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Review Article

Oral Fluid Based Biomarkers in Periodontal Disease: Part 1. Saliva

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***Abstract*:**

Traditional clinical measurements such as probing pocket depth, bleeding on probing, clinical attachment loss; plaque index and radiographs used for periodontal diagnosis are often of limited usefulness as they are indicators of previous periodontal disease rather than present disease activity. A literature search was carried out to find out all the available tests that indicate periodontal disease markers in saliva. All major databases were searched to compile the information on published reports between 1999 and 2014. The list of biomarkers available to date is compiled and presented in a table format. Each biomarker is discussed separately based on the available evidence. Based on the evidence, it can be concluded that several sensitive salivary indicators of periodontitis are available to detect the presence, severity and response to treatment. Further studies are warranted to analyze the sensitivity and reliability of these indicators that might help in developing non-invasive tests that could help in the diagnosis of periodontal disease.

Key Words: Biomarkers, diagnosis, periodontal disease, saliva

Introduction

Saliva, an oral fluid derived from the major and minor salivary glands has been used in the past few decades as a diagnostic fluid. It is secreted mainly by three pairs of major salivary gland and numerous minor salivary glands located at various oral mucosal sites.[1] The saliva derives additional constituents from serum, gingival crevicular fluid (GCF), and oral mucosal transudate, making it appealing as a potential diagnostic fluid reflective of circulating levels of these biomarkers in blood. It contains a highly complex mixture of substances and biomarkers that are used for diagnosing local and systemic diseases, or monitoring the effect of treatment.[2] The use of saliva as a diagnostic fluid has been hindered, mainly because of our lack of understanding of the biomolecules present in saliva and their relevance to disease etiology, combined with the lack of high-sensitivity detection systems. Currently, with improved efficiency and accuracy of the technology, salivary diagnostics has been made into a clinical and commercial reality. Also collection of saliva is safe, non-invasive, and simple, and can be collected repeatedly with minimum discomfort to the patient.

Periodontitis is a multifactorial chronic non-reversible inflammatory disease affecting the supporting structures of dentition, initiated and propagated through a complex interaction between periopathogens and the host defense system.[3] It starts with a microbial infection, followed by a host mediated destruction of periodontal tissues caused by hyper activity of leukocytes and generation of cytokines, eicosanoids and matrix metalloproteinases.[3-5] Clinically, the disease progress with loss of attachment to root surface, formation of a deep pocket, alveolar bone resorption, and subsequent loss of tooth. It is the most common disease affecting the oral cavity after dental caries and the major cause of tooth loss, thereby affecting the quality of individual's life. Therefore, early diagnosis and control of the disease is the paramount goal for clinicians.[6]

Traditional clinical measurements, such as probing pocket depth, bleeding on probing, and clinical attachment loss, which are used for periodontal diagnosis, are often of only limited usefulness because they are indicators of previous periodontal disease rather than present disease activity.[7] Knowing the disease activity might help in early intervention in patients with the disease. This review of the literature focuses the attention on the biochemical markers in saliva that appear to be promising in the future for periodontal diagnosis, as well as some contemporary diagnostic tests available.

Materials and Methods

Two authors independently searched the Medline, EMBASE, Cochrane Library, Web of Science, Google Scholar and Scopus databases for relevant studies. The search was carried out by using a combined text and the MeSH search strategies: Using the key words "saliva" or "salivary" and "biomarker" and "periodontitis" or "diagnostic or prognostic indicator."

Publications in English between 1999 and 2014, which estimated saliva as a marker in the diagnosis, assessment of the severity or response to treatment is included. Due to the large number of markers used and the lack of a sufficient number of studies dealing with each maker, the accuracy and predictability of the marker is not considered in this systematic review. The markers are listed in a table with relevant references (Table 1).

