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Role of Proteomics in the Diagnosis of Periodontal Diseases

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Abstract

Periodontal disease is considered as a polymicrobial inflammatory disease that damages tissues through complex interaction between bacteria and the host defence system. The onset, progression and severity of periodontal disease are mediated by various protein molecules. Biomarkers are an adjunct to the clinical parameters thereby improving the diagnostic precision in early detection of periodontitis

Keywords: Proteomics, Periodontitis, Gingivitis, Saliva, Gingival Crevicular Fluid

Introduction

Plaque is the main etiological agent to cause gingivitis which is characterized by the inflammation of the gingiva. Removal of the plaque reverses this phenomenon and if it left untreated it will progress into periodontitis.1 Periodontitis has been described as a complex and multifactorial disorder associated with a dysbiotic plaque mass and characterized by progressive loss of tooth supporting apparatus.2 The onset, progression and severity of periodontal disease are mediated by various protein molecules. Periodontal tissues constitute multi-compartmental groups of interacting cells and matrices that render continuous support, attachment, proprioception and physical protection for teeth.3 Small amounts of GCF are reportedly present in healthy gingiva as a transudate, whereas large amounts are observed in inflamed regions as an exudate.4-7 Components of Gingival crevicular fluid (GCF) and saliva are considered to be the potential biomarkers to investigate proteomic changes that are associated with the initiation , progression as well as resolution of periodontal disease.

Bleeding on probing is considered as the earliest sign of gingival inflammation but way before it there are certain biomarkers that can be traced in GCF and saliva and that can guide us to detect the disease way before its clinical manifestations.

Oral fluids comprise of local and systemic mediators of periodontal disease. Biomarkers are an adjunct to the clinical parameters thereby improving the diagnostic precision in early detection of periodontitis. 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. Human saliva and GCF in oral diagnostics are of great importance and researchers are concentrating on it.

