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Role of Proteomic Biomarkers in Periodontal Diseases - A Review

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Abstract **Review Article**

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 premalignant and malignant oropharyngeal lesions as well as infectious diseases of the oral cavity can be performed by analyzing an array of constituents within saliva. Although there is a large body of literature on gingival crevicular fluid biomarkers, this review limits itself to saliva analysis. 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), genetic Biomarkers such as DNA and mRNA of host origin, bacteria and bacterial products, ions, steroid hormones and volatile compounds. This review highlights recent advances in the development of salivary proteomic, genomic and microbial biomarkers for periodontal diagnosis, and focus on potential applications and advancements to stimulate additional research.

Keywords: Biomarkers, proteomic, periodontal diseases, salivary markers.

INTRODUCTION

Periodontitis is a group of inflammatory diseases that affect the connective tissue attachment and supporting bone around the teeth whose initiation and progression depend on the presence of virulent microorganisms capable of causing disease [1]. It has been a great challenge in periodontology to determine biomarkers for screening and predicting the early onset of disease (prognostic tests) or evaluating the disease activity and the efficacy of therapy (diagnostic tests) [2]. A biomarker is a substance used to indicate a biological state and is an objective measure to evaluate the present and future disease activity. It is defined as – A substance that is measured objectively and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Various biological media like saliva, serum, and gingival crevicular fluid are used to determine biomarkers in periodontal health and disease. A single biomarker will not be able to predict periodontal disease activity and severity. So combinations of biomarkers are used to predict the disease activity [3].

NEED FOR BIOMARKERS

Current clinical diagnostic parameters that were introduced more than 50 years ago continue to function as the basic model for periodontal diagnosis in clinical practice today. They include probing pocket depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs that quantify alveolar bone levels [4]. Under diagnosis of periodontal therapy leads to failure of periodontal treatment. For that researchers phrased biomarkers that indicated the presence or absence of periodontal disease [5]. Optimal innovative approaches would correctly determine the presence of current disease activity, predict sites vulnerable for future breakdown, and assess the response to periodontal interventions. A new paradigm for periodontal diagnosis would ultimately affect improved clinical management of periodontal patients.

Advantages of traditional diagnostic techniques: Easy to use, Cost effective, Non invasive, Measures disease severity. Limitations of traditional periodontal diagnostic techniques: All clinical diagnostic techniques provide information about past disease

activity and are unable to diagnose present disease activity.

CLASSIFICATION OF BIOMARKERS

The importance of saliva to our everyday activities and the inherent medicinal values it possesses is often taken for granted. When disruptions in the quality or quantity of saliva occur, there will often be detrimental effects on oral and systemic health. Saliva is a mirror of oral health and also a reservoir of analytes from systemic sources that reach the oral cavity through various passageways [6]. Ancient doctors of traditional Chinese medicine considered saliva and blood to be 'sibling biofluids' that come from the same origin. It is believed that changes in saliva are indicative of the wellness of the individual.

The potential diagnostic importance of gingival fluid was recognized more than six decades ago and serious investigations of the dynamics of GCF production began with the epoch making reports of Brill and co-workers in 1950's. Gingival crevicular fluid (GCF) has been employed in the analysis of periodontitis, taking into account indicators and markers of connective tissue and bone destruction therefore has often been used as a useful indicator in determining the severity of periodontal diseases.

Curtis *et al.,* [6]. stated that "markers of disease" might encompass three separate categories:

1) Indicators of current disease activity;
2) Predictors of future disease progression;
3) Predictors of future disease initiation at currently healthy sites.

