



Role of Saliva as Biomarkers for Periodontal Diagnosis

KEYWORDS

Saliva, Periodontal Diagnosis, marker

Dr. Rachna Maheshwari

MDS, Senior Lecturer, Department of Prosthodontics including Crown and Bridge and Implantology, Kothiwal Dental College & Research Centre, Mora Mustaqueem, Kanth Road, Moradabad-244001, Uttar Pradesh

Dr. Suruchi Hans

MDS, Reader Department of Periodontics Maharaja Ganga Singh Dental College & Research Center 11, L.N.P, Hanumangarh Road Sri Ganganagar-335001, Rajasthan

Dr. Jigyasa Duhan

MDS, Professor, Department of Endodontics, Government dental college, Rohtak (Haryana)

Dr. Preeti Baweja

MDS, Professor JCD Dental College, Sirsa (Haryana)

ABSTRACT Saliva is used to describe the combined fluids present in the mouth. However in strict sense this word refers only to the hypnotic, watery fluid secreted by the major and minor salivary glands. Saliva is not only a pleasant lubricant which makes oral functions such as speech, mastication and swallowing easier, but also a fluid with many important functions in the maintenance of oral, general and metabolic health of the mouth as a whole. Saliva is a complex fluid which serves many important functions in the oral cavity that also contributes to the quality of life of an individual. In the recent past, research on saliva have been directed towards better understanding of the potential of saliva as an aid in the diagnosis of several diseases where presence/absence or a change of one or more of its constituents can act as a marker. Saliva is a fluid that can be easily collected non-invasively, contain locally derived and systemically derived markers of periodontal disease hence may offer the basis for patient specific diagnosis for periodontal disease. This review article emphasis on Role of saliva as a Biomarker for Periodontal Diagnosis

According to the AAP glossary of terms 1987, Saliva is defined as the tasteless clear fluid secreted by the major and minor salivary glands. Of all the organs in the craniofacial oral-dental complex, it is perhaps the salivary glands and their remarkable secretory product, saliva that forge the strongest link between oral and systemic health. Saliva may not be one of the popular body fluids since it lacks the 'drama' of blood, the 'sincerity' of sweat and the 'emotional appeal' of tears. Like many other things in life, salivation is taken for granted. It is only when the salivary flow is reduced or lost, its importance is felt.

Three major salivary glands produce about 95% of the total salivary volume and numerous minor salivary glands produce minute amounts of saliva. Saliva consists of 99% water, and varying amounts of proteins, enzymes, mucoproteins, blood group substances, hormones, carbohydrates, lipids, nitrogen containing compounds, lactoferrin and inorganic substances. Saliva is not only a pleasant lubricant which makes oral functions such as speech, mastication and swallowing easier, but also a fluid with many important functions in the maintenance of oral and general health. Rather than just providing lubrication, saliva is important for the metabolic health of the mouth as a whole. Some systemic diseases and hormonal changes can alter the flow and composition of saliva, so that in many cases saliva analyses have diagnostic value.

In recent years saliva has attracted much attention, it has been suggested that saliva might be substituted for plasma in the areas of pharmacokinetic studies and drug monitoring. The diagnosis of active phases of periodontal disease, and the identification of patients at risk for active disease, represents a challenge for both clinical investigators and clinicians. In general, clinical parameters including probing depth, attachment level, bleeding on probing

(BOP) plaque index (PI) and radiographic loss of alveolar bone are used to assess disease severity¹. It has long been realized that a rapid and simple diagnostic test that can provide a reliable evaluation of periodontal active disease would be of value to both clinicians and patients.

Saliva is a fluid that can be easily collected, contains locally-derived and systemically derived markers of periodontal disease, and hence may offer the basis for a patient specific diagnostic test for periodontitis. The collection of gland-specific saliva can allow differences in the amount of fluid and constituents of each gland to be determined. Salivary diagnosis is a late bloomer becoming into a flower as more people appreciate saliva as a "mirror of the body", reflecting fluid levels of natural substances. But there is still much unfinished business in salivary research. Saliva is a fluid that can be easily collected non-invasively, contain locally derived and systemically derived markers of periodontal disease hence may offer the basis for patient specific diagnosis for periodontal disease.

Periodontal Diagnosis by Clinical Monitoring-

The diagnosis of active phase of periodontal disease and identification of patients at risk of active disease represents a challenge for both investigators and clinicians. In general these are the clinical parameters that are used to assess the disease severity¹-

- Probing depth
- Bleeding on probing
- Attachment level loss
- Plaque index
- Radiographic loss

Recently, genetic analysis has also been suggested as a means to identify individuals who are at increased risk for

more severe periodontitis². However, as of yet, no clinical or laboratory test is routinely employed in the monitoring of patients with periodontal disease. Clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation.