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.[89] Among the LDH isoenzymes, LDH4 and LDH5 dominated in whole saliva samples and are predominantly produced by gingival fibroblasts.[90] A study by Nomura *et al.*[6] showed that LDH4 and LDH5 were dominant in samples of whole saliva and can be used as a parameter for the screening of periodontal disease. A reduction in salivary LDH was observed in a study after ultrasonic scaling and could be used as a prognostic indicator.[8] Salivary LDH has also been used as a screening test to detect the presence of periodontitis in pregnant women.[9] The predictive value of periodontal disease progression by assessment of salivary LDH and the total count of *Porphyromonas gingivalis, Prevotella intermedia* was also established by Nomura *et al.*[2] Yoshie *et al*[21] showed that salivary LDH levels reflect inflammation and destruction of periodontal tissue, suggesting it as a clinically useful marker following periodontal therapy.

Table 1: Salivary biomarkers in periodontal diseases.

	References
Enzymes	
LDH	de la Peña *et al.*;[8] Nomura *et al.*;[6] Kugahara *et al.*;[9] Nomura *et al.*[2]
ALP	Totan *et al.*;[10] Kibayashi *et al.*;[11] Kugahara *et al.*;[9] Dabra and Singh.[12]
MMP-8	Górska and Nedzi-Góra; [13] Costa *et al.*;[14] Gursoy *et al.*;[15] Gursoy *et al.*;[16] Gursoy *et al.*;[17] Meschiari *et al.*;[18] Yildirim *et al.*[19]
MMP-1	Pietruska *et al.*;[20] Yildirim *et al.*[19]
Aminotransferase	Totan *et al.*;[10] Yoshie *et al.*;[21] Nomura *et al.*[2]
Amylase	Sanchez *et al.*;[22] Haririan *et al.*;[23] Sanchez *et al.*[24]
Arginase	Ozmeriç *et al.*;[25] Gheren *et al.*;[26] Pereira *et al.*[27]
Lysozyme	Ito *et al.*;[28] Surna *et al.*[29]
Chitinase	Van Steijn *et al.* [30,31]
Dipeptidyl peptidase	Aemaimanan *et al.*[32]
Alanine aminopeptidase	Aemaimanan *et al.*[32]
B-glucuronidase	Lamster *et al.*[33]
Myeloperoxidase	Meschiari *et al.*[18]
Elastase	Pauletto *et al.*[34]
Esterase	Bimstein *et al.*[35]
Proteins	
Lactoferrin	Groenink *et al.*;[36] Fine *et al.*;[37] Jentsch *et al.*;[38] Komine *et al.*;[39] Berlutti *et al.*;[40] Glimvall *et al.*;[41] Rocha Dde *et al.*[42]
HGF	Wilczynska-Borawska *et al.*;[43] Rudrakshi *et al.*;[44] Lönn *et al.*[45]
IL-6	Aurer *et al.*;[46] Teles *et al.*;[47] Costa *et al.*[14]
CRP	Aurer *et al.*;[46] Aurer *et al.*;[48] Shojaee *et al.*[49]
TIMP	Gursoy *et al.*;[15] Isaza-Guzman *et al.*[50]
Cystatins C, S, A, SN	Lie *et al.*;[51] van Gils *et al.*[52]
Neopterin	Ozmeriç *et al.*[53]
α-2-macroglobulin	Ozmeriç *et al.*[53]
α-1-antitrypsin, keratin, complement C3. Fibronectin, albumin, epidermal growth factor, vascular endothelial growth factor	Nomura *et al.*;[2] Wong[1]
Other markers	
8-OHdG	Sugano *et al.*;[54] Sawamoto *et al.*;[55] Takane *et al.*;[56] Canakçi *et al.*;[57] Canakci *et al.*;[58] Sezer *et al.*[59]
OPG	Buduneli *et al.*;[60] Costa *et al.*;[14] Al-Sabbagh; [61] Tabari *et al.*[62]
NO	Aurer *et al.*;[63] Reher *et al.*;[64] Ozer *et al.*;[65] Khorsavi Samani;[66] Parwani *et al.*;[67] Poorsattar Bejeh Mir;[68] Han *et al.*;[69] Sundar *et al.*[70]
Melatonin	Cutando *et al.*;[71] Gómez-Moreno *et al.*;[72] Kennaway; [73] Srinath *et al.*;[74] Almughrabi *et al.*[75]
Urate	Sculley and Langley-Evans; [76] Diab-Ladki *et al.*;[77] Sculley and Langley-Evans[78]
Ascorbate	Sculley and Langley-Evans; [76] Diab-Ladki *et al.*;[77] Sculley and Langley-Evans[78]
Cortisol	Ishisaka *et al.*;[79] Ansai *et al.*;[80] Nayak *et al.*;[81] Refulio *et al.*[82]
Igs (G, A, M, S IgA)	Hagewald *et al.*;[83]; Olayanju *et al.*[84]
Ca	Kojima *et al.*;[85] Erdemir and Erdemir; [86] Kiss *et al.*[87]
PAF	McManus and Pinckard[88]

LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, MMP-8: Matrix metalloproteinase-8, MMP-1: Matrix metalloproteinase-1, HGF: Hepatocyte growth factor, IL-6: Interleukin 6, CRP: C-reactive protein, TIMP: Tissue inhibitor of matrix metalloproteinase, 8-OHdG: 8-hydroxydeoxyguanosine, OPG: Osteoprotegerin, NO: Nitric oxide, CA: Calcium, PAF: Platelet activating factor, Igs: Immunoglobulins

Matrix metalloproteinase

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.[91] 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.[15,92] MMP is the most potent proteinase to initiate the destruction of Type I and III collagen.[15] 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.[93] 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.[93,94] Ramseier *et al.*[95] showed that a combination of salivary MMP-8 and certain anaerobic periodontal pathogens such as *P. gingivalis* or *Treponema denticola* present in subgingival biofilms could predict the status of periodontal disease.

Esterase

Levels of salivary esterase has been found higher in periodontitis patient than in healthy subjects.[35,96] Furthermore, a positive correlation between salivary esterase and formation of calculus was found.[97] Esterase levels were reduced after periodontal treatment. Hence, monitoring esterase levels may be indicative of efficacy of periodontal treatment.[98]

Lysozyme

Lysozyme activity in saliva combats plaque accumulation, which is the main culprit of periodontal disease.[99-101] Therefore, reduced levels of this enzyme may be suggestive of future periodontal disease.[29]

Chitinase

Chitinase plays a role in the defense against chitin containing pathogens. Studies showed that this enzyme was raised in the saliva of periodontitis patients and decreased after treatment.[30]

Aspartate aminotransferase

Studies demonstrated the usefulness of the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), in the diagnosis, prognosis, and treatment of periodontal disease.[6,10] Moreover, they also showed that salivary AST, ALT and LDH levels were significantly decreased after oral prophylaxis.[21] Nomura *et al.*[2] evaluated AST, ALT and LDH levels in patients with treated periodontitis and progressive periodontitis. The levels of these biomarkers tended to be higher in subjects who developed periodontitis. They concluded that ALT and *P. gingivalis* combination is the most promising diagnostic tool for predicting periodontal disease progression.

Alkaline phosphatase

Alkaline phosphatase (ALP) has also been used as a possible indicator for gingival inflammation and bone resorption. It has been found that untreated adult periodontitis exhibited higher level of ALP in whole saliva than in healthy controls.[9-12]

Lactoferrin

Lactoferrin was intensely up-regulated in mucosal secretions during gingival inflammation and also detected at a high concentration in saliva of patients with periodontal disease compared with healthy patients.[36]

Cysteine

Cystatins are cysteine protease inhibitors that can modulate tissue destruction in periodontal diseases. Volatile sulphur compounds seem to be directly involved in the pathogenesis and maintenance of periodontal tissue lesions. The concentration of some of the sulfur compounds such as cysteine, cysteinylglycine, and glutathione is significantly higher in periodontitis patients.[102] The increase of cysteine in periodontitis could be related to some proteolytic activity of bacteria in the oral cavity. The increased concentration of these sulfur compounds present in saliva of patients and their strong correlation with the periodontal probing pocket depth, make these molecules suitable as markers for the severity of periodontitis.[103] An increased cystatin activity in whole saliva of gingivitis and periodontitis subjects was reported by enhanced synthesis of some acinar proteins.[104,105] The cysteine level showed a significant reduction in whole saliva after periodontal treatment.[106] Studies have revealed that smoking is associated with lower cystatin activity and output of Cystatin C increased during gingival inflammation.[51,52]

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.[107,108] 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.