Over 65 components have been examined as possible markers for progression of periodontitis. These components fall into three general categories:

1. Host derived enzymes and their inhibitors
2. Inflammatory Mediators and host response modifiers
3. Tissue breakdown products

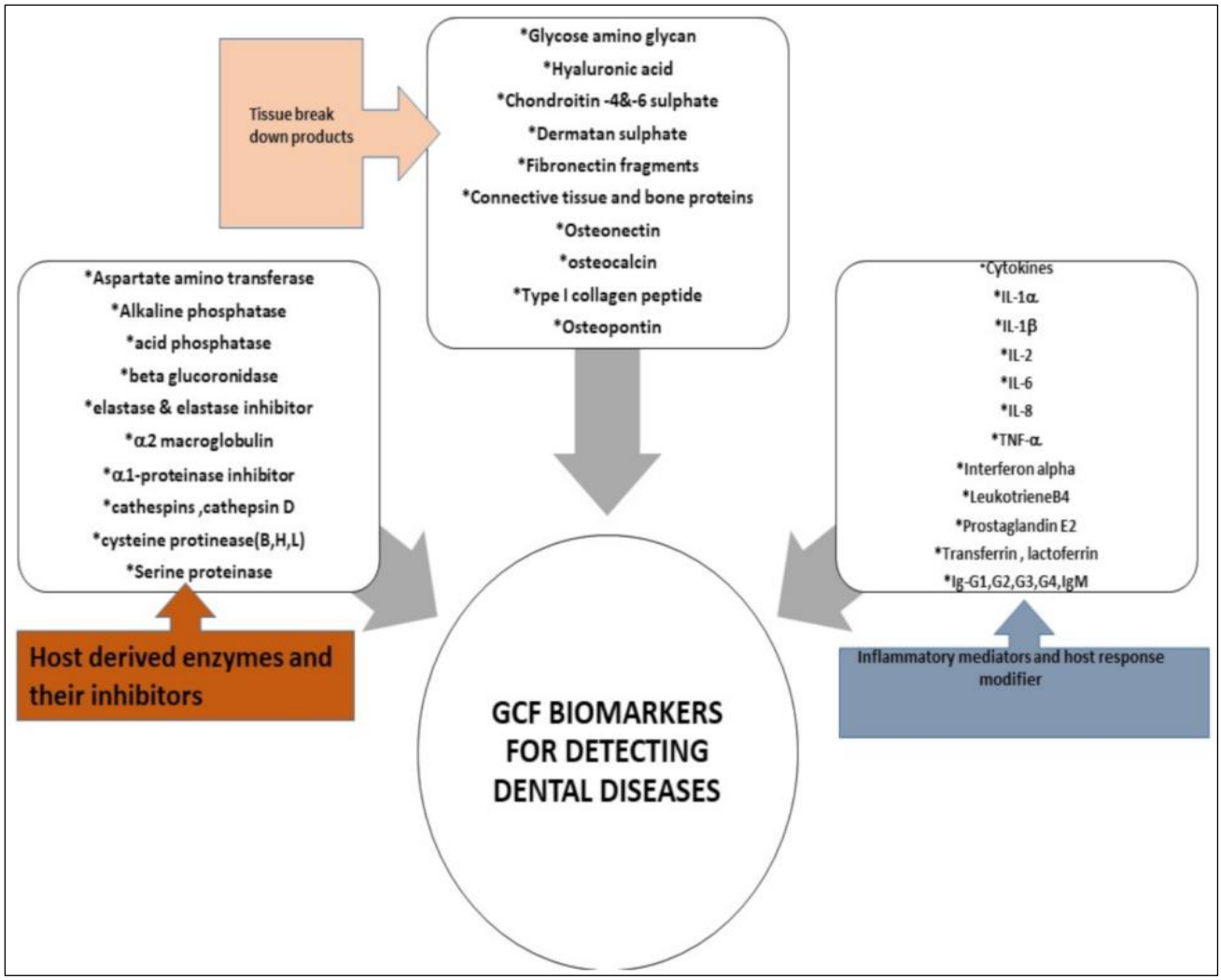

Table 1: Classification of Biomarkers

Proteomic biomarkers	**Genetic biomarkers**	**Microbial biomarkers**	**Other biomarkers**
Cystatins,αglucosidase,	Cathepsin Cgene	*Aggregatibacter actinomycetemcomitans,*	Calcium,
Acid phosphatase,	Mutation,	*Campylobacter rectus, Mycoplasmas,*	Cortisol,
Alkalinephosphatase, Aminopeptidase, Lactoferrin, Translactoferin, IgM, MMP-	Collagen gene mutation,	*Porphyromonas gingivalis,*	Hydrogensu lphide,
13, MMP-8, MMP-9, Cathepsin B, Osteonectin, Osteocalcin, Osteopontin, Elastase	IL-1 polymorphisms,	*Prevotella intermedia, Peptostreptococcus*	Methylmerc aptan,
Platelet-activating factor, Epidermal growth factor, Platelet-derived growth factor,	IL-10 polymorphisms,	*Micros,*	Pyridine.
Esterase,	Tumor necrosis factor,	*Prevotella nigrescens,*	
Pyridinoline crosslinked carboxy –terminal telopeptide,	Polymorphisms.	*Treponema denticola,*	
Fibronectin, sIgA (secretory IgA) Gelatinase, IgA, Trypsin, Vascular endothelial		*Tannerella forsythia.*	
growth factor, IgG		*Treponema socransky.*	

Proteomic biomarkers:

In periodontology, proteomes – the complete protein pool of an organism, are vital for understanding periodontal ligament physiology and regulation and to diagnose disease related protein markers. Proteomics– the large scale analysis of proteins– has become one of the most important disciplines for gene function characterization, building functional linkages between protein molecules, and to comprehend the mechanisms of biological processes. ―PROTEOMICS is a relatively new field; and is defined as the total protein content of a cell or that of an organism. The word "proteome" is a blend of "protein" and "genome", and was coined by Marc Wilkins. The proteome is the entire complement of proteins, including the modifications of a particular set of proteins. Proteomics offers a new approach to the understanding the changes occurring as oral micro-organisms adapt to environmental change within their habitats in the mouth[7]. Proteomic studies analyses the structure and function of various proteins and the protein-protein synergism of an organism. Any minor defects either in protein structure, its function or alteration in expression pattern can be detected using proteomic techniques. Recent progress in tissue isolation, protein separation, quantification, sequence analysis and structural interaction using proteomic techniques offers great promise for bringing about a change in periodontal physiology and pathology into the modern era. The following flow chart illustrates the major steps from the separation of the fractionated proteins till the determination of its sequence analysis.

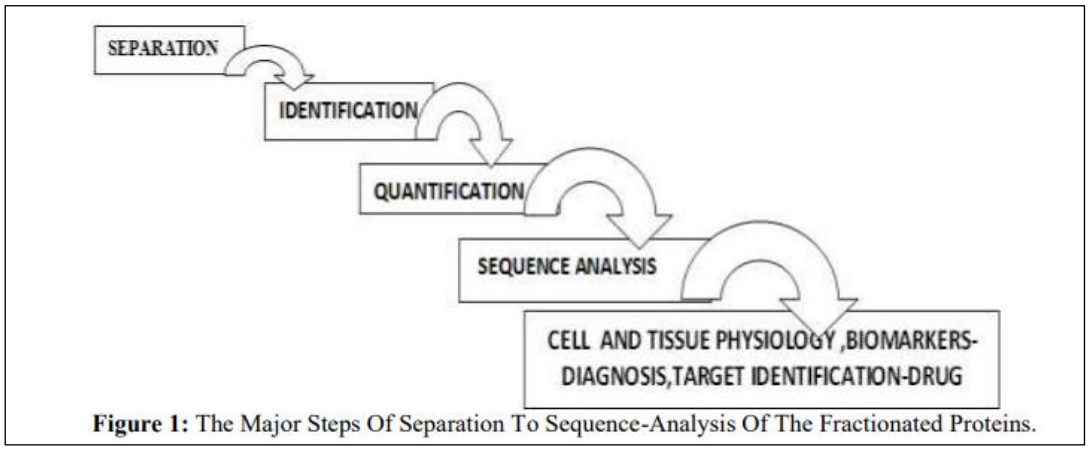

Figure 1: The Major Steps Of Separation To Sequence-Analysis Of The Fractionated Proteins.