Disadvantages of clinical monitoring –

- 1) It is time consuming subject to considerable measurement errors and is often poorly tolerated by the patients.
- 2) These measurements provide information about disease severity and do not provide measurements of disease activity.
- 3) Information is about the past pathology but not the present condition or the probability of alterations occurring in the future.

MARKERS IN SALIVA WITH POTENTIAL IMPORTANCE FOR PERIODONTAL DISEASE:

ENZYMES-

Enzymes present in saliva can be produced by cells in the salivary glands, oral microorganisms, polymorphonuclear leucocytes (PMNs), epithelial cells and GCF entering oral cavity³. Studies have examined enzyme activity in saliva in relation to periodontal status and in response to periodontal treatment.

Nakamura & Slots (1983) studied the enzymatic activity of mixed whole saliva and parotid saliva in 10 healthy, 10 Adult periodontitis and 4 Localized juvenile periodontitis patients. Mixed whole saliva from Adult Periodontitis patients demonstrated the highest enzyme activity as compared to healthy controls. **Zambon et al. (1985)** evaluated changes in the levels of enzymes in whole mixed saliva of Adult Periodontitis patients before and after treatment which included scaling, root planing and tetracycline therapy. This treatment resulted in reduced salivary levels of caprylate esterase lipase, leucine, valine and cysteine aminopeptidases, trypsin, β galactosidase, β glucuronidase and β -glucosidase.

Comparable results were reported by **Uitto et al. (1990)**, who collected saliva from subjects with a healthy or diseased periodontium to assay collagenase activity which was higher in the periodontitis patients than the controls. Children with **Down's syndrome** have an increased prevalence and severity of activity of salivary collagenase in 9 affected children, 9–17 years of age, was higher than controls suggesting its role in early periodontal tissue and alveolar bone destruction⁴.

a) Collagenase & related Matrix Metalloproteins-

Collagenase is a family of matrix metalloproteins (MMPs) which degrade collagen. MMP 8 is found in the inflammatory cells such as PMNs, macrophages and can be detected in the saliva^{4b}. MMP 9 is also found in Polymorphonuclear leukocytes. These can be immuno detected using monoclonal/polyclonal antibodies with the ELISA technique (**Ingman 1996**), Western blot (**Killi 2002**).

b) Salivary Gelatinase-

Makela et al. (1994) determined that the concentration of MMP-9 (92 kDa gelatinase) was significantly higher in whole saliva of periodontitis patients compared with healthy subjects.

c) Lysozymes-

Antibacterial enzyme found in the body secretions notably tears and saliva. The concentration of lysozyme in Patients with periodontitis demonstrated decreased lysozyme concentration compared to controls (**Markkanen et al. 1986**).

d) Lactoferrin-

Antibacterial agent found produced by inflammatory cells. **Soumalainen et al. (1996)** evaluated myeloperoxidase (MPO), lysozyme and lactoferrin activity in relationship to periodontal disease. Following periodontal therapy, these values declined and approached those observed in healthy controls.

e) PEROXIDASE-

Salivary peroxidase activity significantly increases with inflammation and reduces after oral hygiene measures⁵.

f) Kallikrein-

Kallikrein hydrolyzes kininogen to release vasoactive kinin peptide such as bradykinin⁶. These peptides are important mediators of inflammation.

IMMUNOGLOBULINS

The predominant immunoglobulin in saliva is secretory IgA (sIgA) which is derived from plasma cells in the salivary glands. sIgA maintains homeostasis in the oral cavity. sIgA controls the oral microbiota by reducing the adherence of bacterial cells to the oral mucosa and teeth⁷.

Immunoglobulin Isotypes in Saliva

sIgA is actively secreted by the salivary glands, therefore studies have attempted to determine if a relationship exists between salivary levels of sIgA and periodontal status.

Guyen et al. (1982) reported that higher levels of IgA were present in whole saliva collected from patients with gingivitis and periodontitis. **Sandholm et al. (1984)** evaluated that Salivary IgA, IgG, and IgM levels were higher in the JP patients as compared with the healthy siblings and controls. **Bokor et al. (1997)** reported that the concentration of IgA in mixed unstimulated saliva was lower in subjects with more gingival inflammation. **Reiff et al. (1984)** examined changes in the concentration of immunoglobulins in saliva following the treatment. A decrease in salivary levels of both IgA and IgG was observed after periodontal therapy.