Major biomarkers of proteomics which are found in GCF and Saliva

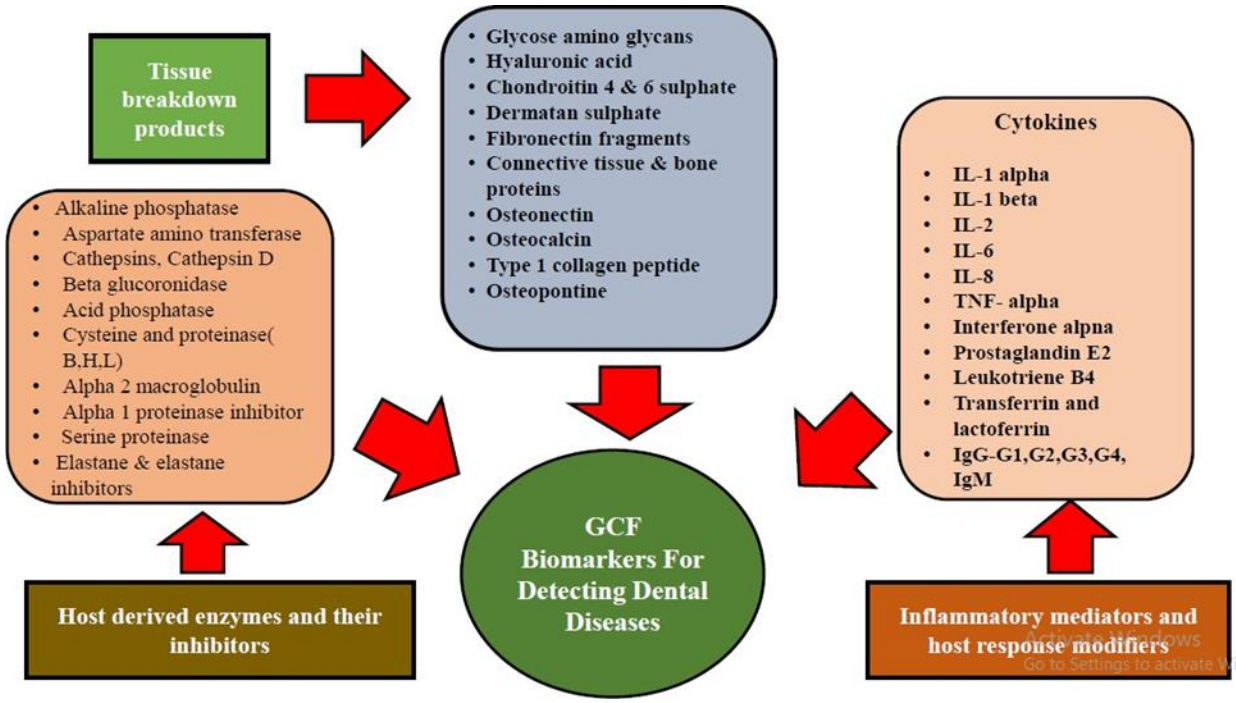

Figure 1. Showing GCF biomarkers

Salivary components

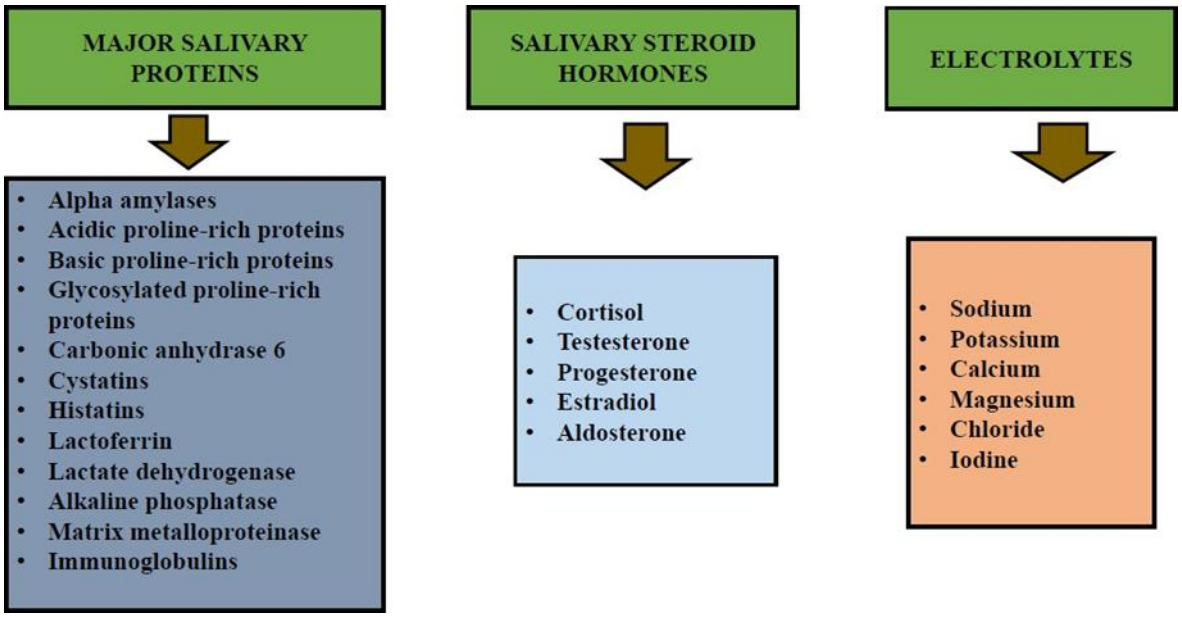

Figure 2. Showing various salivary biomarkers

Structural proteomic aims to map out the structure of protein complexes or proteins present in a specific cell organelle. It attempts to determine the location of proteins and characterize protein-protein interactions.[8] Some structural information arises from analysis of unknown proteins in which a protein-bound ligand or co-factor is discovered.

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.[9] Several large-scale proteomics technologies have been developed to generate comprehensive, cellular protein– protein interaction

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maps.[10] One of the most commonly used technologies is the yeast two-hybrid system, an ex vivo assay that detects binary physical interactions.

Functional proteomics is a broad term for many specific directed proteomic approaches. This approach allows selected group of proteins to be studied and characterized thereby providing important information about protein signalling, disease mechanisms or protein-drug interactions. 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 issues.[11]

Elucidation of biological functions of unknown proteins,

Cellular activity at molecular level

Proteome technologies:

A range of techniques are now available for the analytical separation and identification of proteins from complex mixtures. One and two dimensional gel electrophoresis, or high- performance liquid chromatography (HPLC) are the most common separation methods, while mass spectrometry (MS) is now the gold standard for protein identification.

