Innovations

Salivary Proteomics – A Comprehensive Literature Review

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Abstract: *Salivary diagnostics is a potent and imminent field utilized for molecular diagnostics which aids in the diagnosis of oral and systemic diseases. As saliva is a clinically informative biological fluid useful for novel approaches and for expanding perspectives in clinical diagnosis, disease monitoring and treatment planning. Due to its ease and non-invasive collection process it is widely used as a molecular biomarker and also as an extensive potential diagnostic tool. Here, we briefly summarized the components of salivary proteomics and their applications and latest advances using salivary biomarkers for disease diagnosis.*

Key words: *saliva, biomarker, diagnosis, disease*

Introduction:

Saliva is a biologic body fluid which plays an important role in oral cavity, has mechanical, protective and homeostatic characteristics, the important function of saliva includes lubrication; buffering action and clearance; maintaining tooth and mucosal integrity and found to have effective antibacterial and antiviral activity as well as taste and digestion.Saliva is constituted majorly by 99% of water and 1% of proteins and salts (electrolytes, immunoglobulins, various proteins, harmones and enzymes). (1)90% of saliva is secreted by major salivary glands whereas only 10% by minor salivary glands. It is termed as "mirror fluid"of health and disease which was curiously utilizedas a diagnostic vehicle for "omics" research. In the last 10 years, saliva has become the object among various researchersand studies, where it is considered as a biological specimen. (2)The main supremacy of using saliva as a

diagnostic fluid is due to non-invasiveness, economical collection, safe, easy and adequate volumes of salivary samples for assay are easily obtainable.

Proteomics is the study of the "proteome" which is widely used to analyse bodily fluids. Salivary proteomic research has received a lot of intentness in recent days because of its efficient diagnostic potential in diagnosing various oral and systemic diseases. There are approximately 64% of human oral tissues studied to date. Recently proteomic platforms have identified the human salivary proteome, characterising about 3000 differentially expressed proteins and peptides, many of them of microbiological origin but their biochemical properties remains uncertain.(1, 3)Proteomic studies of human saliva are mainly characterized by four major salivary families of specific secretory proteins: proline-rich proteins (PRPs), statherins, cystatins and histatins that differ significantly from other host defence salivary proteins. The analysis of proteomics basically discriminates between physiological and pathological conditions."Salivaomics" studies the salivary biomolecules which consists of salivary proteome, microbiome, transcriptome, metabolome, and micro-RNA. As there are various researches are validated there is no "gold standard" technique established for saliva collection and assay for proteomics research and also these technologies are immensely complex, expensive and has limited convenience. (2, 4)

Studying the proteins in saliva helps in defining the protein complex in different salivary glands, pathological alterations related to them and to understand the function of new proteins and peptides in oral cavity. (2)The aim of this current review is to briefly describe the discrete aspects onproteomic researches and other –omic sciences imposed on human saliva and its potential use as a diagnostic biomarkeron most demanding and challenging panorama.

Salivary proteins:

Sir Francis Crick described that proteins are diverse group of genome-encoded biological macromolecules found in all cells in the body, they are covalently linked linear chains of 20 amino acids. Wilkins et al and Wasinger et al described Proteome as a mixture of proteins, where the name states that the contraction of "**PRO** teins" encoded by a given "gen **OME**", in a cell or tissue type. Proteomics profiling is the study of protein transcriptional profiles and their interactions of expressed proteins across cells, organisms, and even ecosystems, offering essential insights to understanding the function of proteins and their roles in systemic health. (2, 4, 5)

Component of salivary proteome:

On comparing with other body fluids it is found that whole saliva has a specific type of characteristics where 3000 protein components are detected in saliva. 90% weight of about 200 protein components are secreted by major salivary glands and remaining 10% by other components. Saliva enters the blood stream through passive diffusion or active transport, thereby forming bridging between salivary and serum protein concentrations.(2)Majority of the salivary secretory proteins are polymorphic and various post-translational modifications (PTMs) occur before secretion, such as glycosylation, phosphorylation, exo- and endoproteolytic cleavages. Age and salivary flow rate plays an important role in proteome identification, in the recent studies it was found that salivary alterations and differentiation in the specific peptides which should be considered during analysis. (4)

