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Review Article

Salivary biomarkers – Emerging era in periodontics

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ABSTRACT

Periodontitis is a chronic inflammatory noncommunicable disease that affects all parts of the periodontium and causes irreversible damage. A biological sign of either pathogenic or normal processes is called a biomarker. Finding biomarkers is helpful for tracking the advancement of pathological conditions as well as for disease prevention, diagnosis, and prognosis. Effective treatment depends heavily on early disease identification. The disease process's severity and potential implications are lessened by early detection and treatment. Traditional clinical criteria are frequently inadequate in the field of periodontology to identify sites of active disease, track the effectiveness of therapy, or gauge the degree of vulnerability to future. Medical researchers are committed to identifying molecular illness biomarkers that indicate a concealed deadly threat prior to the disease becoming complicated in order to overcome this obstacle. Saliva is a vital physiological fluid that contains a complex mixture of chemicals. As a diagnostic tool, it is becoming more and more popular.

1. Introduction

A variety of diagnostic instruments are now available to medical professionals, such as blood tests, imaging studies, genetic testing, and monitoring technologies, such as electronic health records, wearable technology, and remote monitoring systems.[1] Sampling, as used in healthcare, is the process of gathering and examining biological or non-biological material. This material can come from a variety of sources, such as blood, tissue, or other body fluids, and is used to help with disease diagnosis, monitoring, or treatment. It is impossible to overestimate the importance of sampling in the diagnosis and monitoring of medical conditions because it is a critical first step in supplying invaluable information about the underlying cause of a patient's symptoms, the course of a disease, and the effectiveness of a certain medication.[2] The severity and potential problems of the illness process are decreased by early identification and treatment. In an effort to surmount this obstacle, scientists studying medicine are focused on identifying molecular disease indicators that indicate a deadly threat hiding beneath the surface of an illness before it becomes worse. Traditional clinical criteria in periodontics and implant dentistry are frequently inadequate for identifying active disease sites, quantifying the response to therapy, or assessing the degree of susceptibility to future disease progression.[3,4]

As a reflection of oral and systemic health, saliva is a useful source of clinically relevant data because it contains biomarkers specific to the particular physiological aspects of periodontal peri-implant disease. Qualitative changes in the composition of these biomarkers may be useful for diagnosing patients with increased disease susceptibility, identifying sites of active disease, predicting sites of future disease, or acting as surrogate end points for tracking the efficacy of therapy.[5]

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On the other hand, intrusive procedures represent major problems for modern healthcare monitoring and diagnosis. Penetrating the skin or bodily tissues in order to gather monitoring or diagnostic data is known as an invasive procedure. Invasive procedures entail a risk of consequences and can be painful, uncomfortable, and a significant source of information. For example, the diagnosis and monitoring of specific illness conditions typically need biopsies, an intrusive process that involves the removal of a tissue sample for study. Due to the possibility of bleeding, infection, or harm to surrounding tissues, patients who undergo biopsy diagnostic must recuperate for a considerable amount of time.[6] Non-invasive biofluids, like saliva, have sparked a lot of interest recently as a way to reduce the invasiveness of diagnostic and monitoring procedures. It has long been known that saliva, an oral fluid with an abundance of proteins and genetic components and easy access through a completely noninvasive procedure, offers a viable remedy for these constraints. Saliva offers a readily available, noninvasive diagnostic medium for an increasing number of clinical conditions and disorders.[7] Traditional clinical criteria are frequently inadequate in the field of periodontology for identifying the sites of current disease, tracking the effectiveness of therapy, or gauging the degree of susceptibility to future disease progression. Saliva contains biomarkers unique to the physiological features of periodontal disorders, making it a significant source of therapeutically relevant information as a mirror of dental and systemic health.[8]

2. Analysis and Sampling of Saliva

A rapidly expanding area of study and therapeutic use is salivary analysis, which examines the different biomolecules found in saliva, such as RNA, proteins, metabolites, DNA, and microbiota. These biomolecules can provide important details about a person's health, such as the existence of illnesses, how well a treatment is working, and risk factors for certain medical disorders.[9]

2.1. Collection of saliva

Several procedures, such as passive drooling, saliva collecting tools, mouth rinses, and swabs, can be used to obtain saliva samples.

2.1.1. Passive drooling

The most popular technique for gathering saliva is passive drooling, which entails having the subject drool saliva into a collection tube. This technique is easy to use, non-invasive, and doesn't need any specialised tools.[10]

2.1.2. Collection devices

Devices for collecting saliva are made to minimise contamination and gather a consistent volume of saliva. Usually, these gadgets are made of a glass or plastic tube with a funnel-shaped end that is inserted between the gums and cheek. Preservatives or stabilisers are also included in some devices to reduce the amount of the target biomolecules that degrade.[11]

2.1.3. Oral rinse or swab technique

Using an oral rinse or swab technique involves washing the mouth with a solution or using a collecting device to swab the oral cavity in order to collect saliva. These techniques are less intrusive than passive drooling or saliva collection tools, and they can be helpful in gathering samples from those who have trouble secreting enough saliva.[12]

3. Analysis of Salivary Samples

It is crucial to process salivary samples according to best practices in order to guarantee high-quality salivary analysis. For processing salivary samples, filtering, centrifugation, and solid-phase extraction are the most often utilised techniques.

