



## PROTEOMICS: A NEW DIMENSION TO DIAGNOSIS OF PERIODONTAL DISEASE

<b>Dr. Rizwan Sanadi</b>	Professor, Department of Periodontics, Y.M.T Dental College and Hospital.
<b>Dr. Kalyani Ramteke</b>	PG student 2 <sup>nd</sup> year, Department of Periodontics, Y.M.T Dental College and Hospital.
<b>Dr. Susmita Bhakkand</b>	PG student 1 <sup>st</sup> year, Department of Periodontics, Y.M.T Dental College and Hospital.

**ABSTRACT** Periodontitis is the result of complex interrelationship between infectious agents and host factors. Proteins are vital parts of living organisms as they are integral components of physiological metabolic pathways of cells. Various protein molecules determine the onset, progression and severity of periodontal disease. The study of proteins as biomarkers in periodontal diseases has got increased attention during the last few years. The proteins involved in pathogenesis of periodontal disease can be used as biomarkers. The knowledge of various proteins involved in periodontal disease pathogenesis can be used in the diagnosis, prevention and treatment of periodontal disease.

**KEYWORDS :** Proteins, Periodontitis, Biomarkers

### Introduction:

Proteins are vital parts of living organisms, as they are integral components of the physiological metabolic pathways of cells. **Marc Wilkins**, an Australian geneticist in 1996 created the word "proteome" which was blend of 'protein' and 'genome'. Proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system.

The term "proteomics" was first coined in the year 1997 by **James** to make analogy with genomics, the study of genes. Proteomics is the study of all proteins including their relative abundance, distribution, posttranslational modification, functions and interactions with other macromolecules, in a given cell or organism within a given environment and at a specific stage in the cell cycle.<sup>1</sup>

The two primary areas which dental proteomics have really shown promising outcomes are salivary diagnostics and gingival crevicular fluid (GCF) diagnostics that is oral fluid diagnostics or oral fluid biomarkers. Human saliva contains proteins that can be informative for disease detection and surveillance of oral health.

### Major differences between genomics and proteomics<sup>2</sup>:

GENOMICS	PROTEOMICS
1. Genomics is the study of genome of an organism.	1. Proteomics is the study of proteome of an organism.
2. Genomics includes mapping, sequencing and analysis of genome.	2. Proteomics include characterization of all protein of an organism or study of structure and function of proteins.
3. Genomics can be broadly classified into: · Structural genomics: is the study of the structure of all gene and its relative position on the chromosomes. · Functional genomics: study of function of all genes or the role of these genes regulating metabolic activities of the cell.	3. Proteomics can be classified into: · Structural proteomics: is the study of the structure of proteins and their location in the cell. · Functional proteomics: study of function of all proteins which primarily include protein-protein interaction and interaction of proteins with other biomolecules. · Expression proteomics: is the study of identification and quantification or expression level of proteins of the cell at different developmental stages or at different environmental condition.

4. Techniques in genomics include Gene sequencing strategies like directed gene sequencing, whole genome short gun sequencing. · Construction of ESTs (Expressed Sequence Tags), · Identification of single nucleotide polymorphisms.(SNPs) · Analysis and interpretation of sequenced data using different databases and software.	4. Techniques in proteomics include Protein extraction, electrophoretic separation, digestion of separated proteins into small fragments using trypsin. Mass spectroscopy to find out amino acid sequences and finally protein identification using standard databases. · Protein 3D structures prediction using software. · Protein expression study using protein microarray.
5. Trust areas in Genomics: Genome sequencing project of many organisms including Human Genome Project.	5. Proteome database development like SWISS-2D PAGE and software development for computer aided drug design.

### Types of Proteomics

#### Structural proteomics:

Structural proteomics is based on structural information of total repertoire for three-dimensional images for all proteins in an organism. In any newly sequenced genome, 30% - 50% of gene, encode proteins with unknown molecular or cellular function. This arises from analysis of unknown proteins such as protein-bound ligand or cofactor and is useful for functional description.

Structural genomics attempts to map the total repertoire of protein folds in the hope of providing three-dimensional image for all proteins in an organism and to infer protein functions. Functional and evolutionary protein relations which were not visible at sequence level are now possible with the advent of structural proteomics.

#### Interaction proteomics:

The functions of biological systems are dependent on interactions between their components. These interactions are ultimately determined by genetic elements and selection processes. The sequencing of complete genomes provides information on the proteins responsible for cellular regulation.