Rationale of periodontics: Apart from analysing the structure and function of biological systems, the objective of proteomics is to analyse the varying proteomes of an organism at different times, in order to highlight the differences between them. Among the many objectives of proteomics, the discovery of novel protein biomarkers has been a major driving force in the development of proteome characterization methodologies.

Proteomics and dentistry: Proteomics is a relatively new postgenomic science with tremendous potential and

has become one of the most important research frontiers in modern dentistry. Proteomics has quickly become one of the most exciting research frontiers in modern dentistry. The two primary areas which dental proteomics have really shown are salivary diagnostics i.e. oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel . Human saliva contains proteins that can be informative for disease detection and surveillance of oral health. Comprehensive analysis and identification of the proteomic contents in human whole and ductal saliva is a necessary first step toward the discovery of saliva protein markers for human disease detection in particular for oral cancer and Sjogren's syndrome.

Proteomics in periodontal ligament fibroblasts: To understand PDL physiology and disease related protein markers, the analysis of the entire complement of PDL fibroblast proteome is of utmost importance. Although immunological methods have been tried to study the protein expression of PDL fibroblast, but this is limited to only previously identified proteins for which antibodies are available. Characterization of periodontal ligament (PDL) fibroblast proteome is an important tool for understanding PDL physiology and regulation and for identifying disease-related protein markers. PDL fibroblast protein expression has been studied using immunological methods, although this technique is limited to previously identified proteins for which specific antibodies are available. A total of 117 proteins have been identified from PDL fibroblasts which can serve as a reference map for future clinical studies as well as basic research [8].

The tissue and cell complexity in the periodontium require the submission of more global experimental approaches for determining expression profiles. Proteomic armamentarium contains a broad array of technical approaches. For analysis of dissected periodontal tissues, sections through periodontium or cultured periodontal cells, fractionation of cells, and matrix followed by protein separation are the initial steps to proteomic study. The enumerated methods of proteome analysis are below. (a) ELISA (Enzyme Linked Immunosorbent Assay) which is a tried and tested method for isolation and quantification of protein. (b) In 1995, Randall Nelson pioneered the use of immunoassays with mass spectrometry (MSIA). To determine the set of proteins that have undergone posttranslational modification, antibodies can be developed which are specific to the modifications and can only recognize certain proteins. (c) Recently, another approach has been developed called PROTOMAP (Protein Topography and Migration Analysis Platform) which combines Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDSPAGE) with shotgun proteomics to enable detection of changes in gel migration such as those caused by proteolysis or posttranslational modification. (d) More recent techniques such as matrix-assisted laser desorption/ionization have been employed for rapid determination of proteins in particular mixtures. (e) For analysis of complex protein mixtures derived from biological samples, two-dimensional polyacrylamide gel electrophoresis remains an important technology. (f) Nongel based proteome separation techniques to overcome the limitations of two-dimensional electrophoresis while preserving the ability to resolve complex protein and peptide mixtures before mass spectrometry analysis were developed. (g) Capillary electrophoresis is an alternative to both two dimensional electrophoresis for protein separation and to chromatography for peptide separation [9].