Epithelial keratins

Along with other constituents, saliva contains epithelial cells from the lining of the oral cavity, but input of crevicular or pocket epithelial cells to the total number of salivary epithelial cells is not known.[109] McLaughlin *et al.*[110] studied the keratin level in GCF and demonstrated that the keratin concentration was significantly higher at sites exhibiting signs of periodontitis compared with healthy sites.

Platelet activating factor

A correlation have been found between salivary platelet activating factor, a potent phospholipid inflammatory mediator, level and the extent of periodontal disease and post-treatment.[111]

Hepatocyte growth factor

Ohshima *et al.*[112] demonstrated a correlation between salivary hepatocyte growth factor (HGF) level and the number of deep pockets exceeding 4 mm.[112] HGF acts as mitogen and antiapoptic factor for various kinds of epithelial cells. Fibronectin is a glycoprotein, which mediates adhesion between cells. *P. gingivalis* fimbriae bind to salivary fibronectin resulting in reduced salivary fibronectin in periodontitis.[44,45]

Osteoprotegerin

Osteoprotegerin (OPG) is a glycoprotein that inhibits osteoclast differentiation and promoting bone-resorption. The salivary receptor activator of nuclear factor kappa-B ligand/OPG ratio may be helpful in the screening and diagnosis of periodontitis.[62] OPG concentrations were elevated in patients with periodontitis.[14]

8-hydroxydeoxyguanosine

The neutrophils play a central role in the initial host inflammatory response to the periodontal pathogens, which leads to enhanced oxidative stress. Oxidative stress induces DNA damage, including oxidation of nucleosides. 8-hydroxydeoxyguanosine (8-OHdG) is an oxidized nucleoside that is excreted in the bodily fluids with DNA. Takane *et al.*[113] have demonstrated that the mean 8-OHdG level in saliva is a useful marker to screen periodontal disease. The level of 8-OHdG can be also used as a prognostic indicator to monitor the progression of periodontal disease.[56] Canakçi *et al.*[57,58] studied the 8-OHdG levels in saliva and mitochondrial DNA deletions in gingival tissue of patients with chronic periodontitis. They established that the salivary 8-OHdG level may signify premature oxidative mitochondrial DNA damage in diseased gingival tissue and could serve as a marker of periodontitis. The 8-OHdG levels in saliva reflect the load of periodontal pathogens and could be a useful biomarker for assessing periodontal status accurately, and for evaluating the efficacy of periodontal treatment.[55,59]

Nitric oxide

Nitric oxide (NO), which is synthesized from L-arginine by NO synthase, plays a protective role in infectious diseases. NO has been linked to etiopathogenesis of inflammatory periodontal disease and is expressed in saliva.[114] Salivary NO levels can be utilized as a good indicator of the inflammatory status of the periodontium, and evaluating its levels in saliva by Griess reaction on a photoelectric colorimeter is a reliable, accurate and faster method to estimate the level of inflammation in periodontal tissues.[67,69] A higher level of salivary NO was observed in patients with periodontitis in comparison to the healthy individuals and can be used as a valuable screening tool for periodontitis.[66,68]

Immunoglobulin

Immunoglobulins (Igs) have an influence on oral microbiota as they interfere in adherence and bacterial metabolism. Higher concentrations of Ig A, Ig G, and Ig M have been found in periodontal disease as compared with healthy patients[83] and their concentration drops significantly following treatment.[115]