1. Two-Dimensional Gel Electrophoresis (2D-GE)
2. Fluorescence 2D Difference Gel Electrophoresis (2DDIGE)
3. Isotope-Coded Affinity Tag (ICAT)
4. Stable Isotope Labelling with Amino Acids in Cell Culture (SILAC)
5. 18O Stable Isotope Labelling
6. Isobaric Tag for Relative and Absolute Quantitation (iTRAQ).
7. Liquid Phase IEF Fractionation Methods.
8. Large-Scale Western Blotting Proteome Analysis
9. Multidimensional Protein Identification Technology (MudPIT)
10. Mass-Spectrometry Based Proteomics
11. Protein Micro-array Technology

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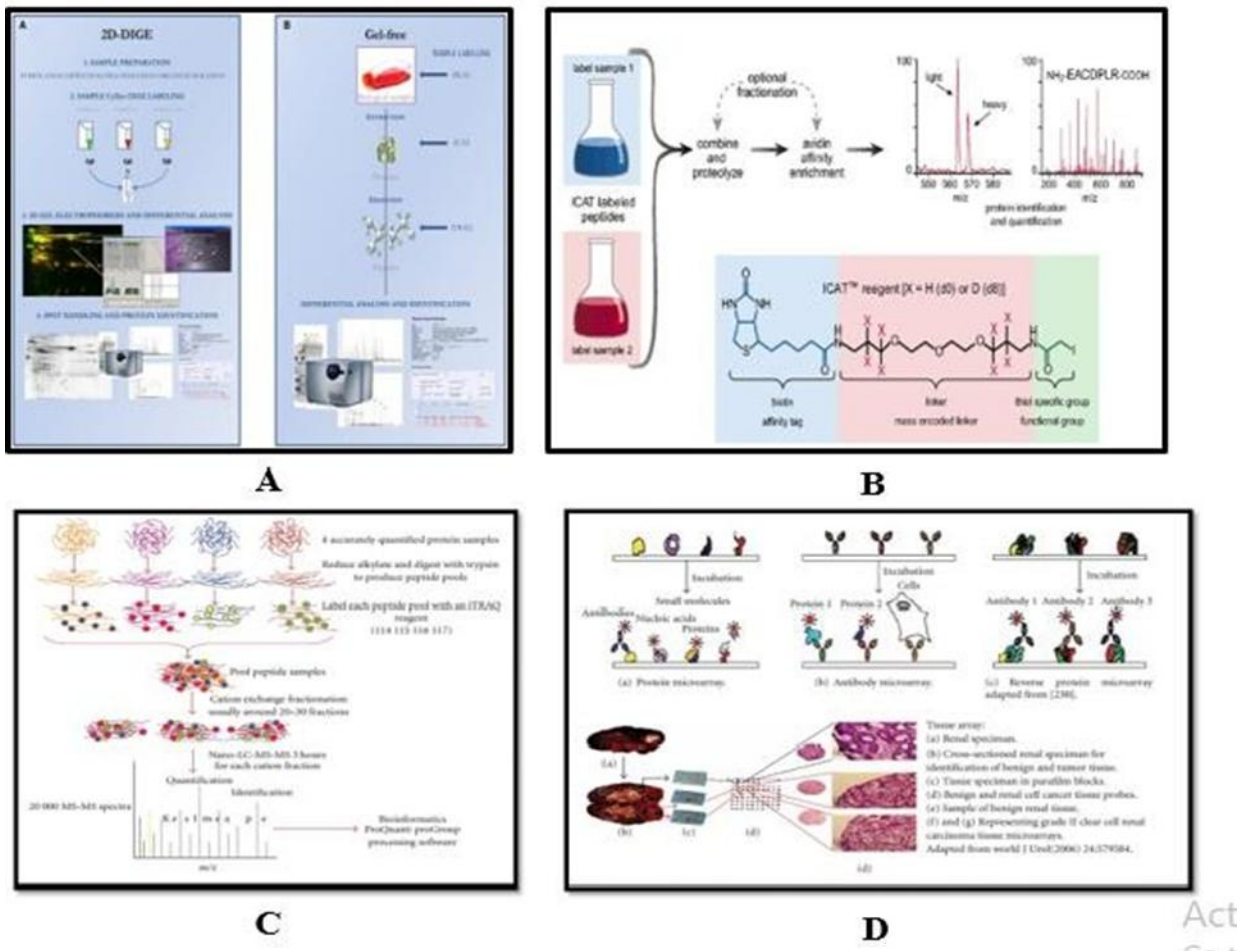

Figure 3. (A) Two-Dimensional Difference Gel Electrophoresis, (B) Isotope Coded Affinity Tag, (C) iTRAQ Work Flow, (D) Application of functional Protein microarray and Tissue array

