Journal of Academy of Dental Education, 1-7, DOI: 10.18311/jade/2018/20883
ISSN (Print) : 2348-1595
ISSN (Online) : 2348-2621

Saliva- A Tool for Diagnosis in Periodontal Disease

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Abstract

Periodontitis, a chronic inflammatory condition involving tooth supporting structures that results in connective tissue loss and progressive bone loss. Careful diagnosis and treatment plan is inevitable for the successful clinical outcome. Investigation of biomarkers specific to certain periodontal disease in saliva gains more clinical interest in the present era. The gratification towards saliva has been increased over GCF due to its ease and non-invasive methods of collection compared to GCF. Saliva contains numerous biomarkers which is an emerging chair side diagnostic tool that will play a major role for the future investigators. This article aims at reviewing the biomarkers present in the saliva pertaining to particular periodontal diseases and its activity.

Keywords: Periodontitis, Salivary Biomarkers, Salivary Enzymes

Date of Receipt: April 2018 Date of Submission: March 2018 Date of Acceptance: May 2018

1. Introduction

Successful periodontal therapy relays on early diagnosis and severity of a disease[1]. Periodontitis, which is a most common periodontal disease category, is a irreversible inflammatory chronic condition that persuades to formation of probing pocket depth, progressive attachment loss, with loss of collagen fibres and can progress to bone loss if left untreated[2]. Traditional diagnostic parameters fails to assess patients who are highly susceptible to periodontitis. The diagnostic use of saliva has attracted the attention of clinical investigators due to its non-invasiveness and ease of collection.

2. Emerging Diagnostic Tool-Saliva

Saliva plays a major role in lubrication, chewing, swallowing, digestion and protection of oral mucosa[4]. Local and general health of the human body is reflected as an essential biomarker in the saliva. Although gingival crevicular fluid plays a potential role in early diagnosis of periodontal disease progression[3], the invasive and complex collecting procedure offers a technical challenge to the clinician.

3. Gratification of Saliva over Gingival Crevicular Fluid[5]

1. Painless collection process
2. Less distress to patient
3. Simple to collect and non-invasive
4. Convenient for multi sampling
5. Cost effective for screening large population
6. Cheap technology for laboratorial diagnosis
7. Easy to store
8. Less time consuming
9. Less blood contamination

4. Salivary Biomarkers in Periodontal Diseases

A biomarker is a substance used as an indicator for early disease detection and disease progression in diagnosis

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of oral diseases[6]. The bacterial products released during periodontal disease progression activate the immune response and triggers the various inflammatory cells such as polymorphonuclear neutrophils, monocytes, macrophages. Thus resulting in the release of various cytokines (prostaglandin E2, tumor necrosis factor and interleukins) and inflammatory markers in serum and saliva. Consequently the release of matrix metalloproteinases and collagen destructive enzymes leads to the extra cellular matrix degradation, a trespasser for periodontal disease progression.

The various salivary biomarkers[2] includes

1. Markers affecting dental biofilm

A. Specific marker

B. Non specific

2. Systemicbiomarkers to periodontal infection
3. Markers of periodontal soft tissue inflammation
4. Markers of alveolar bone loss
5. Salivary enzymes and ions
6. Growth factor as a salivary biomarker
7. Epithelial keratins
8. Hormones
9. Inflammatory cells and bacteria
10. Volatiles and oxidative stress marker

4.1 Markers Affecting Dental Biofilm

4.1.1 Specific Marker

Immunoglobulin interferes in adherence of bacterial metabolism in chronic and aggressive periodontitis. Increased concentration of immunoglobulin in patients with periodontitis has been clinically evident. Higher salivary constituents of various immunoglobulins such as IgA, IgG, IgM specific to certain periodontal pathogens are observed as a salivary parameter in patients with periodontitis compared to Non-periodontitis individuals. Especially, level of IgA has been greatly detected and their levels decreased significantly following non-surgical periodontal therapy.

4.1.2 Non Specific Biomarker (Table 1)

Table 1: Nonspecific biomarkers in periodontitis

Protein Markers	Nature of the Biomarker	Mechanism	Activity in Periodontitis
Mucins[7]	Glycoproteins(MG1,MG2) helps in maintaining the viscoelasticity of saliva	Interferes with attachment of A. actinomycetemcomitans	MG2 level declined in saliva with increased periodontal pathogens
Lactoferrin[2,7]	Iron binding glycoprotein	Inhibit the growth of bacterial by lowering the iron content	i)Upregulated in gingival inflammation ii)Higher concentration in periodontal disease
Histatin[2,7]	Salivary protein with antimicrobial properties	i) Neutralizes enzymes and lipopolysaccharides in gram negative bacteria ii) Inhibits the histamine expressed	Decreases the role of histamine
Fibronectin[7]	Glycoprotein	Improves the attachment and colonisation of bacteria	Additive role in repair and healing process
Cystatins[7]	Proteolytic enzyme released by bacteria inflammatory cells, osteoclast, fibroblast	Collagenolytic activity causing tissue destruction	Modulating enzyme activity in periodontium
Platelet Activating Factor (PAF)[7]	Stimulates the phospholipid and triggers the inflammation	Release of inflammatory mediators	Positive correlation between PAF and periodontal inflammation
Proline[7]	Amino acid	Degradation of amino acid	Both positive and negative correlation exist

