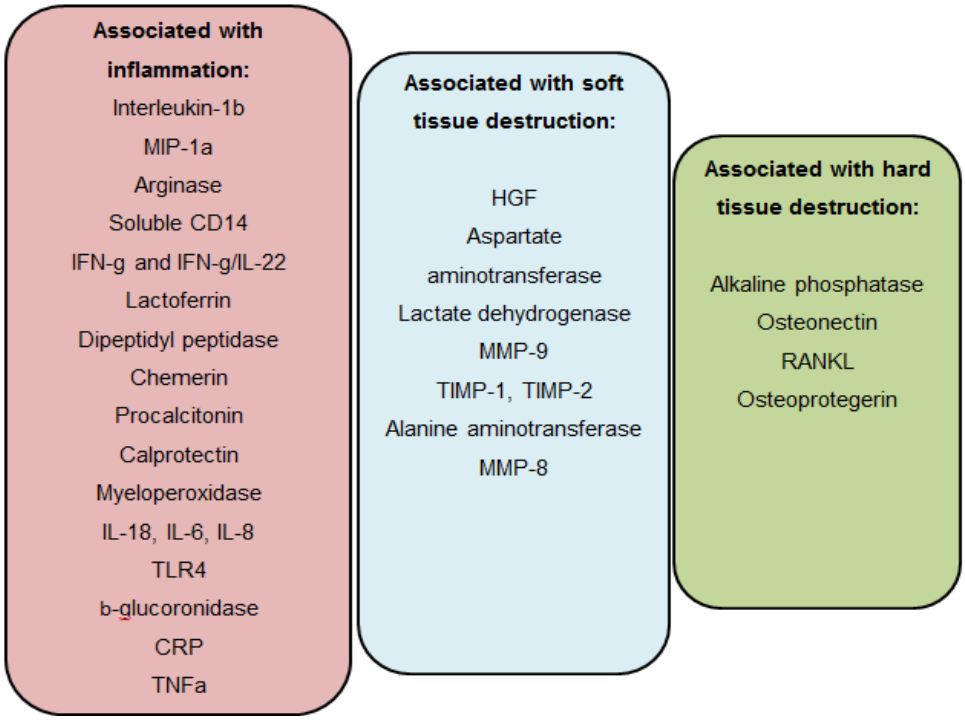
CLASSIFICATION OF SALIVARY BIOMARKERS OF PERIODONTITIS

Salivary biomarkers can originate from both bacteria and the host. As periodontitis progresses inflammation, soft tissue destruction and hard tissue destruction occur sequentially and release associated proteins or metabolites in to the saliva. Therefore host derived biomarkers are categorized according to whether they play a role in inflammation, soft tissue destruction or hard tissue destruction[11]

Bacteria derived salivary biomarkers
DNA DERIVED
Porphyromonasgingivalis
Prevotella intermedia
Tannerella forsythia
Treponema denticola

Campylobacter rectus
Pseudomonas aeruginosa +Acinetobacter spp.
Peptostreptococcus micros
Fusobacterium nucleatum
Aggregatibacteractinomycetemcomitans

PROTEIN DERIVED
Dipeptidyl peptidase
Host derived salivary biomarkers



Bacteria derived salivary biomarkers
Tannerela forsythia: It is a non-motile, spindle shaped, highly pleomorphic rod and a gram negative obligate anerobe. This species produces several proteolytic enzymes that are able to destroy immunoglobulins and factors of the complement system. It also induces apaptotic cell death.

Porphyromonasgingivalis: P. gingivalis is a non-motile, pleomorphic rod and gram negative obligate anerobe. It is an aggressive periodontal pathogen. Its fimbriae mediate adhesion and its capsule defends against phagocytosis. This species produces a series of virulence factors, including many proteases (destruction of immunoglobulins, complement factors and heme sequestering proteins; degradation of host cell collagenase inhibitors). This species can inhibit migration of PMNLs across an epithelial barrier and affects production or degradation of cytokines by mammalian cells. P.gingivalis also has the capacity to invade soft tissues.
Prevotella intermedia: They are short, round ended, non motile, gram-negative rods. They grow anaerobically. These species are less virulent and less proteolytic than P.gingivalis.
Actinobacilllusactinomycetumcomitans: They are small short straight curved rod with rounded ends. It is non-motile and gram negative. It possesses a number of virulence factors, including lipopolysaccharide (endotoxin), leukotoxin (form pores in neutrophil granulocytes, monocytes and some lymphocytes) and a protease (cleaves IgG). The leukotoxin plays a significant role in pathogenicity.