3.1. Immunoassays for salivary analysis

Immunoassays are frequently used to identify and measure specific compounds in saliva, such as antibodies, cytokines, and hormones. The specific binding that occurs between an antibody and its antigen is used in immunoassays to identify and measure the target analyte in a complicated mixture. Salivary analysis can be performed using a variety of immunoassay techniques, such as fluorescence immunoassays, radioimmunoassays, and ELISAs.[13]

3.2. Enzymatic assays for salivary analysis

For the identification and measurement of small molecule metabolites like glucose, lactate, and urea in saliva, enzymatic assays are frequently employed. The target metabolite is precisely converted by enzymes in enzymatic tests into a detectable product, such as hydrogen peroxide, which can be measured using a colorimetric or fluorescent assay.[14]

3.3. Electrochemical methods for salivary analysis

Because electrochemical techniques are inexpensive, very sensitive, and have a quick response time, they are frequently utilised in salivary analysis. Salivary analysis can be conducted using conventional electrochemical techniques such as amperometry, potentiometry, and voltammetry.[15]

3.4. Chromatographic techniques for salivary analysis

Saliva biomolecules are frequently separated and quantified using chromatographic techniques such as gas chromatography (GC) and high-performance liquid

chromatography (HPLC). Chromatographic techniques are frequently costly and time-consuming, requiring intricate sample preparation and analysis procedures.[16]

3.5. Mass spectrometry for salivary analysis

Saliva contains biomolecules that can be identified and quantified using mass spectrometry (MS), a potent analytical tool. High sensitivity, specificity, and accuracy are just a few of the benefits that MS offers for salivary analysis.[17]

4. Salivary Markers for Periodontal Diseases

Chronic periodontitis is the most prevalent form of destructive periodontal disease.[18] For clinical investigators and practitioners alike, diagnosing active phases of periodontal disease and identifying patients at risk for the disease present challenges. Researchers must develop novel diagnostic tests that prioritise early recognition of the microbial challenge to the host. Ideal novel approaches would accurately identify the presence of current disease activity, predict sites vulnerable for future breakdown, and evaluate the response to periodontal interventions. Researchers studying the potential application of oral fluids, like saliva, for disease evaluation are now studying periodontal disease diagnosis.

Oral fluid, or saliva, is a reflection of the body. It could be used to track the development of particular diseases as well as general health. Biomarkers are indicators of health that can be used to track illness onset, course, response to treatment, and outcome.[19] They can be produced by healthy persons or by those with certain systemic disorders. Although they do not measure disease activity, clinical parameters like probing depth, attachment level, bleeding on probing, plaque index, and radiographic assessment of alveolar bone loss provide information on the severity of periodontitis. In contrast, host response analysis, genetic analyses, and microbiological tests have been proposed as ways to monitor and identify patients at increased risk for periodontitis.[20]

5. Markers Affecting Dental Biofilm

5.1. Specific markers

5.1.1. Immunoglobulins

Saliva contains significant particular defence components called immunoglobulins (Ig). Secretory IgA, or sIgA, is the main immunoglobulin found in saliva. It is produced by the salivary glands' plasma cells. Saliva also contains comparatively smaller amounts of IgG and IgM. IgA, IgG, and IgM affect bacterial adhesion or impede bacterial metabolism to affect the oral microbiome. IgA is divided into two subclasses: IgA1 and IgA2.[21] Whereas IgA2 is more prevalent in external secretions including tears, saliva, and milk, IgA1 predominates in serum.[22]

The diagnostic potential of certain salivary immunoglobulins that target periodontal infections has also been investigated. According to research by Eggert et al., the saliva of patients receiving treatment for periodontitis had greater levels of IgA and IgG against the periodontal pathogens Porphyromonas gingivalis and Treponema denticola than did the saliva of control participants.[23] Patients with advanced periodontitis had higher quantities of salivary IgG against Aggregatibacter actinomycetemcomitans, according to Sandholm et al.[24]

5.1.2. Salivary enzymes

Oral microbes, polymorphonuclear leukocytes, salivary glands, and oral epithelial cells originating from gingival crevicular fluid (GCF) can all create salivary enzymes.