Proteomic biomarkers: Periodontal disease is a bacteria-induced chronic inflammatory disease affecting the soft and hard supporting structures encompassing the teeth. Assessment of periodontal disease is based on clinical parameters and radiographs. Though efficient, these traditional techniques are limited, as the current status of the disease alone can be determined. They do not have the capacity to diagnose susceptible patients who are at risk for disease progression. Development in the use of oral fluids as biological samples for measuring the present disease state, has made saliva and other oral-based fluids the vanguard of technology. Oral fluids comprises of local and systemic mediators of periodontal disease, which comprises of microbial, host response, and markers specific for bone resorption. Though most biomarkers in oral fluids exhibit inflammatory mediators, specific collagen downgrading and bone turn over related molecules have become apparent measures of periodontal disease. In oral diagnostics, it has been a great challenge to determine biomarkers for screening and predicting the early onset of disease or evaluating the disease activity and the efficacy of therapy. An oral diagnostic tool should provide relevant information for differential diagnosis, localization of disease and severity of infection. It should serve as a basis for treatment planning and act as a means of assessing the effectiveness of therapy. Thus keeping these criterias in mind researchers have made an effort to understand the underlying science of the disease, with an aim to find improved ways to diagnose and treat the disease before any serious outcome. Human saliva in oral diagnostics is of great importance and researchers are concentrating on it. Saliva is considered as an important Periodontal diagnostic tool since variable amounts of blood, serum and its products, GCF, epithelial cells as well as immune cells, microorganisms, products obtained from bacterial degradation, lipopolyscaaharides, bronchial products and other foreign substances are present in whole saliva. Saliva, has high potential for the surveillance of general health and disease. It represents a promising diagnostic fluid for the screening of various oral diseases. It is a fluid that comprises of constituents of exocrine glands and gingival crevicular fluid (GCF). Moreover, saliva is effortlessly available and easily collected without any cumbersome procedure. Matrix Metalloproteinases (MMP 2 and 9), Immunoglobulin (Ig), Esterases,

Lysozyme, Lactoferrin levels in saliva are valuable for predicting the progression of periodontitis. Cytokines like C- reactive protein, pentraxin-3, TNF, and other interleukins which are involved in disease pathogenesis are essential for diagnosing periodontal diseases. Apart from this, numerous proteomic markers, like acid phosphatase, alkaline phosphatase, histatins, kallikreins, cystatins, kininogens, aminopeptidases, glucosidase, galactosidase and glucuronidase, and various bone remodelling proteins (Osteopontin, Osteonectin, Osteocalcin) are noted in the diagnosis of periodontal disease. Apart from periodontal diseases, salivary defence systems which comprises of salivary proteins play a significant and vital role in maintaining the health of the oral cavity and preventing caries. Significant amount of salivary phosphopeptides (PRP1/3, histatin-1 and statherin) were associated with the absence of dental caries, affirming the value of these peptides in nurturing tooth integrity.

Proteomics and Periodontal Pathogens: The oral environment contains various colonies of microorganisms comprising of bacteria, fungi, protozoa, and viruses. Oral ecology studies have reviewed the complexity of the interactions that these microorganisms have with their host in both health and disease. In spite of this, dental caries and periodontal diseases are still worldwide oral diseases, resulting in a high level of morbidity among humans. Proteomics offers a new approach to comprehending these holistic changes, as oral micro-organisms adapt to environmental change within their habitats in the mouth. Various microorganisms are present in subgingival plaque, of which only few play a etiological role in the pathogenesis of periodontal diseases in the susceptible host. Specific microorganisms involved in the periodontal pathogenesis are Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola, and Aggregatibacter actinomycetemcomitans. BANA activity (benzoylDLargininenaphthylamide) was exhibited by the members of the ―red complex of periodontal pathogens (T. forsythia, P. gingivalis, and T. denticola) are strongly correlated with periodontal activity.

Proteomics and Tissue Engineering: Tissue engineering has evolved in recent years, as efficient means for treating various pathological conditions. This scientific knowledge mainly constitutes stem cell procurement, storage, differentiation as well as transplantation which is performed by using specific biomarkers such as proteins. However, the absence of knowledge regarding these prospective markers for stem cells and their specific differentiation remains a considerable limitation for these applications. In future, the proteomic and transcriptomic analyses may pave way to obtain new and hopefully fundamental insights into the protein expression, and cellular biology of mesenchymal stem cell.