Myint et al. (1997) assessed levels of IgA to bacteria in dental plaque in parotid saliva from HIV+ and HIV- subjects with healthy gingiva, chronic gingivitis, chronic marginal periodontitis and necrotizing ulcerative periodontitis. When the HIV+ group was compared with the HIV- group, regardless of periodontal status, total salivary IgA concentration was higher in the HIV+ patients. It was thus concluded that salivary IgA response to bacteria in dental plaque may have been influenced by acute periodontal infection.

Specific Immunoglobulins in Saliva

Specific immunoglobulins in saliva directed towards periodontal pathogens have also been examined for their diagnostic potential.

Eggert et al. (1987) reported that saliva from treated periodontitis patients had higher IgA and IgG levels than did saliva from control subjects. **Tynelius-Barthall & Ellen (1985)** found similar trends measured changes in salivary antibodies to *Actinomyces viscosus* and *Actinomyces naeslundii* in 6 patients with gingivitis prior to and after treat-

ment. **Anil et al. (1995)** reported higher IgA and IgG levels in Non Insulin Dependent Diabetes Mellitus patients as compared to non diabetic patients. This can be attributed to an altered immune response found in diabetic due to greater antigenic challenge present.

Neiminen et al. (1993) reported that concentration of specific IgA and IgG to *A. actinomycetemcomitans* in saliva of patients with advanced periodontitis correlated significantly with corresponding antibody titres in serum of these patients.

OTHER PROTEINS

1) Fibronectin

It is a glycoprotein which mediates adhesion between cells and is also involved in chemotaxis, migration, inflammation and wound healing and tissue repair. Fibronectin levels in saliva do not differentiate between periodontal health and disease⁸.

2) Cyseine proteinases

These are proteolytic enzymes originating pathogenic bacteria, inflammatory cells, osteoclasts and fibroblasts. These enzymes have collagenolytic activity which may cause tissue destruction⁹.

Henskens et al. (1996) evaluated the change in concentration and activity of salivary cystatins in whole and parotid saliva from 20 periodontitis patients undergoing treatment. After periodontal treatment, total cystatin and cystatin C concentration decreased to control levels.

ACTIVATING FACTOR (PAF)

It is potent phospholipid mediator of inflammation which stimulates the activity of production of platelets.

Garito et al. (1995) studied PAF in whole saliva collected from 69 subjects found a significant positive correlation between the level of PAF in saliva and measures of periodontal inflammation. **Rasch et al. (1995)** conducted a longitudinal evaluation of the effect of periodontal therapy (oral hygiene instruction, prophylaxis and scaling/root planning) on salivary PAF levels in 15 chronic Adult periodontitis patients. These levels declined further following Scaling and root planning. It was therefore concluded that PAF might participate in inflammatory events during periodontal tissue injury and disease.

EPIDERMAL GROWTH FACTORS (EGF)

Involved in wound healing and functions with hormone like properties to stimulate epithelial cells. In humans, the parotid gland is a major source of EGF⁹. **Oxford et al. (1998)** found a transient increase in salivary EGF levels in response to periodontal surgery. **Hormia et al. (1993)** observed higher EGF secretion rates in unstimulated and stimulated saliva in 17 Juvenile periodontitis patients as compared with healthy age and gender matched controls.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

It is also known as permeability factor or vasculoprotein. It is a multifunctional angiogenic cytokine important in inflammation and wound healing. This cytokine was found to be a component of whole saliva¹⁰.

INFLAMMATORY CELLS

The number of leukocytes in saliva varies from person to person, and cell counts vary for an individual during the course of the day. The majority of salivary leukocytes enter the oral cavity via the gingival crevice¹².

Klinkhammer et al. (1968) standardized collection and counting of leukocytes in saliva and developed the Oroganulocytic Migratory Rate (OMR). In an experimental gingivitis model, the number of granulocytes in saliva increased before the appearance of clinical gingivitis^{13, 14}. **Raeste et al. (1978)** studied that OMR was determined with sequentialal mouthrinse sampling in periodontitis patients and controls. The results indicated that the OMR reflects the presence of oral inflammation.

SALIVARY IONS

Calcium ion is most intensely studied as a periodontal marker for periodontal disease in saliva. A high concentration of Calcium was correlated with good dental health in young adults, but no relationship was detected with periodontal bone loss as measured from dental radiographs¹⁵. **Sewon et al. (1990 & 1995)** demonstrated that salivary Calcium and the saliva Calcium to phosphate ratio were higher in periodontitis-affected subjects in comparison to healthy controls. He also examined a higher concentration of Calcium in whole stimulated saliva from the periodontitis patients.