Host derived salivary biomarkers associated with inflammation:
Interleukin 1-beta: A proinflammatory cytokine is important in pathogenesis of periodontal disease stimulates the induction of adhesion molecules. It includes widespread gene expression of cyclo-oxygenase 2, inducible nitric oxide synthetase and metalloproteinases, which results in activation of osteoclasts and bone resorption and down regulation of type1 collagen expression in bone. IL-1b is more potent in stimulating bone resorption and is more occurring in periodontitis. It plays a key role in inflammation and innate immunity activation, and synergizes with other pro-inflammatory mediators that contribute to inflammatory changes and tissue damage[5].

Macrophage inflammatory protein- 1 alpha: Macrophage inflammatory protein (MIP)-1a is a member of the cysteine–cysteine chemokine family. MIP-1alpha is a potent chemoattractant for monocytes and macrophage, which is secreted by inflammatory cells and is primarily associated with cell adhesion and migration. MIP-1alpha helps in the recruitment of leucocytes through the epithelium at early as well as later stages of inflammation. It stimulates monocytes and/ or osteoclast progenitor cells to become active osteoclasts in a dose-dependent manner. MIP-1a has been detected in saliva of adolescents who had aggressive periodontitis compared with controls[12].

Arginase : Human saliva has been shown to possess enzymatic activities, one of which is derived from arginase. Arginase is known to be an arginine-depleting enzyme belonging to the l-arginine/nitric oxide pathway. Arginase makes use of L-arginine to produce urea and ornithine, whereas NOS uses L-arginine to produce nitric oxide. Nitric oxide is produced in response to periodontal pathogens and local inflammatory alterations. Due to its antimicrobial activity, it is considered an important molecule against some infections. Furthermore, considering that L-arginine is used as a common substrate by both arginase and NOS, it is believed that an increase in arginase production would lead to a reduction in the production of nitric oxide, consequently increasing the susceptibility to bacterial infection[13].

Soluble CD14: sCD14 play a role in inflammatory diseases by controlling the immune system level of response. It has been demonstrated that sCD14 is a regulatory factor capable of modulating cellular and humoral immune responses by interacting directly with T and B cells, decreasing antigen and mitogen-induced proliferation. SCD14 has been involved in periodontal inflammation as playing a crucial role in the subsequent periodontal destruction mediated by Lipo poly saccharide[14].

Chemerin: Chemerin is a newly discovered adipokine, which is associated with inflammatory markers such as CRP, IL-6, and TNF-α. Chemerin have anti-inflammatory properties in vascular endothelial cells via preventing the TNF-α-induced vascular cell adhesion molecule (VCAM)-1 expression and subsequent monocyte adhesion through inhibiting the activation of NF kB and p38. High concentrations, chemerin has an inflammatory effect by activation of endothelial nitric oxide synthase (eNOS). Chemerin has anti-inflammatory effects in low concentrations and pro-inflammatory effects in high concentrations. Chemerin can also be determined in inflammatory fluids, since it leads to chemo taxis of the monocytes and the macrophages to the site of inflammation. In addition to the pro-inflammatory cytokines, chemerin also causes an irreversible tissue damage by increasing the MMP levels. Increased salivary chemerin levels in the patients with periodontitis can indicate that this marker is more specific in distinguishing destructive periodontal disease from gingivitis. Therefore, chemerin can be used for screening purposes in large populations for detection of destructive periodontal disease[15].

Interferon gamma: It is a signature cytokine of the adaptive immune response. Its main function is to promote antigen-presenting cell binding of antigen by up-regulating major histocompatibility complex class I and class II expression. Interferon gamma also plays a major role in B-cell maturation, and, accordingly, in immunoglobulin secretion. In periodontal disease, interferon gamma is present at high levels in periodontal lesions, and is associated with progressive lesions or severe forms of periodontitis[16].