The regulation of cell metabolism involves protein interaction domains which regulate the association of polypeptides with each other and with phospholipids, small molecules, or nucleic acids. Several large-scale proteomics technologies have been developed to generate comprehensive, cellular protein-protein interaction maps.<sup>3</sup>

#### Functional proteomics:

Types of proteins that indicate the function of proteins or how they are

assembled into the molecular machines and functional network that regulate cell behavior determine the functional proteomics. It is "focused to monitor and analyse the spatial and temporal properties of the molecular networks and fluxes involved in the living cells". It concentrates on the following two tissues<sup>1</sup>:-

- (I) Elucidation of biological functions of unknown proteins
- (ii) Cellular activity at molecular level.

#### Proteins of periodontium:

Periodontal proteomic markers range from salivary protein markers like Immunoglobulin G to bone remodeling protein markers. These can be specific/nonspecific. Specific markers are immunoglobulins which characterize the presence of chronic or aggressive periodontitis. Among nonspecific markers are enzymes, proteins, mucins, histatin, lactoferrin, lysosomal peroxidase and so forth. In addition, blood, GCF, serum, serum products, electrolytes, microorganisms, epithelial and immune cells, bacterial degradation products, lipopolysaccharides and periodontal fibroblasts can be used for proteome analysis.<sup>5</sup>

#### Possible potential periodontal biomarkers are as follows:<sup>3</sup>

##### 1. Immunoglobulins: (IgA, IgG, Ig M, and sIgA)

Immunoglobulins act as an innate defence mechanism of the periodontium by interfering with adherence and metabolism of bacteria. The concentrations of salivary immunoglobulins (IgA, IgG, and IgM) are specific to periodontal pathogens and they are higher in affected individuals. Screening of saliva (non-invasive technique) is a useful technique, especially for IgA, as it identifies individuals who have the potential to develop periodontal disease.

##### 2. By-Products of Tissue Breakdown: (Collagen Telopeptides, Proteoglycans, Osteocalcin, Fibronectin Fragments, and Bone Collagen Fragments)

Osteocalcin, osteonectin, collagen telopeptidases and bone collagen are proteome biomarkers for bone homeostasis. These are connective tissue derived molecules and are associated with local bone metabolism confined to periodontitis and systemic conditions like osteoporosis or metastatic bone cancers.

##### a) Pyridinoline Cross-Linked Carboxyterminal Telopeptide of Type I Collagen

Pyridinoline, deoxypyridinoline, N-telopeptides, and C-telopeptides are a class of degradation molecules which are released systemically during degradation of collagen matrix and bone resorption, due to post-translational modification of collagen.

It emerged as valuable proteome markers for bone turnover and are very specific for periodontal disease. These markers differentiate the active periodontal or peri-implant bone destruction from latent periodontal disease.

##### b) Osteocalcin

It is the most abundant noncollagenous protein in bone which has specific calcium binding property. It is synthesized mainly by osteoblasts and thus has a dominant role in bone remodelling. **Kunimatsu et al.** in the year 1993 reported "a positive correlation between GCF osteocalcin N-terminal peptide levels and clinical parameters in a cross-sectional study of periodontitis and gingivitis patients. On evaluation of a combination of the biochemical markers osteocalcin, collagenase, prostaglandin E<sub>2</sub>,  $\alpha$ 2-macroglobulin, elastase and alkaline phosphatase, increased diagnostic sensitivity and specificity values of 80% and 91%, respectively, were reported by **Nakashima et al.** in 1996.

##### c) Osteopontin (OPN)

It is a noncollagenous calcium binding glycosylated phosphoprotein in bone matrix and is produced by several cells including osteoblasts, osteoclasts and macrophages. In 2001, **Kido et al.** demonstrated that OPN level in GCF is significantly correlated with progression of periodontal disease.

##### 3. Host Factors

Host response includes monocytes, PMNs, macrophages, IL-1, TNF- $\alpha$ , and PGE<sub>2</sub>. Host cells include immune cells, interleukins and periodontal ligament fibroblasts. Host derived enzymes include matrix metalloproteinases (MMPs), elastase, aspartate aminotransferase, cathepsin B and acid phosphatase.<sup>4</sup>

##### a) Host Cells

Periodontal inflammation occurs in the gingival tissue in response to plaque bacterial biofilms. The cellular components of GCF include 70–80% granulocytes, 10–20% monocytes/macrophages, 5% mast cells and 5% T lymphocytes. Thus the pathophysiological status of the periodontium in a site-specific manner can be assessed by proteome analysis of GCF samples.<sup>3</sup>

##### b) Inflammatory Cells

**Friedman and Klinkhammer** developed the Orogranulocyte Migratory Rate (OMR) by standardized method of collecting and counting leukocytes in saliva. In a study by **Khashu et al.** in the year 1978, the OMR was determined with sequential mouth rinse sampling in periodontitis patients and controls. Results indicated that OMR reflected the presence of oral inflammation and thus this measure could be used as a laboratory test.<sup>4</sup>

##### c) Macrophages

Interleukins and prostaglandins are important inflammatory mediators released by macrophages and PMNs, by the chemo attractant effects of lipopolysaccharides present in bacterial cell wall.<sup>3</sup>

Increasing IL levels increase the risk of periodontal disease by 4-5 fold. A difference of concentration of PGE<sub>2</sub> in GCF was shown in patients with gingivitis and periodontitis (**Offenbecher et al.** 1986)<sup>6</sup>.