SERUM MARKERS-CORTISOL

Emotional stress has been established as a risk factor for periodontitis^{16,17,18,19}. One mechanism proposed to account for the relationship is that elevated serum cortisol levels associated with emotional stress exert a strong inhibitory effect on the inflammatory process and immune response^{20, 21, 22}. Recently, salivary cortisol levels were used to evaluate the role of emotional stress in periodontal disease. Higher salivary cortisol levels were detected in individuals exhibiting severe periodontitis, a high level of financial strain, and high emotion-focused coping, as compared to individuals with little or no periodontal disease, low financial strain, and low levels of emotion-focused coping²³.

BACTERIA

Microorganisms in dental plaque can survive in saliva, and can utilize salivary components as a substrate. It was shown that saliva could serve as a growth medium for oral *Streptococcus* species and *A. viscosus*²⁴.

Asikainen et al. (1991) recovered actinomycetemcomitans from subgingival sites, it was also found in 69.9% and 35.9% of the samples of stimulated and unstimulated saliva, respectively.

Umeda et al. (1998) examined the presence of *P. gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens* and *T. denticola* in whole saliva and in periodontal pocket samples.

Schaeken et al. (1987) examined the effect of plaque accumulation on the salivary counts of some dental plaque microorganisms in 20 subjects who refrained from oral hygiene for 7 days. The large increase in the number of bacteria on the teeth was reflected by an increase in salivary counts of *Actinomyces* species. A highly significant correlation was found between *S. mutans* level in dental plaque and the salivary level of these microorganisms. **Rosenberg et al. (1989)** described a microbial rinse test (Oratest). In this study Oratest was found to be a simple method for estimating oral microbial levels. In a companion study²⁵, Oratest results were correlated with plaque index and gingival index scores, and the authors stated that this test provides a reliable estimate of gingival inflammation.

VOLATILES

Volatile sulphur compounds, primarily hydrogen sulfide and methylmercaptan, are associated with oral malodor²⁶.²⁷. Salivary volatiles have been suggested as possible diagnostic markers and contributory factors in periodontal disease. Pyridine and picolines were found only in subjects with moderate to severe periodontitis^{28, 29}. Furthermore, saliva seems to be a useful medium to evaluate oral malodor. A significant association between the BANA scores from saliva and oral malodor was found³⁰.

CONCLUSION

Saliva is a complex fluid which serves many important functions in the oral cavity that also contributes to the quality of life of an individual. Research on saliva has seen a paradigm shift over the years. In the earlier part of the century, attention was focused on studying the quantitative properties of saliva. As newer methods of analysis developed, the focus duly shifted to the analysis of the composition of saliva. In the recent past, efforts have been directed towards better understanding of the potential of saliva as an aid in the diagnosis of several diseases where presence/absence or a change of one or more of its constituents can act as a marker. Until just recently, dentistry seemed to have had a monopoly on saliva, with some overlap from health professionals dealing with procedures that resulted in decreased or total lack of salivary flow.

Whole saliva samples contains desquamated epithelial cells from oral mucosa can be extracted and used for gene characterization may be utilized for gene mapping for identification of systemic disease polymorphism that will give a further insight into pathophysiology of disease. The combination of identification of disease gene polymorphism and understanding the expression of genes will be an important tool for new therapies not only for salivary glands disease but systemic disease as it is said- Mouth is a mirror of the systemic diseases.