Interleukin 18: IL-18 is a pro-inflammatory cytokine and amplifies immune responses by inducing other cytokines viz. IL-1β, TNF-α and IL-8 . It can stimulate neutrophil migration and osteoclastic activity and is important in the removal of intracellular pathogens and viruses. The role of IL-18 in periodontal disease has been suggested because of their induced level in gingival crevicular fluid, saliva, serum and gingival tissue samples. IL-18 is synthesized intracellularly as a biologically inactive precursor and requires caspase-1 to cleave it into the active IL-18 molecule. This process could occur after TLR4 is activated by lipopolysaccharides. Inflammation involves a series of pathways which results in the expression of extracellular damage-associated molecular patterns (DAMPs). DAMPs are endogenous molecules released specifically and rapidly at times of cellular stress, injury or necrosis, the presence of which induces host tissue inflammation[16].

Interleukin-6:.It is a proinflammatory cytokine that dictates the transition from acute to chronic inflammation by changing the nature of leukocyte infiltrate (from polymorphonuclear neutrophils to monocytes/macrophages), exerts stimulatory effects on T and B cells, and induces antibody formation, favoring a chronic inflammatory response. In addition, IL-6 stimulates osteoclast activity and bone resorption, inducing osteoblastic production of downstream effectors like RANKL[17].

Tumor necrosis factor-alpha: TNF-a is a proinflammatory and immunoregulatory cytokine central to the pathogenesis of various inflammatory conditions. It causes bone resorption through its ability to stimulate IL-1

and granulocyte macrophage-colony stimulating factor (GM-CSF), inhibit bone collagen synthesis, induce collagenases, and stimulate osteoclast differentiation in the presence of M-CSF. After periodontal treatment the concentrations of salivary tumor necrosis factor-alpha were reported to decrease, there is no evidence that the levels of salivary tumor necrosis factor-alpha correlate with clinical changes in the periodontium either during disease progression or after treatment . Currently, therefore, it seems unlikely that salivary tumor necrosis factor-alpha can be regarded as a good candidate biomarker for periodontal disease[18].

C reactive protein: C-reactive protein (CRP), produced by liver, is a systemic marker released during acute phase of an inflammatory response. Circulating CRP reaches saliva via GCF or salivary glands. Studies reported high levels of CRP in association with chronic and aggressive periodontal diseases.Various observations were made which revealed that higher the levels of CRP, the more severe are the periodontal disease. In addition, elevated serum CRP is a strong independent risk factor for the development of cardiovascular disease (CVD), which establishes a link with periodontal disease. Therefore, salivary CRP may represent a novel method for diagnosing and monitoring CVD and periodontal diseases[17].

Host derived salivary biomarkers associated with soft tissue destruction

Matrix metalloproteinase-8: Matrix metalloproteinase (MMP) are zinc-dependent endopeptidases and a leading enzyme in degradation of extracellular collagen matrix. They are derived mainly from polymorphonuclear leukocytes during acute stages of periodontal disease.The specific proteolytic enzyme secreted by neutrophils and macrophages, the Collagenase-2 also called MMP-8 plays an important role in the pathogenesis of periodontal disease.MMP is the most potent proteinase to initiate the destruction of Type I and III collagen.This critical feature makes MMP-8 important in the pathogenesis of periodontal disease. MMP-8 is up-regulated not only in affected tissues, but also in the secreted, disease affected oral fluids such as saliva and GCF due to the permeability of the sulcular epithelium. Salivary MMP-8 have been found to be four times higher in subjects with periodontitis.This indicates that elevated levels of MMP-8 is reflective of the collagen degradation phase of periodontitis and may be useful for monitoring disease activity[17].

Hepatocyte growth factor: Hepatocyte growth factor (HGF) is a multifunctional cytokine involved in embryonic development and the repair and regeneration of various tissues/organs and their protection from injury. It exhibits mitogenic and antiapoptotic activities, and enhances the motility of different cell types, including not only hepatocytes but also epithelial cells and vascular endothelial cells. Following tissue damage, HGF is expressed in mesenchymal cells (i.e. fibroblasts, mononuclear cells. megakaryocytes), while its high-affinity receptor c-Met is expressed by almost all epithelial cells, endothelial and erythroid progenitor cells. Remarkably, HGF is also involved in the development of periodontal disease. Recently it was shown that HGF levels in unstimulated whole mixed saliva are directly correlated with probing depth and the percentage of sites positive for bleeding on probing in the general population[19].

Aspartate aminotransferase: AST is an enzyme normally confined to the cell, which is released to the gingival crevicular fluid and saliva upon cell death in the active phase of periodontal disease[20].

