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RESEARCH ARTICLE

PROTEOMICS IN DENTISTRY- A REVIEW.

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Abstract

Proteomics is the study of proteins, which constitute the building blocks of living matrix, on a large scale. Applications of proteomics have revolutionized various spheres of dental diagnostics, treatment and research. The review evaluates the role of proteomics in the study of dental hard and soft tissues, oral fluids and dental material research. Proteomics has helped in the early diagnosis of various diseases by detecting various biomarkers in oral fluids. The study of proteomics in dentistry is still in the nascent stage and lot of work still has to be done to realize the true potential of proteomics in future treatment evaluation, disease prevention and prognosis of interventions.

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Introduction:-

Proteins, the building blocks for living matrix exist as three dimensional structures because of sequences involving the twenty different amino acids linked by peptide bonds [1].

Proteomics, the large scale analysis of proteins has been used for gene function characterization, comprehending biological mechanisms and building personal linkages between protein molecules. The term proteome was coined by Marc Wilkins in 1994 to describe the entire complement of proteins expressed by genome, cell tissue or organism [2].

Proteomics have been extensively used for studying human body fluids like blood, urine, saliva, gingival crevicular fluid, semen as well as tissues like gingiva, pulp, dentin, enamel etc with great success. In dentistry, salivary protein studies have shown great promise in disease conditions like caries, periodontitis and oral cancer [3].

Two approaches are usually used for analysis of oral fluids. In the bottom up approach known as "short gun proteome" direct digestion of a biologic sample using a proteolytic enzyme that cleaves at well defined sites creating a complex peptide mixture is used. This is analyzed by liquid chromatography and tandem spectrometry. (LC-MS/MS or LC-MALDI MS/MS). Top-down proteomics involves separating intact proteins from complex biological samples using traditional separation techniques such as liquid chromatography or 2-D gel electrophoresis followed by differential expression analysis using spectrum analysis or gel imaging platforms. Bottom up method is used for more samples while top down proteomics is used for hundreds of different complex biological samples [4]. The paper aims to bring to the forefront the tremendous potential of proteomics especially as a diagnostic tool for dental conditions including oral cancer.

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Fluid Proteomics:-

The development of state of art mass spectrometry (MS) with sensitivity, speed and global protein analysis has transformed classical protein chemistry. Oral fluids like whole saliva (WS) and gingival crevicular fluid (GCF) with their minimal patient discomfort and noninvasive collection technique have given a big impetus for proteomic analysis [5].

The term salivomics was first introduced in 2008 on the basis of specific biomolecules appearing in saliva that included DNA, m-RNA, micro RNA, proteins, metabolites and oral microbiota extractions[6].

Salivary transcriptomic biomarkers appear to be upregulated in various human malignancies like lung cancer[7], ovarian cancer[8] and oral squamous cell carcinoma[9]. Overall over one thousand salivary protein biomarkers have been identified by proteomic approaches including secretory IgA, lactoperoxidase, statherin, proline rich glycoprotein, truncated cystatin S, cystatins, lysozyme and histatin-5[10].

GCF as a bio monitoring fluid plays role in diagnosis of oral diseases especially gingivitis and periodontitis. GCF contains serum transudate, broken products of histological epithelial or connective tissue, subgingival microbial plaque, extracellular proteins and host inflammatory mediator and cells [11]. Its limited amount compromises the biochemical and proteomic analysis and the severity of inflammation in periodontium affects its collection [12]. It has a non invasive collection technique. Various inflammatory factors have been isolated from GCF including cytokines, phosphatase, proteinase, proteins and local tissue degradation products. These factors are reported to be possible diagnostic markers in periodontitis and can be used to evaluate progression of periodontitis [13]. Right production of proteases is necessary for proper tooth development. Two proteases, early protease enamelysin and late protease kallikrein is secreted into enamel matrix in developing tooth[14]. The importance of extracellular matrix protein can be illustrated by dramatic changes in dental phenotypes observed in targeted knockout of enamel matrix genes (amelogenin, ameloblastin, matrix metalloproteinase, dentin sialophosphoprotein) encoding corresponding proteins in mice[15]. Dentin a vital tissue located between pulp and enamel has regenerative and neurogenic properties. It has 70% minerals, 20% organic content and 10% proteins. Odontoblasts deposit dentin, an organic matrix that contains collagen, noncollagenous proteins, phospholipids and growth factors. Proteomic tools are used for detailed profiling of GCF and the proteins identified are actin keratin, histones, annexins, proteins S100-A9, apolipoprotein A1, salivary gland antimicrobial, albumin and cystatin B[16].