Human salivary proteomic platform:

Salivary biomarker discovery for disease diagnosis and treatment planning is based on the assessment of their source of variability. Thus, the assessment of this, exploits saliva as a diagnostic and prognostic biomarker. Proteomic platform can be broadly classified into top-down and bottom-up platforms. Top-down platform analyse the naturally occurring intact protein structure with reduced sample alteration, which includes qualitative and quantitative assessment of protein samples. (5, 6)They are time consuming and expensive which can only demonstrate a limited proteomic subset. Bottom-up platform is considered popular, where the samples are pre-digested and the break down peptide fragments are analysed. But most of the proteins are subjected through post-translation modification, wherein there is chance of missing specific molecular information. Shotgun proteomics can be considered as the common bottom-up strategy. As these are high-throughput techniques which can analyse thousands of protein components, provides better biomarker with higher specificity and sensitivity. However, there are few challenges associated with most of these high ended techniques.(6, 7)

Saliva as a diagnostic fluid:

Saliva carries a thriving number of proteins, which represents the pathological status of the oral and systemic health, thus computing appealing biomarkers. Saliva omics was coined after the expanding evolution in molecular biology, genomics, and proteomics, which reveals the importance of salivary biomarkers in diagnosis. Better treatment planning is based on accurate diagnosis. It can be used in detection of various pathological conditions such as Caries, periodontal diseases, and oral malignant lesions that can be diagnosed with saliva tests also systemic diseases such as diabetes mellitus, hypertension, cardiovascular diseases, stress and infections can

be potentially diagnosed using saliva. It was once commonly used in drug detection and forensic sciences. (2, 4, 8, 9)

Oral Diseases:

Dental caries is a common oral disease, formed by demineralization of the inorganic structures, causing pain and inflammation. Several microorganisms are meant to play a vital role in destruction process. Saliva is used to identify these microorganisms, as it contains structures such as molecules that causing remineralisation. There are numerous studiesanalyzing biomarkers using saliva. In relation to dental caries salivary lactobacilli and Streptococcus mutants are the dominant microorganism, used as a biomarker. (1, 8, 10). Pune N. Paqué in 2021 studied the disease status of gingivitis, caries with healthy individuals for analysing the potentiality of salivary bacterial and protein markers and found that interleukins and eotaxin/CCL11 as a potential salivary biomarkers for identifying non-invasive caries.(11)Zaid Majeed Khan in 2021 studied the salivarytotal protein expression and immunoglobulin using gel electrophoresis among 33 dental caries patients and concluded that there were structural alterations in the proteins. (12)

Periodontal diseasesis an inflammatory condition of gingival and periodontium. The main etiological factors include gram negative bacteria and several immunoglobulins' also relative oxidative stress can lead to severity of the disease. (6)Sarah Reddahi in 2022 in Morocco studied the Levels of salivary cytokines including IL-1β, IL-6, MMP-8, and IL-10 in 30 periodontitis patients and found that higher concentration of IL-1β and IL-6. (13)Myung-Seop Shin in 2018 applied Shotgun proteomics to detect salivary proteins from 207 periodontitis subjects and concluded that salivary S100A8 and S100A9 were plausible biomarkers for periodontitis. (14)Shamala.S in 2023 compared the salivary thiocyanate levels in Light and Heavy Smokers with Chronic Periodontitis and concluded that there was increased levels of SCN was observed in heavy smokers with chronic periodontitis. (15)