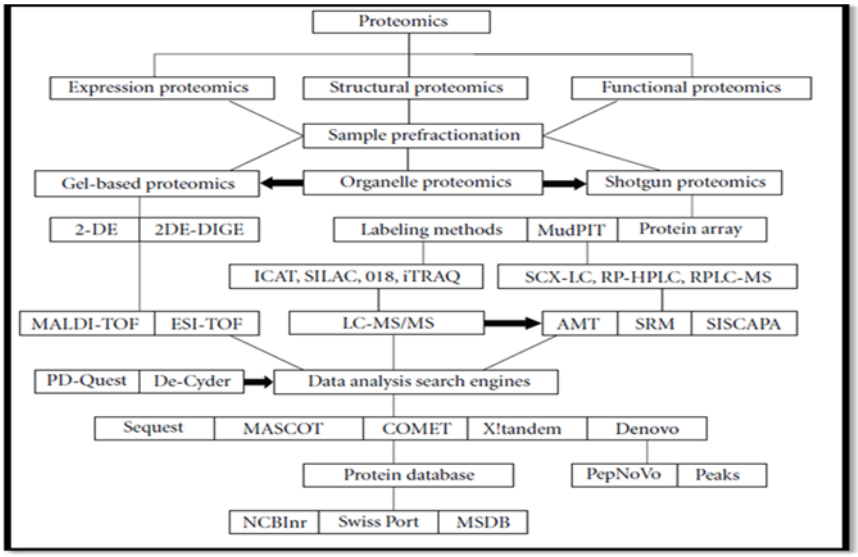

Figure 4. Various Proteomic Strategies

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Steps of proteomic analysis:

Cell and tissue fractionation- For analysis of dissected periodontal tissues, sections through periodontium or cultured periodontal cells, fractionation of cells and matrix followed by protein separation are the first steps required prior to protein analysis.

Protein separation- Typically, proteomics involves separating the very large number of proteins in cells or tissue prior to analysis by mass spectrometry, followed by recognition and characterization with bio-informatics approaches. Protein separations are performed at the protein or peptide level. Fractionation of samples can be performed according to a wide variety of parameters including post-translational modifications, or, for example, from their subcellular organelle localization.[12]

Mass spectrometery- Mass spectrometer-based proteomic analysis is now being used more frequently in studies of interest to dental scientists including, for example, the analysis of Streptococcus mutans and the analysis of osteoblastic differentiation.[13-14] Mass spectrometry is particularly useful when limited sample material is available and when femtomole sensitivity is required.

Quantification and sequence analysis- The comparison of different proteomes using two dimensional electrophoresis is technically demanding and can be invalidated by intergel variability. One approach to reduce this variation is to separate two or more protein samples labelled with different fluorescent dyes in one single gel (two-dimensional difference gel electrophoresis).[14]

Contribution of proteomics to our understanding of periodontal health and disease:

The knowledge of various proteins involved in periodontal disease pathogenesis helps in diagnosis, prevention and treatment of periodontal diseases. Proteins are fairly large molecules made up of strings of amino acids linked like a chain. Proteome is the entire set of proteins, present in an organism encoded by the genome which varies with time and distinct circumstances, that a cell or organism undergoes. 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.[15] In a broader sense, the term 'proteomics' refer to cataloguing proteins of a biological subject and the monitoring of reversible post-translational modification of proteins by specific enzymes, i.e. phosphorylation, glycosylation, acylation, phrenylation, sulfurization, etc. In many ways, proteomics is similar to genomics. Proteomics helps in understanding of alteration in protein expression during different stages of life cycle or under stress condition Also, proteomics helps in understanding the structure and function of different proteins as well as protein-protein interactions of an organism. In general, proteomic approaches can be used for proteome profiling, for comparative expression analysis of two or more protein samples, for the localization and identification of post-translational modifications and for the study of protein– protein interactions. Proteomic technologies play an important role in drug discovery, diagnostics and molecular medicine because it is the link between genes, proteins.[16]

Through this proteonomic changes a paradigm shift in periodontal science has occurred which is used to

1. monitor onset of disease,
2. monitor status of disease in regard to health,
3. monitor response to treatment,
4. monitor outcome.

The major challenge for research workers in periodontology is to embrace proteomics approaches when appropriate and start to apply them to critical, unresolved questions and disease. such as molecular and biologic understanding for the various cell populations of periodontium. Thus a more in-depth knowledge of cellular and matrix protein component of periodontium provides an excellent commencement for future advance.[2]

This review compiles the basics of periodontal proteomics, currently used proteomic methods, proteomic biomarkers specific to periodontal structure, and applied proteomics in oral health and disease.