Lactate dehydrogenase: Lactate dehydrogenase (LDH) is a ubiquitous enzyme that plays a significant role in the clinical diagnosis of pathologic processes. Salivary LDH was found to be the most useful enzyme for the screening of periodontitis. Studies showed increased LDH activity in the saliva of subjects with increased probing depth than in individuals with healthy periodontium[17].

Tissue inhibitors of matrix metalloproteinases-2: The activities of MMPs in body tissues, such as the periodontium, are regulated at one level by tissue inhibitors of metalloproteinases (TIMPs). This well-studied family of inhibitors consists of four members (TIMPs 1–4). TIMP-1, -2 and -4 are secreted extracellular proteins and TIMP-3 is bound to the extracellular matrix. All can inhibit MMPs. In addition to proteinase inhibition, TIMPs can exert other functions including, but not limited to, MMP transportation and stabilization, MMP focalization to the cell surface, inhibition of angiogenesis and promotion of bone-resorbing activity. The most common inhibitor, TIMP-1, is secreted by the regional cells of the periodontium (fibroblasts, keratinocytes and endothelial cells) and by the migratory cells of the inflammatory infiltrate (monocytes/macrophages). Under natural conditions, inhibitors of MMPs are required for the normal physiological remodeling of connective tissue. An imbalance between the levels of active MMPs and their tissue inhibitors can lead to excessive degradation of extracellular matrix proteins[21].

Host derived salivary biomarkers associated with hard tissue destruction

Osteoprotegerin: Osteoprotegerin (OPG) is a glycoprotein that inhibits osteoclast differentiation and promotes bone-resorption. The salivary receptor activator of nuclear factor kappa-B ligand/OPG ratio may be helpful in the screening and diagnosis of periodontitis.OPG concentrations elevates in patients with periodontitis[17].

Alkaline phosphatase: ALP is a non-specific enzyme associated with the calcification process, although its exact function remains unknown. Associated with the cell plasma membrane, this enzyme appears to play a role

in the transport of substances from the intracellular compartment across the cell membrane to the extracellular region. Alkaline phosphatase (ALP) has also been used as a possible indicator for gingival inflammation and bone resorption[21].

Strong and weak salivary biomarkers in prediction of periodontal disease according to their classification.

Bacteria derived salivary biomarkers	
Porphyromonasgingivalis Prevotella intermedia Tanerella forsythia	Strong biomarkers
Fusobacterium nucleatum Aggregatibacteractinomycetemcomitans	Questionable biomarkers
Host derived salivary biomarkers associated with inflammation	
IL-1b, MIP-1a, Arginase	Strong biomarkers
IL-6, IL-8, TNF-a	Questionable biomarkers
Host derived salivary biomarkers associated with soft tissue destruction	
MMP-8, HGF	Strong biomarkers
Alanine aminotransferase, TIMP-1	Questionable biomarkers
Host derived salivary biomarkers associated with hard tissue destruction	
Alkaline phosphatase, Osteonectin, RANKL	Potential biomarker
Osteoprotegerin	Questionable biomarker

III. CONCLUSION

Saliva is an exocrine secretion of the salivary glands, consisting of water, electrolytes, enzymes, Ig, mucosal glycoproteins and numerous antimicrobial proteins, growth factors and regulatory peptides. With the advent of highly sensitive techniques, traces of markers can be accurately established in saliva. The present review demonstrates that saliva is a valid source for investigating periodontal cytokines, with many advantages over other sources such as gingival crevicular fluid. Based on all the studies reported and discussed in this review we now have substantial information suggesting that a limited number of protein biomarkers may be effective in diagnosis and management of periodontitis. Most biomarkers in GCF and saliva are indicators of inflammatory events that precede the destruction of the alveolar bone. Current evidence suggests that of the molecules with cytokine-like activity studied thus far, interleukin-1- beta and hepatocyte growth factor are the most robust salivary biomarkers for periodontal disease. Several markers related to inflammation, connective tissue destruction and bone remodeling are elevated in chronic periodontitis. Inhibitors of proteinases are reduced in saliva in chronic periodontitis. Specific markers (macrophage inflammatory protein-1a) are associated with aggressive forms of periodontitis. These biomarkers thus can be helpful in diagnosing and predicting the risk of periodontal disease in a larger population to prevent the future occurrence of the disease.

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