Hard Tissue Proteomics:-

Enamel, the hardest tissue in the body is predominantly inorganic with 96% minerals, 1% protein and rest water. Major enamel proteins recognized are amelogenin, enamelin, ameloblastin, enamelin and tuftelin. A total of 42 proteins have been identified during enamel formation by two dimensional electrophoresis (2-DE) and MS. ERp29 is a protein involved in secretory protein synthesis and calcium binding protein (calbindin) and play a role in tooth mineralization [17]. Park et.al[18] used liquid chromatography-tandem mass spectroscopy (LS-MS/MS) proteomic approaches to reveal proteins in human dentin after third molar dentin were cut, isolated, demineralized and extracted protein were separated by SDS-PAGE method (Sodium-Dodecyl-Sulfate, Polyacrylamide Gel Electrophoresis). They identified 233 total and 68 common proteins including a wide variety of collagenous and non-collagenous proteins like DSPP, biglycan, osteopontin and osteocalcin. In another study by Jagr et.al[19] extracted proteins were analysed by gel electrophoresis (SDS-PAGE) and two gel electrophoresis digested with trypsin and separated by liquid chromatography /tandem mass spectroscopy. They identified 289 proteins of which 90 were previously unknown. Nine proteins identified denovo in humans have a variety of functions like cytoskeletal protein binding, immune transport, calcium ion binding and formation of ECM. These findings provide insight into regenerative capacity of dental tissues. Salmon et.al [20] did a proteomic study of alveolar bone harvested from extraction sites and cementum obtained from apical portion of extracted third molars. The tissues were denatured followed by protein extraction, reduction, alkylation and analysed by nano Acquity HPLC system and LTQ-FT ultra. 318 proteins were identified in AB&DC and 105&83 protein exclusive to AB&DC was noted. These candidate biomarkers should provide impetus to periodontal regeneration techniques.

Dental Soft Tissue Proteomics:-

The dental pulp resides in a rigid chamber compromising dentine, enamel and cementum which provide strong mechanical support and protection from microbial rich environment. The dental pulp is derived from neural crest cells. Tooth development, nourishment, defence, sensitivity, regeneration and repair are main functions of pulp.[21] Pakkonen et.al[22] analysed the gene and protein expression of pulp tissues from sound and carious human

teeth using c DNA microarray and 2-D gel electrophoresis to evaluate their usefulness in pulp biology. c DNA microarray reveals several differentially expressed genes with a high expression in both tissues. 2-D electrophoresis followed by MS/MS technique helped in identifying 96 proteins. McLachlan et.al[23] analyzed oligonucleotide microarrays containing ~ 15000 human sequences using probe total RNA from both normal and healthy tooth. 445 genes with 2-fold or greater difference in expression level with 85 genes abundant in health and 360 abundant in disease.

Periodontal ligament has abundance of Collagen-1 and plays a key role in anchorage, homeostasis and in regeneration and repair of periodontium in response to disease and mechanical trauma. Reichenbeg et.al [24] applied proteomic analysis coupled with mass spectrometry to human periodontal ligament fibroblasts and detected 900 spots and identified 117 protein spots originating in 74 different genes.

Wu[25] used two dimensional gel electrophoresis mass spectroscopy and m peptide mass fingerprinting in analyzing protein profiles of periodontal ligament cells undergoing mineralization. 61 proteins in periodontal ligament cells showed an 1.5 fold change in intensity of which 9 differentially expressed proteins cytoskeleton products, cytoskeleton associated proteins, nuclear proteins and cell membrane bound molecules. Wei et.al[26] investigated the differential profile of human DPCs(DNA-protein cross links) undergoing odontogenic induction for 7 days using two dimensional gel electrophoresis coupled with matrix assisted laser adsorption ionization mass spectroscopy and found 23 spots related to early odontogenic differentiation. Eckard et.al[27] used terminal amine isotope labeling of substrates (TAILS) for in-depth characterization of human dental pulp proteome from its N-terminome and identified 17 missing protein candidates for the Chromosome centric Human Proteome Project (c-HPP).

Dental Material Proteomics:-

Dental materials interact with the oral environment and hence should be highly biocompatible. A study by Heil et.al[28] compared the biologic response of both primary peripheral blood monocytes and THP-1 cell lines to form common components of dental materials known to be released into the oral environment; nickel ions, 2-hydroxyethyl methacrylate(HEMA), triethylene glycol methacrylate (TEGDMA) and Bis-GMA. The study supported the use of THP-1s as a model for ranking cytotoxicity of dental material components. Derhami et.al[29] studied the molecular basis of biocompatibility of titanium as an implant material. A comparison of response of skin fibroblasts to two different supporting surfaces: commercially pure Titanium(CPTi) and tissue culture polystyrene(TCPs) was analyzed. Among the major cellular proteins, fibronectin and cytoskeletal protein(non-muscle myosin heavy chain type-A) were expressed at lower levels by fibroblasts grown on CPTi compared to TCPs. Dorkhan et.al[30] examined the adherence of clinical strains of S.Oralis to Ti with smooth or moderately rough surface topography and to determine the effect of saliva or serum derived coating on the process. The adherence of LA11 and 89 C stain to the moderately rough surfaces coated with saliva was more than twice that seen on smooth saliva coated surface proving that surface topography is maintained by salivary coating. Lagocka et.al[31] evaluated the dynamics of unreacted TEGDMA monomer elution from new generation of flowable bulk fill composite resin (SDR Dentsply). Polymerised specimens were treated with four solutions (100% ethanol, 75% ethanol, distilled water and 100% methanol) with different concentration to evaluate dental pulp toxicity of unreacted TEGDMA monomer. After 31 days total concentrations of TEGDMA were 100% ethanol -16 micro gram/g, 75% ethanol, -9.4 micro gram/g, distilled water -9.4 microgram/g and methanol -7 microgram/g. In an aqueous environment SDR composite exhibits high chemical stability compared to other solutions.

Conclusion:-

Proteomics have undoubtedly helped in analyzing dental hard and soft tissue, fluids and materials with release of pertinent information that can help in filling the lacunae of several aspects of oral and dental health. Proteomic studies should be sustained for interpolating the pathogenicity of dental conditions as well as analyzing the structural stability and biocompatibility of dental materials.

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