Melatonin

Melatonin is a hormone, which is involved in the control of the circadian rhythm, but also acts as an antioxidant and immune modulator.[116] Periodontitis may be triggered by a shortage of antioxidants to balance increased oxidative stress. Melatonin acts as an antioxidative, anti-inflammatory, and bone-preserving agent suggesting a role in periodontal disease.[117] Studies have shown decreased salivary melatonin levels in periodontitis patients.[71,74,118] Salivary melatonin levels may be related to periodontal inflammation possibly due to its antioxidant abilities, and its estimation in saliva act as a risk indicator for the severity of periodontal disease.[119]

Cortisol

Stress has been advocated as a risk factor for periodontitis. Studies showed a positive relationship between periodontitis and the cortisol level in saliva. Elevated levels of serum cortisol associated with stress exert an inhibition on the immune response to inflammation. Salivary cortisol levels were used to evaluate the role of stress in periodontal disease.[80,120,121]

Calcium

Calcium (Ca) ion present in saliva has been intensively studied for its correlation with periodontal disease. Elevated levels were correlated with good dental health in young subjects, but no relation was detected with periodontal disease or bone loss as measured from dental radiographs.[122] However in another study, Ca and Ca to phosphate ratio were higher in periodontitis patients compared with controls.[123] The authors concluded that the high level of Ca in saliva was characteristic of periodontitis.

Bacteria

Periodontitis is an inflammatory disease initiated through interactions with colonizing of periodontal pathogens subgingivally.[124] Longitudinal studies have evaluated periodontal pathogen counts in saliva and their connection to periodontal disease. *P. gingivalis*, *Actinobacillus actinomycetemcomitans*, *Tannerella forsythia*, *P. intermedia*, and *T. denticola* have been attributed as prognostic biomarkers for disease progression.[125,126] However, these studies did not specify, which bacterial species can be used for identification of individuals at risk of disease progression. Saygun *et al.*[127] reported that salivary counts

of *P. gingivalis, T. forsythia* and *P. intermedia* appear to have the potential to identify the presence of periodontitis.[127] Similarly, von Troil-Lindén *et al.*[128] studied the salivary levels of *A. actinomycetemcomitans, P. gingivalis, P. intermedia, Campylobacter rectus*, and *Peptostreptococcus micros* and related their levels to clinical periodontal status in 40 subjects with varying degrees of periodontitis. Furthermore, Nomura *et al.*[2] studied the salivary counts of periodontal bacteria in patients with treated periodontitis and progressive periodontitis and reported statistically significant increase in *P. gingivalis* and *P. intermedia* levels in progressive periodontitis group. Conversely, *T. forsythia* did not show the same increase. Association between the gingivitis and the presence of *Mycoplasma* species in saliva has also been reported.[129] Association between oral microbial levels and Plaque and gingival index scores were reported, and it was concluded that the test can serve as an indicator of gingival inflammation. The existence of bacteria in saliva provide information about the bacterial challenge by periodontal tissue, in the initiation of disease and tissue response.[130]

Conclusion

Saliva, an exocrine secretion of the salivary glands, consists of water, electrolytes, enzymes, Ig, mucosal glycoproteins and numerous antimicrobial proteins, growth factors and regulatory peptides.[131] Development of innovative diagnostic tests to detect active phases of periodontal disease and to identify individuals at higher risk for future disease occurrence is the focus of numerous clinical investigations. With the advent of highly sensitive techniques, traces of markers can be accurately established in saliva. Saliva contains locally and systemically derived mediators of periodontal disease, including pathogens, host-response, and bone-specific markers. Most biomarkers in GCF and saliva are indicators of inflammatory events that precede the destruction of the alveolar bone.[132] As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be collected non-invasively. With the advantages of an easy, safe, cost-effective, and non-invasive diagnostic approach, saliva shows a high potential for monitoring periodontal disease. New developments in proteomics of saliva and gene transfer technologies applied to the salivary glands will facilitate development of biomarkers with diagnostic and/or prognostic value.

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