GCF:

Various cellular components of GCF are epithelial cells, neutrophils, monocytes/ macrophages, lymphocytes, T-lymphocytes, B- lymphocytes.

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Inflammatory mediators like total and sub group of immunoglobulin IgG, Prostaglandin E2, C-reactive protein, leukotriene B4, cytokines such as IL-1, IL-2, IL-4, IL-6, IL-8, IL10, TNF-alpha, Interferon alpha.

Host derived enzymes & products like Alkaline phosphatase (ALP) is a very important indicator of bone formation and its presence in the GCF indicates inflammation and destruction of periodontal tissues. The severity of the periodontal disease is positively correlated to the level of ALP. Lactate dehydrogenase (LDH) activity is elevated with increasing probing depth. Aspartate aminotransferase (AST) is a nonspecific marker for cell death and necrosis and its activity is associated with the severity of periodontitis. Finally, cathepsin-B aids in distinguishing periodontitis from gingivitis by serving as a predictor of attachment loss. GCF also contain various bone-related biomarkers to reflect the disease status of periodontal tissues.

Various bone specific proteins like Osteocalcin (OC) is the most specific biomarker of osteoblast function. An increase in OC level in GCF is associated with high rates of bone turnover and seen during increased periodontal disease activity. Calprotectin alters the immune response by inhibiting the immunoglobulin production and also plays a role in neutrophil recruitment and activation. Higher levels of calprotectin levels are reported in periodontitis patients. Osteopontin (OPN) is mainly produced by osteoblast and macrophages and its raised levels are found associated with periodontal disease. Similarly, osteonectin is an important biomarker associated with the periodontal disease status and its increasing levels correlates with the increase in pocket depth. Bone sialoprotein (BSP) is synthesized by active osteoblasts and lay in extracellular bone matrix it seems to express osteoclast activity.

Tissue breakdown products like Glycosaminoglycan, Hydroxyproline, Fibronectin fragments, connective tissue & bone proteins, polypeptide growth factors etc are present.

Saliva:

1. In saliva there are certain electrolytes are present such as sodium, potassium, calcium, magnesium, chloride, bicarbonate, iodine and phosphate.
2. Major salivary proteins like alpha amylases is antibacterial and helps in digestion of proteins. Acidic proline-rich proteins provide lubrication, mineralization and tissue coating. Histatins are antifungal, antibacterial and aids in wound healing. Alkaline phosphatase is indicator of bone metabolism. Matrix metalloproteinase helps in degradation of extracellular matrix.

Current Trends in Proteomics:

1. Organelle Proteomics and Subcellular Fractionation- Organellar proteomics aims to describe the full complement of proteins of subcellular structures and organelles. Identification of the proteins contained in subcellular organelles has become a popular proteomics endeavour. When compared with whole-cell or whole-tissue proteomes, the more focused results from subcellular proteomic studies have yielded relatively simpler datasets from which biologically relevant information can be more easily extracted. Subcellular fractionation consists of two major steps, disruption of the cellular organization (homogenization) and fractionation of the homogenate to separate the different populations of organelles.
2. Post-translation Modification Analysis (PTM)- PTMs of proteins are considered to be one of the major determinants regarding organisms complexity. To date, at least more than 200 different types of PTMs have been identified of which only a few are reversible and important for the regulation of biological processes. Specific functions are usually mediated through PTMs, such as phosphorylations, acetylations, or glycosylations, which places additional demands on the sensitivity and precision of the method. One of the most studied PTMs is protein phosphorylation, because it is vital for a large number of protein functions that are important to cellular processes spanning from signal transduction, cell differentiation, and development to cell cycle control and metabolism.
3. Proteome Analysis of Unsequenced or Non-model Organisms- The application of proteomics and related technologies for the

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analysis of proteome is severely hampered by the lack of publicly available sequence information for most of the un-sequenced organisms. Despite the precision of the mass information yielded by the SELDI technique, a significant number of proteins were found to have no similarity to known peptides, an aforementioned weakness of proteomics studies in non-model organisms.

