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REVIEW ARTICLE

PROTEOMICS: AN INSIGHT IN MOLECULAR DIAGNOSIS.

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Abstract

Proteins are the integral components of the physiological metabolic pathways of cells. Periodontitis is the result of complex interrelationship between infectious agents and host factors. The onset, progression and severity of periodontal disease are mainly mediated by various protein molecules. The proteome map, that is, complete catalogue of the matrix and cellular proteins expressed in alveolar bone, cementum, periodontal ligament, and gingiva, is to be explored for more in-depth understanding of periodontium. The knowledge of various proteins involved in periodontal disease pathogenesis can be used in the diagnosis, prevention and treatment of periodontal diseases.

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Introduction:-

Proteins are the building blocks of human cells. Almost every organic molecule in the body is either a protein or the result of a protein's activity. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. The word "proteome" is a blend of the words "protein" and "genome" created in the mid-1990s by Marc Wilkins, an Australian geneticist.¹ The term "proteomics" was first coined in 1997 to make an analogy with genomics, the study of the genes. In simple terms, proteomics is defined as the study of all proteins including their relative abundance, distribution, posttranslational modifications, 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.² Periodontal tissues comprise groups of interacting cells and matrices that provide continuous support, attachment, proprioception and physical protection for the teeth. The complex interactions of

cells and matrix within compartmental groups make the molecular understanding of periodontium³. Visual examination, tactile examination, periodontal pocket depth, clinical attachment level, and various periodontal indices are basis of periodontal diagnosis in day to day clinical practice. To develop screening and diagnostic modalities for early detection of periodontal disease it is important to understand the underlying science and molecular basis of tissue complexity of periodontium. Evolvement with time has brought biomarkers, proteomics, genomics, and metabolomics in the forefront for periodontal diagnosis as well to assess response to therapy⁴. Better in depth understanding of periodontium, its proteome map, that is, complete catalogue of the matrix and cellular proteins, is required. Proteome analysis of bone and dental structure (enamel, periodontal ligament, and cementum) and oral fluid diagnostics (saliva and GCF) are the primary areas where dental proteomics has shown promising outcomes⁵.

Genomic or Proteomic:-

Proteomics is a relatively new 'post-genomic' science with tremendous potential. While an organism's genome is more or less constant, the proteome differs from cell to cell and from time to time as distinct genes are expressed in distinct cell types. This means that even the basic set of proteins which are produced in a cell needs to be determined. Hence proteomics is considered the next step in the study of biological systems⁶.

Proteomics and Dentistry:-

The two principal areas in which dental proteomics have shown promising results are salivary diagnostics i.e. oral fluid diagnostics or oral fluid biomarkers and proteomics of bone and enamel structures, especially dental enamel.⁷ 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 first essential step toward the discovery of saliva protein markers for human disease detection in particular for oral cancer and Sjogren's syndrome. Specific salivary proteomic biomarkers have been identified for three key features, namely the pathogenic process inflammation, collagen degradation and bone turnover. Recently, by using proteomic approach, a reference proteome map of human whole saliva allowing for the resolution of greater than 200 protein spots in a single two-dimensional polyacrylamide gel was deduced. The proteomic approach identifies candidates from human whole saliva that may prove to be of diagnostic and therapeutic significance.^{8,9}

Types of Proteomics:-

Structural Proteomics:

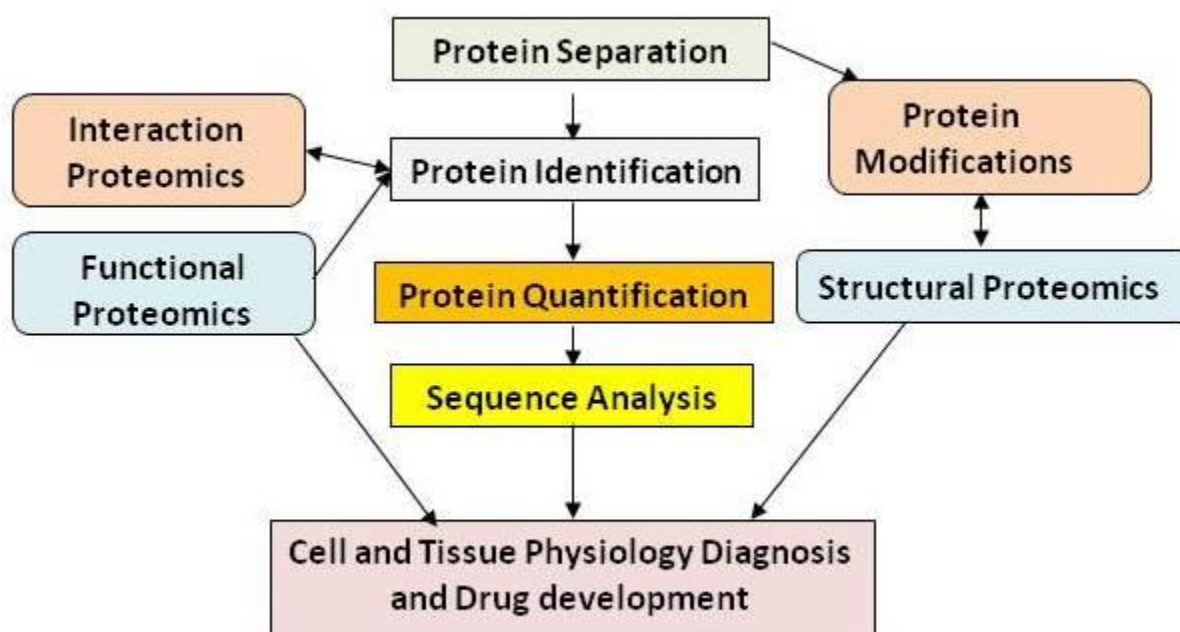
Structural proteomics includes the identification of all the proteins on a genome-wide scale, determining their structure function relationships, and describing three-dimensional structures of the proteins. Structural genomics attempts to map the total repertoire of protein folds in the hope of providing three-dimensional images for all proteins in an organism and to infer protein functions.¹⁰