4.2 Systemic Biomarker - C-Reactive Protein

Periodontal inflammation causes stimulation of circulatory cytokines such as tumour necrosis factor α, interleukin-1 which inturn induces the liver to produce C-reactive protein into the circulation. Salivary C-reactive protein is appreciated in chronic and aggressive periodontitis and act as inflammatory marker[2].

4.3 Markers Present in Inflamed Periodontium

Prostaglandin E2 is the major mediator of periodontal disease and act as a potent biomarker of soft tissue inflammation (figure 1). PGE2 stimulates the fibroblast and osteoblast to produce MMP'S which is again a potential biomarker of tissue destruction[2].

4.4 Markers Expressed in Alveolar Bone Loss

MMPs are responsible for tissue destruction and remodelling. Periodontal collagens are broken down by collagenases. MMP8 levels are most prevalent in active disease progression of patients with gingivitis and periodontitis. Also in patient with peri-implantitis the peri-implant sulcular fluid demonstrates an elevated level of MMP8. The need for future longitudinal studies in the salivary level of MMP8 in periodontitis patient is inevitable[2,7].

Gelatinase (MMP9) is a collagenase produced by neutrophils and has a pivotal role in degradation of collagenous intercellular ground substance. MMP9 increased in patients with periodontitis has been reported in the literature by Teng et al[8].

Collagenase (MMP13) is another collagenolytic MMP found to be elevated in patients with periodontitis and peri-implantitis[7].

Increase in concentration of telopeptide, osteocalcin, osteopontin in GCF of patients with progressive periodontal disease has been reported[7].

4.5 Salivary Enzymes and Ions

The major salivary enzymes which act as potential biomarkers include lysozyme and peroxidase. Lysozymes are capable of breaking chemical bond in bacterial cell wall and hydrolyze the glycosidic linkages and distrupts the bacterial cell wall. Low level salivary lysozyme with high level of salivary peroxidase is considered as a factor for periodontal disease[7].

Increased salivary calcium demonstrated in the saliva of patient with periodontal disease has been reported in the literature by Sewon et al[9].

4.6 Growth Factor as a Salivary Biomarker

Growth factor act as remarkable biomarker in inflammation and wound healing. Higher levels of growth factor were detected in whole saliva of patient with periodontitis[7].

4.7 Epithelial Keratins

Morgan et al., demonstrated the epithelial cells lining the mucosa can be demonstrated in patients saliva with periodontitis[10].

4.8 Hormones

High salivary cortisol level has been demonstrated in patients with periodontitis along with emotional stress, poor oral hygiene, smoking[7].

4.9 Inflammatory Cells and Bacteria

Increased orogranulocytic migration rate in the presence of oral inflammation has been reported by Raeste et al[11]. Klinkhammer et al., developed the orogranulocytic migration rate[12].

Asikainen et al., Umeda et al., demonstrated the presence of microorganisms in the whole saliva[13]. Dejong et al., concluded saliva as a culture medium for Streptococcus and Actinomyces in his study[14].

4.10 Volatiles and Oxidative Stress Marker

Hydrogen peroxide, methyl mercaptans are the known volatile sulphur compounds which act as a diagnostic marker in periodontal disease.

This results due to excessive free radicals or diminished antioxidant system. Oxidative stress leads to DNA damage including oxidation of nucleosides (8-hydroxy deoxy guanosine) which is a potent biomarker of oxidative stress. Biomarkers ellicited in periodontitis has been summarized in (Table 2).