Bioinformatics for proteomics:

The major bottlenecks in proteomics research today are related to data analysis to create an environment where computer scientists and biologists and the people who collect data can work closely together, so they can develop the necessary analytical tools that will help interpret the data. Processing and analysis of proteomics data is indeed a very complex multistep process. We can now generate huge amounts of data, and currently there is an enormous challenge to figure out how to actually analyse this data and generate real biological insights

1. Protein Identification and Validation. This step consists of the assignment of MS/MS spectra to a database search using one of several engines available (e.g., Sequest, Mascot, Comet, etc.). One of the difficulties related to the use of sequest for peptide identifications is the lack of methods to globally evaluate the quality of data and the lack of methods to access global changes created by filtering schemes and/or database changes. Peptide identification via database searches is very computationally intensive and time- demanding. Protein Prophet database tool combines probabilities assigned to peptides identified by MS/MS to compute accurate probabilities for the proteins present.
2. Data Repositories. Importance of data repositories is to store, retrieve, and exchange data and results. Typically, proteomics experiments are carried out in isolation by one single laboratory often in an uncoordinated way, thus making sharing and comparison of results tedious if not impossible. Sharing and exchange of data and results requires the definition of standard formats for the data at all levels (including raw mass spectrometric data, processed data, and search results) as well as a better definition (and/or standardization) of the parameters used for the data processing or the database searches.[17]

The Future of Proteomics:

1. Customized Drugs- One of the most promising developments to come from the study of human genes and proteins has been the identification of potential new drugs for the treatment of disease. This relies on genome and proteome information to identify proteins associated with a disease, which computer software can then use as targets for new drugs.
2. Development of Biomarkers- 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 non-invasive, its use in clinical diagnostics has only recently been demonstrated.
3. Computational Method- A computer technique which attempts to fit millions of small molecules to the three-dimensional structure of a protein is called "virtual ligand screening". The computer rates the quality of the fit to various sites in the protein, with the goal of either enhancing or disabling the function of the protein, depending on its function in the cell.

Various studies done in proteomics separately in GCF and Saliva

1.	Saliva	Haigh BJ et al identified changes in the salivary proteome associated with active periodontitis. They took Quantitative proteomics (two-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis) to investigate whole saliva from individuals with severe periodontitis and their proteomic profiles before and after periodontal treatment were compared.[18]

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2.	Saliva	Wu Y et al compared the proteomic profile of whole unstimulated saliva of subjects with generalized aggressive periodontitis (GAgP) with that of healthy volunteers. This study revealed at least 11 differential proteins and suggested that the approach applied herein might be helpful to aid understanding of the aetiology of GAgP.[19]
3.	Saliva	Sanchez GA et al compared the salivary concentrations of IL-1B and PGE2 in relation to periodontal status and their changes after periodontal treatment. This study revealed that the high sensitivity and specificity of salivary IL-1B and PGE2 in identifying periodontitis suggests their potential use as biomarkers for diagnosis of periodontitis presence and its severity.[20]
4.	GCF	Choi et al. searched for potential protein biomarkers for periodontitis in gingival crevicular fluid using LCtandem MS (LC-MS-MS). Azurocidin, an antibiotic protein of azurophil granules with chemo tactic activity, was identified as up regulated in gingival crevicular fluid.[21]
5.	GCF	Baliban RC et al in 2011 conducted a study to identify possible novel biomarkers in gingival crevicular fluid (GCF) samples from 12 chronic periodontitis (CP) and 12 periodontally healthy individuals using high-throughput proteomic analysis. This study revealed that the proposed methods for large-scale comprehensive proteomic analysis may lead to the identification of novel biomarkers of periodontal health or disease.[22]
6.	GCF	AHS Huynh et al in 2014 conducted a study to compare the proteome composition of gingival crevicular fluid obtained from healthy periodontium, gingivitis and chronic periodontitis affected sites wherein fifteen males

Conclusion:

Proteomics is a relatively new post-genomic science with tremendous potential and consists of mass screening of proteins and the analysis of various genomes via their protein complements. The diagnosis of dynamic phase of disease, identifying patient at risk for periodontal disease, and focusing on early identification of microbial confront to host are tranquil for clinical investigations been increasing interest in exploring protein biomarkers to get optimal, best possible, novel, and non-invasive approaches. Proteomic technologies have provided great insights in understanding the inflammatory and tissue destructive processes that govern periodontal infections. Clinical proteomics offers the promise of biomarker discovery and early detection, diagnosis and prognosis of disease, but major challenges still remain. Further advances in technology are needed to eliminate proteomics deficiencies and augment its contributions to the medical and dental field

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