REFERENCE

- Polson A. M., Goodson J. M. Periodontal diagnosis-current status and future need. *J Periodontol* 1985; 56:25-34. | 2) Kornman K. S., Crane A., Wang H. Y., Di Giovine, F. S., Newman M. G., Pirk FW, Wilson T. G. Jr, Higginbottom FL, Duff, G. W. The interleukin-1 genotype as a severity factor in adult periodontal disease. *J Clin Periodontol* 1997; 24: 72-77. | 3) Chauncey, H. H. Salivary enzymes. *J Am Dent Assoc* 1961; 63: 361-369. | 4) Ingman T., Sorsa T., Kontinen Y. T., Liede K., Saari H., Lindy O., Suomalainen K. Salivary collagenase, elastase and trypsin like proteases as biochemical markers of periodontal tissue destruction in adult and localized juvenile periodontitis. *Oral Microbiol Immunol* 1993; 8:298-305. | 5) Smith A. J., Smith G., Basu M. K., Walsh T. F. Changes in salivary peroxidase activity observed during experimentally- induced gingivitis. *J Clin Periodontol* 1984; 11, 373-378. | 6) Fujimoto Y., Moriya H., Moriwaki, C. Studies on human salivary kallikrein. *J Biochem*1973;74: 239-246. | 7) Marcotte H., Lavoie M.C. Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiology and Molecular Biology Reviews* 1998;62:71-109. | 8) Lamberts B. L., Pederson, E. D., Bial, J. J. & Tombasco, P. K. (1989) Fibronectin levels of unstimulated saliva from naval recruits with and without chronic inflammatory periodontal disease. *J Clin Periodontol* 16, 342-346. | 9) Cutler C. W., Kalmr J. R., Genco C. A. Pathogenic strategies of the oral anaerobe, p.gingivalis. *Trends in Microbiology* 1995;3: 45-51. | 10) Thesleff I., Viinikka L., Saxen L., Lehtonen E., Perheentupa J. The Parotid gland is the main source of human epidermal growth factor. *Life Sciences* 1988; 43: 13-18. | 11) Taichman N. S., Cruchley A. T., Fletcher L.M., Hagi-Pavli E. P., Paleolog E. M., Abrams W. R., Booth V., Edwards R. M., Malamud D. Vascular endothelial growth factor in normal human salivary glands and saliva: a possible role in the maintenance of mucosal homeostasis. *Laboratory Investigation* 1998; 78: 869-875. | 12) Schiott R C., Loe H. The origin and variation in number of leukocytes in the human saliva. *J Periodontal Res* 1970;5: 36-41. | 13) Friedman L. A., Klinkhammer J. M. Experimental human gingivitis. *J Periodontol*1971; 42: 702-705. | 14) Skougaard M. R., Bay I., Klinkhammer J. M. Correlation between gingivitis and orogranulocyte migratory rate. *J Dent Res* 1969; 48: 716-718. | 15) Sewon L., Makela M. A study of the possible correlation of high salivary calcium levels with periodontal and dental Saliva and periodontal diagnosis 465 conditions in young adults. *Arch Oral Bio* 1990;35: 211-212. | 16) Breivik T., Thrane P. S., Murison R., Gjermo P. Emotional stress effects Non immunity,gingivitis and periodontitis. *Eur J Oral Sci* 1996; 104: 327-334. | 17) Genco R. J., Current view of risk factors for periodontal diseases. *J Periodontol*. 1996; 67: 1041-1049. | 18) Linden G. J., Mullally B. H., Freeman R. Stress and the progression of periodontal disease. *J Clin Periodontol* 1996; 23: 675-680. | 19) Axtelius B., Soderfeldt B., Nilsson A., Edwardsson S., Attstrom R. Therapy- resistant periodontitis. Psychosocial characteristics. *J Clin Periodontol* 1998; 62: 482-491. | 20) Chrousos G. P., Gold, P. W. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *J Amer Med Assc* 1992; 267: 1244-1252. | 21) Johnson E. O., Kamilaris T. C., Chrousos G. P., Gold P. W. Mechanisms of stress: A dynamic overview of hormonal and behavioral homeostasis. *Neuroscience and Biobehavioral Review* 1992; 16: 115-130. | 22) Fantuzzi G., Ghezzi, P. Glucocorticoids as cytokine inhibitors: role in neuroendocrine control and therapy of inflammatory diseases. *Mediators of inflammation* 1993; 2: 263-270. | 23) Genco R. J., Ho A. W., Kopman J., Grossi S. G., Dunford R. G., Tedesco L. A. Models to evaluate the role of stress in periodontal disease. | *Ann Periodontol* 1998; 3, 288-302. | 24) De Jong M. H., Van der Hoeven J. S., Van Os J. H., Olijve J. H. Growth of oral streptococcus species and actinomyces viscosus in human saliva. *Applied and Environmental Microbiology* 1984; 47, 901-904. | 25) Tal H., Rosenberg M. Estimation of dental plaque levels and gingival inflammation using a simple oral rinse technique. *J Periodontol* 1990; 61: 339-342. | 26) Rosenberg M., McCulloch C. A. G. Measurement of oral malodor: current methods and future prospects. *J Periodontol* 1992; 63, 776-782. | 27) Tonzetich J. Production and origin of oral malodor: A review of mechanisms and methods of analysis. *J Periodontol* 48:13-20. | 28) Kostelc J. G., Preti G., Zelson P. R., Stoller N. H., Tonzetich J. Salivary volatiles as indicators of periodontitis. *J Periodontal Res*1980; 15: 185-192. | 29) Kostelc J. G., Zelson P. R., Preti G., Tonzetich J. Quantitative differences in volatiles from healthy mouths and mouths with periodontitis. *Clin Chem* 1981; 27: 842-845. | 30) Kozlovsky A., Gordon D., Gelernter, I., Loesch W. J., Rosenberg, M. Correlation between the BANA test and oral malodor parameters. *J Dent Res* 1994; 73: 1036-1042 |