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 different technique used for this includes yeast two-hybrid system, microassays, and affinity purification. This technology has been used for many different biological systems including, for example, identification of novel matrix metalloproteinase substrates that act to regulate inflammation.^{11,12}

Functional Proteomics:-

Types of proteins that indicate the function of proteins or how they are assembled into the molecular machines and functional networks that regulate cell behaviour determine the functional proteomics.¹³ It is "focused to monitor and analyse the spatial and temporal properties of the molecular networks and fluxes involved in the living cells"^{13,14}. It concentrates on the following two issues: (i) elucidation of biological functions of unknown proteins, (ii) cellular activity at molecular level^{14,15}.



Proteomics and Periodontics:-

Periodontal ligament 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.¹⁶

Periodontal Pathogens:-

Periodontal diseases are still worldwide human ailments, resulting in a high level of morbidity and an economic burden to the society. Proteomics offers a new approach to the understanding of holistic changes occurring as oral microorganisms adapt to environmental change within their habitats in the mouth. *Porphyromonas gingivalis* is a periodontal pathogen, is known to undergo a transition from its commensal status in healthy individuals to a highly invasive intracellular pathogen in human patients suffering from periodontal disease.¹⁶

Extensive proteomic research is done on *P. gingivalis*. • Whole cell quantitative proteomics, along with mutant construction and analysis were conducted to investigate how *P. gingivalis* adapts to species community. The results have confirmed that some 403 proteins were down regulated and 89 proteins were upregulated. The proteins such as HmuR which is up-regulated can be necessary for community structure.¹⁷

- ❖ Whole-cell proteomic analyses were conducted to investigate the changes from an extracellular to intracellular lifestyle for *Porphyromonas gingivalis* and found that a total of 385 proteins were over expressed in internalised *P. gingivalis* relative to controls.¹⁸
- ❖ Hendrickson EL et al found that there is shift in the production of cytotoxic fatty acids by intracellular *P. gingivalis*, which suggests that the interior of host cells provides a more energy rich environment compared to the extracellular milieu.¹⁸
- ❖ Yoshimura M et al carried out a similar study on proteome analysis of *P. gingivalis* which was placed in subcutaneous chamber of mice showed that PG1385 protein is involved in the virulence of these bacteria.¹⁹
- ❖ The results of these studies suggest that adaptation to an epithelial cell environment induces a major shift in the expressed proteome of the organism.

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, lipopolysaccharides, bronchial products and other foreign substances are present in whole saliva. 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 diseases²⁵. 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. Numerous proteomic markers, like acid phosphatase, alkaline phosphatase, histatins, cystatins, kallikreins & kininogens, aminopeptidases, aspartate transaminase, glucosidase, galactosidase and glucuronidase and various bone remodeling proteins (Osteopontin, Osteonectin, Osteocalcin) are well known in periodontal diagnosis.²⁰

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.²¹

Genetic single nucleotide polymorphisms:-

Several researchers have focused on genetic single nucleotide polymorphisms in the study of periodontal disease. A genetic susceptibility test is available for severe chronic periodontitis (Interleukin Genetics, Waltham, Massachusetts). It works by detection of two types of IL-1 genetic alleles, IL-1 α + 4845 and IL-1 β + 3954 30. Individuals identified as "genotype positive," or are found to have both of these alleles, are more likely to have the phenotype of overexpression of this gene.²²

The Future of Proteomics:-**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. For example, if a certain protein is implicated in a disease, its 3D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. This is the basis of new drug-discovery tools, which aim to find new drugs to inactivate proteins involved in disease. As genetic differences among individuals are found, researchers expect to use these techniques to develop personalized drugs that are more effective for the individual.²³

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 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 NanoSensor 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.²⁴

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. A good example of this is the identification of new drugs to target and inactivate the HIV-1 protease. The HIV-1 protease is an enzyme that cleaves a very large HIV protein into smaller, functional proteins. The virus cannot survive without this enzyme; therefore, it could be one of the most effective protein targets for killing HIV.^{23,24}

Limitation:-

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.

Conclusion:-

The use of proteomics and gene expression will advance the diagnosis and treatment of various oral pathological conditions. Advances in tissue engineering, drug delivery, gene therapy and biopharmaceuticals will present new therapeutic opportunities. However, its application into the field of dentistry depends on how best oral health care practitioners will incorporate this into their practice as it requires a thorough knowledge of human genetics and application of new diagnostic and therapeutic technologies.

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