Table 2. Summary of studies involving biomarkers in periodontitis

Authors	Aim	Outcome
Baltacioglu et al[17]	Evaluation of lipid peroxidation(malondialdehyde) level and total oxidant/antioxidant in patients serum and saliva	Salivary MDA level, TOS, TAOS are elevated in periodontitis group than control group
Priscila et al[18]	Compared the salivary concentration of matrix metalloproteinase 8, interleukin 6,osteoprotegrin in periodontitis patients with or without diabetes	Salivary IL-6 concentration is increased in both the groups while salivary MMP8 and OPG are elevated in patients with diabetes and periodontal inflammation
Ghallab et al[19]	Investigated the salivary soluble CD44 profiles of smoking and non-smoking patients with and without periodontitis in counter to SRT	Baseline salivary soluble CD44 elevated in smokers compared with non-smoking periodontitis group
Gursoy et al[20]	Concentration of ICTP, MMP8, MMP14 and TIMP1 were analysed to detect potential markers of periodontitis	MMP8,MMP14,TIMP1 and ICTP are highly susceptible in advanced periodontitis patients
Al-Rawi et al[21]	Comparison of relationship between resist in and period onto pathogenic bacterial levels in obese adults, obese patients with type 2 diabetes and healthy controls	obese individuals with type 2 diabetes exhibited high level of salivary resist in
Nomura et al[22]	Evaluated the level of alanine amino transferase,alkaline phosphatase and free haemoglobinas biomarker also the count of P. intermedia, P. gingivalis, T. forsythia in stimulated saliva	Low P. Gingivalis and ALT act as a potential indicator for the progression of periodontal disease
Bertl et al[23]	Assessed the effect of non surgical periodontal therapy on salivary malotonin levels	Decreased salivary malotinin levels are the baseline which progressively increased at the end of the study
Novacovic et al[24]	Investigated the impact of nonsurgical periodontal treatment and salivary antioxidants(albumins, uricacid, superoxide dismutase, glutathione peroxidase) and evaluated their capacity as a biomarker	All the clinical parameters except attachment level were remarkably decreased after SRP
Ramseier et al[25]	Microbially derived biomarkers of plaque biofilm and saliva is determined	MMP-8,9and osteoprotegerin along with red-complex anaerobic bacteria were detected.
Miller et al[26]	Conducted the study to determine the correspondence between periodontal disease and levels of IL-1ß, MMP8, OPG in saliva of patients with moderate to severe periodontitis	Salivary MMP8 and IL-1ß are visible to serve as biomarker of periodontal disease
Banasova et al[27]	Compared oxidative stress salivary markers and feature of salivary DNA to the patients with chronic periodontitis and free control groups	Salivary thiobarbiturics acid reacting substances were higher in periodontitis subjects than controls and no differences found regarding salivary DNA
Sexton et al[28]	Assessed the salivary biomarkers (IL-1ß,IL-8,MIP1α, MMP8, OPG,TNF-α) of periodontitis patients	Salivary biomarker level of IL-1ß, MMP8, OPG and MIP1α reflected the disease severity
Tabari et al[29]	Evaluated the salivary concentration of visfatin level in patients with chronic periodontitis	Increased concentration of salivary visfatin level were significantly higher in periodontitis group
Arroyave et al[30]	Determined the difference in salivary concentration of sRANKL, osteoprotegrin and its ratio in periodontal disease	Higher salivary concentration of sRANKL, osteoprotegrin and its ration in periodontitis patients
Yesica et al[31]	Investigated the salivary levels of 8-hydroxy-2-deoxy guanosine and human neutrophil elastase complex as salivary biomarker in patient with chronic periodontitis	Increased salivary levels 8-hydroxy-2-deoxy guanosine and human neutrophil elastase in chronic periodontitis patient

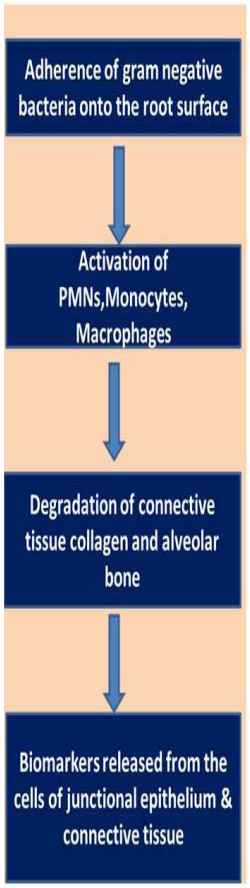

Figure 1. Mechanism of biomarker release due to soft tissue inflammation.

5. Forthcoming Biomarker in Saliva for Periodontitis

5.1 Salivary Proteome

A varying group of proteins manifested by a genome or a cell or a tissue or an organism referred as proteome. Mass spectrometry and shot gun proteomics are techniques that are used to identify proteomes[2].

5.2 Salivary Transcriptome

Transcriptome refers to an RNA molecules present in a single cell or a group of cells. Li et al., demonstrated the elevated RNA molecules in saliva of patients with oral cancer tissues[15].

Zudakov et al., demonstrated the five salivary RNA markers (SPRR1A SPRR3, KRT6A, KRT13, KRT4) lasted till 180days in the saliva and blood samples[16].

6. Conclusion

Thus biomarkers which are present in the saliva act as effective diagnostic tool because of its ease of collection, cost and chair side diagnostic tool. But still a lot more research efforts are necessary to determine the sensitivity and specificity of salivary biomarker and to increase the availability of routine detection methods[32].

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