##### d) Periodontal ligament (PDL) fibroblast:

Proteome analysis in functioning human PDL fibroblast has been studied and has revealed proteins that will broaden the basis for future understanding of PDL cellular activities in health and disease. The proteomic analysis of the total proteins of PDL cells leads to the identification of 117 proteins that correspond to 74 different gene products, creating a proteome map showing a variety of novel as well as expected proteins.<sup>7</sup>

##### e) Matrix Metalloproteinases (MMPs)

The neutrophils are the major cells responsible for release of MMP and more importantly MMP-8 (collagenase-2) and MMP-9 (gelatinase-B) which is a concern to the periodontist, as it is released during acute stages of periodontal disease. Increased levels of MMPs are seen in peri-implant sulcular fluid from peri-implantitis lesions and hence it can be employed as a biomarker in the active phase of peri-implant disease. Gelatinase (MMP-9) level is higher in GCF of patients with chronic periodontitis. Collagenase-3 or MMP-13 is another collagenolytic MMP which also plays a role in peri-implantitis. A study by **Rai et al.** 2008, showed that MMP-8, MMP-2 and MMP-9 are biomarkers of periodontal disease and aid in early detection of periodontitis or gingivitis.<sup>3</sup>

##### (f) Esterase, Lysozyme, Lactoferrins:

The esterase activity of whole saliva is higher in individuals with periodontal disease. Patients with low levels of lysozyme in saliva are more susceptible to plaque accumulation, which is considered a risk factor for periodontal disease. Lactoferrin is strongly up-regulated in mucosal secretions during gingival inflammation and is detected at a high concentration in saliva of patients with periodontal disease.<sup>8</sup>

(**Kathariya and Pradeep** 2010)

##### (g) Aspartate Aminotransferase Enzyme (AST)

AST is a tissue destruction biomarker released from necrotic cells in GCF and is associated with periodontitis severity.

##### (h) Alkaline Phosphatase (ALP)

**Nakashima et al.** demonstrated that high levels of ALP preceded clinical attachment loss and that the total amount of ALP in GCF was significantly higher in active sites.

##### (I) Microbial factors:

###### Porphyromonas gingivalis:

*Porphyromonas gingivalis* is a periodontal pathogen that resides in a complex multispecies microbial biofilm community known as dental plaque. It is often the dominant species of pathogenic bacteria commonly residing along with streptococcus gordonii and fusobacterium nucleatum.

Whole cell quantitative proteomics, along with mutant construction and analysis were conducted to investigate how *P. gingivalis* adapts to this three species community. The results have confirmed that some

403 proteins were down regulated and 89 proteins were upregulated. (Kuboniwa, Hendrickson, Xia, Wang, Xie, Hackett, Lamont 2009).<sup>2</sup>

A study on proteome analysis of *P. gingivalis*, placed in subcutaneous chamber of mice showed that PG1385 protein is involved in the virulence of these bacteria. (Yoshimura M et al 2008).

#### **Aggregatibacter actinomycetemcomitans**

Virulence factors of *A. actinomycetemcomitans* can be delivered into human cells via outer membrane vesicles (OMVs) or by free-soluble surface components with proinflammatory activity. Abundant production of both OMVs and free-soluble surface material is seen in plaque; they form a significant source of inflammatory stimulants along with the planktonic bacteria in the haemopoietic system.<sup>3</sup>

Lactoferrin interacts with *A. actinomycetemcomitans*, which is a causative microorganism in aggressive periodontitis and its colonization may occur more readily in an environment containing lactoferrin with low iron levels.<sup>4</sup> (Alugupalli et al. 1995)

#### **Conclusion:**

Proteomics can provide comprehensive and systematic information about proteins in a wide array of tissues and organs. Current proteomics analyses have the capacity to provide new insights into the repertoire of expressed proteins and some inkling of their interactions, at a more global level than previously considered<sup>5</sup>. 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. However, its application into the field of dentistry depends on how best oral health care practitioners will incorporate this into their practices as it requires a thorough knowledge of human genetics and application of new diagnostic and therapeutic technologies.<sup>5</sup>

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