Salivary Proteomics for Periodontitis: Saliva is considered as an important Periodontal diagnostic tool since variable amounts of blood, serum, serum products, GCF, electrolytes, epithelial and immune cells, microorganisms, bacterial degradation products, lipopolyscaaharides, bronchial products and other foreign substances are present in whole saliva[10]. Matrix Metalloproteinases (MMP 2, 39), Immunoglobulin (Ig), Esterases, Lysozyme, Lactoferrin levels in saliva are valuable for predicting the progression of periodontitis. Numerous other salivary proteases have also been used as diagnostics biomarkers. Various cytokines like C-reactive protein, pentraxin-3, TNF, various other interleukins which are involved in its pathogenesis have come handy in diagnosing periodontal diseases. Young-J in Choi et al studied the GCF in healthy individuals and Periodontitis patients to study biomarkers. He identified azurocidin in the GCF, but not in the saliva, as an upregulated protein in the periodontitis patients. He concluded that azurocidin could be a potential biomarker candidate for the early detection of inflammatory periodontal destruction by gingivitis and some chronic periodontitis. Azurocidin may have an inhibitory role in osteoclast differentiation and, thus, a protective role in alveolar bone loss during the early stages of periodontitis. Melissa M. Grant et al studied the 21-day experimental gingivitis model. The model was designed to enable the study of both the induction and resolution of inflammation. Across the course of experimentally induced gingivitis, He identified 16 bacterial and 186 human proteins. Although abundances of the bacterial proteins identified did not vary temporally, Fusobacterium outer membrane proteins were detected. Proteomics And Stem Cell Research: Large scale mesenchymal stem (MSC) cell proteome analyses have been emphasized in recent MSC research. A review by Hye Won park presents an expandable list of MSC proteins which will function as a starting point for the generation of a comprehensive reference map of their proteome. This proteomic and transcriptomic analyses may allow us to obtain new and hopefully fundamental insights into the protein expression, regulation, and cellular biology of MSC. Clinical Applications: The use of rapid point-of-care oral diagnostics will greatly advance periodontal surveillance and disease diagnosis over the coming years. Novel technologies such as lab-on-a-chip and microfluidic devices have the potential to manage complex oral fluids such as saliva and gingival crevicular fluid, and helps in determining the patient's periodontal disease-risk profile, current disease activity and response to therapeutic interventions. This approach should acts like a catalyst for clinical decision making and monitoring of episodic disease progression in a chronic infectious disease such as periodontitis. While the future of periodontal disease diagnosis using salivary diagnostics looks encouraging, hurdles to these approaches may be encountered in the clinical setting. These novel periodontal diagnostic technologies need to be validated & benchmarked with existing gold

standards of disease, such as alveolar bone levels and clinical attachment levels, in large patient populations.

The Future of Proteomics: The two main research frontiers for application of proteomics in dentistry are salivary diagnostics, or oral fluid biomarkers, and proteomics of bone and enamel. While saliva is accessible and its collection is totally noninvasive, its use in clinical diagnostics has only recently been demonstrated. One team of researchers at UCLA, and others, has shown that oral fluid harbors the same composition of disease biomarkers as blood, but in smaller quantities. These scientists have developed, with support of the National Institute of Dental and Craniofacial Research, a molecular sensor that provides the basis for future development of the "Oral Fluid Nano Sensor Test (OFNASET)." OFNASET is predicted to be a handheld and easy-to-use instrument that clinicians can use to rapidly detect complex salivary protein and nucleic acid targets. The result will be the ability to clinically detect oral cancer before oral signs and symptoms.

Limitations

As protein expression and post-translational modifications are dynamic processes, particularly in the periodontium, identification and quantification of proteins alone are not sufficient to understand functional changes. New technologies will be needed to enable combinations of metabolic labeling and identification as well as quantification and measurement of synthesis rates. Also, Proteomics experiments conducted in one laboratory are not easily reproduced in another.

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