Oral potentially malignant disorders and oral cancer: OPMD'sare the heterogeneous group of conditions which has a higher risk for malignant transformation. Early detection can provide an improved treatment outcome with better prognosis. (1, 2)Pia López-Jornet in 2023 studied salivary adenosine deaminase (ADA), ferritin (FRR) and total proteins (TP) in oral potentially malignant disorders (OPMD) and found that Ferritin and total proteins may construct as a potential salivary biomarkers. (16)Ramsheena Payambrot in 2023 estimated the salivary IL-6 levels in potentially malignant oral disorders (PMDs) (leukoplakia, lichen planus, oral submucous fibrosis) and was interestingly found that there were increased levels of IL-6 in oral submucous fibrosis and other PMD's. (17)Sumaiya

Irfan in 2023 evaluated the salivary endothelin-1 in oral cancer and pre-cancer as a potential biomarker. Concluded that, there was a higher level of salivary endothelin-1 was found in oral squamous cell carcinoma when compared to premalignant. (18)Beeula. A in 2020 compared the salivary antioxidant levels such as Malondialdehyde (MDA), Glutathione (GSH) and Catalase (CAT) among smokers, leukoplakia and oral cancer and found that CAT and GSH showed decreased levels among smokers and oral cancer whereas, increased levels of MDA was observed in smokers, leukoplakia and oral cancer. (19)

Oral microbial infections: Kaori Kobayashi in 2022 studied the anti-influenza-A virus activity in saliva using protein bound sialic acid levels and found to have significantly higher levels as it is a direct regulator of salivary anti-IAV activity. (20)Núbia Carina de Oliveira in 2020 investigated the flow rate, amylase concentration and total proteins in the saliva of hospitalized AIDS complication patients. And there was no difference between amylase enzyme levels and total proteins were observed. (21)Alberto Muñoz-Prieto in 2022 identified 537 proteins from 20 COVID-19 saliva samples and interestingly found that 30 proteins showed differences in abundance between them, where 20 proteins were down-regulated [Cystatin-S, CN SN and SA, BPI fold-containing family B member 2 and carbonic anhydrase 6] and 10 were up-regulated [F-actin-capping protein subunit alpha-1, alpha-actinin-4, protein-glutamine gamma-glutamy 1 transferase, complement C4-A , annexin A4, macrophage-capping protein, heat shock protein HSP 90-beta and plasma protease C1 inhibitor]. (22)

Systemic Diseases:

Saliva is used as a diagnostic utility in various systemic diseases such as in diabetes, hypertension, neurological disorders, cardiovascular, anti-depressives and drug analysis in treatment of systemic diseases.

Diabetes Mellitus and hypertension: Prathibha K.M in 2013 compared the salivary flow rates and salivary physical and biochemical such as salivary glucose, salivary alpha amylase, salivary total proteins and inorganic constituents like sodium, calcium and potassium parameters among diabetic and non-diabetic subjects.And found to have major variations among diabetes subjects. (23)Ramya. V in 2023 studied the Salivary Magnesium Levels in Type 2 Diabetes Mellitus and Hypertensive Patients with Chronic Periodontitis and found that there wasincreased magnesium levels in chronic periodontitis without associated systemic disease and decreased levels was seen in diabetes and hypertension individuals. (24)

Conclusion:

As a salivary analysis is an easy and non-invasive technique, contain sinstantaneous information's describing goverall physiological state of the body. It is routinely usedin clinical laboratories for detection of various proteins, secretory IgA antibodies, hormones, enzymesand for genetic purposes. The application of encompassing technologies such as salivary proteomics and other –omic sciences to saliva will provide an incredulous complexity of capturing diverse and unique metabolic fingerprint and pathways involved. (25)Omic sciences are hallmarks contributein identifying salivary components, including DNA, RNA, proteins, metabolites and microorganisms. These not only restricted to oral diseases also it can be used to analyse systemic diseases such as cardiovascular, neurological, syndromes and carcinomas. However, it is believed that in the near future human saliva will be a pertinent diagnostic fluid for diseasediagnosis and prognosis.

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