5.1.2.1. Lysozyme. it is an antibacterial enzyme that breaks down chemical connections in the cell walls of bacteria. It has the ability to hydrolyze glycosidic connections in the peptidoglycan cell wall of some bacterial species, lysing them. Additionally, it may interact with salivary proteases and monovalent anions to promote bacterial cell lysis. The activation and dysregulation of endogenous bacterial autolysins is likely what causes the destabilisation of the cell membrane caused by this combination. Individuals who have low salivary lysozyme levels are at a higher risk of developing plaque, which is linked to periodontal disease.[25]

5.1.2.2. Peroxidase. It is an enzyme found in saliva that is produced by the salivary glands' acinar cells. This enzyme lowers acid generation in the dental biofilm and eliminates harmful hydrogen peroxide created by oral microbes, which lowers plaque buildup and the development of gingivitis and caries. Saliva from patients with periodontal disease has shown elevated levels of this enzyme.[26]

5.1.3. Salivary ions

5.1.3.1. Calcium. The ion that has been investigated the most as a possible marker for periodontal disease in saliva is calcium (Ca). Sewon et al. found that periodontitis patients' entire stimulated saliva had a greater quantity of calcium. The scientists came to the conclusion that patients with periodontitis had higher Ca concentrations in their saliva. However, it is unclear how important salivary calcium content is in relation to periodontal disease progression. Ca's distribution suggests that there is little chance of using this ion as a marker for periodontal disease.[27]

6. Non Specific Markers

6.1. Mucins

Glycoproteins known as mucins are secreted by many small salivary glands as well as the submandibular and sublingual

salivary glands. The preservation of viscoelasticity in secretions, lubrication, defence against dehydration, and cytoprotection are the physiological roles of the mucins (MG1 and MG2). A. actinomycetemcomitans is known to interact with the mucin MG2, which influences bacterial aggregation and adhesion. Therefore, a decrease in MG2 content in saliva may lead to an increase in A. actinomycetemcomitans colonisation.

6.2. Lactoferrin

Salivary glands create a glycoprotein called lactoferrin, which binds iron and prevents microorganisms from growing by removing it from the surrounding environment. When gingival inflammation occurs, lactoferrin is highly elevated in mucosal secretions and is found in greater concentrations in the saliva of periodontal disease patients than in healthy individuals.[28]

6.3. Fibronectin

A glycoprotein called fibronectin encourages some bacterial species to adhere and colonise while suppressing others. In addition to chemotaxis, migration, inflammation, wound healing, and tissue repair, it facilitates cell adhesion.[29]

6.4. Histatin

Salivary glands in the parotid and submandibular regions secrete a protein called histatin, which has antibacterial qualities. It counteracts the endotoxic lipopolysaccharides found in gram-negative bacteria's membrane. Additionally, histatin inhibits the bacterial and host enzymes responsible for the periodontium's demise. Histatin has antibacterial properties as well as the ability to suppress mast cell histamine production, which impacts the mast cells' involvement in oral inflammation.[30]

6.5. Cystatins

Proteolytic enzymes called cystatins (also known as cysteine proteinases) are derived by inflammatory cells, fibroblasts, osteoclasts, and pathogenic microorganisms. The collagenolytic activity of these enzymes has the potential to degrade tissue. Cystatins are naturally occurring inhibitors of cysteine proteinases, and they may work by adjusting the activity of the periodontal enzymes. Saliva is one of the many bodily fluids and tissues that contain cystatins. Saliva from the submandibular and sublingual salivary glands, as well as to a lesser extent from the parotid gland, were shown to contain cystatins.[31]

6.6. Platelet activating factor

Inflammation is effectively mediated by the phospholipid platelet activating factor (PAF). A positive association between periodontal inflammation and PAF was discovered by Garito et al. Similar results were found in a number of other investigations, however none of the authors addressed the data' possible diagnostic importance.[32]

6.7. Aminoacids

Numerous research have looked at the relationship between periodontal health and salivary levels of free amino acids. Elevated amounts of various amino acids, particularly proline, seem to be present in some cases. These amino acids most likely show up in entire saliva due to proline-rich salivary protein breakdown or bacterial metabolism. The same researchers discovered no variations in salivary amino acid concentrations between patients with progressing periodontitis and controls in another investigation. The scientists came to the conclusion that there is no diagnostic value for periodontal disease associated with the amounts of amino acids in oral secretions, especially GCF.[33]

7. Growth Factors

7.1. Epidermal growth factors

Epidermal growth factor (EGF) stimulates epithelial cells by acting like a hormone and has a role in the healing of oral wounds. The primary human source of EGF is the parotid gland.[20]

7.2. Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), sometimes referred to as vasculotropin or vascular permeability factor, is a multipurpose angiogenic cytokine that plays a key role in wound healing and inflammation. It was discovered that this cytokine was present in entire saliva. Patients with periodontitis had entire saliva that had higher amounts of VEGF.[20]

8. Epithelial Keratins

Saliva contains epithelial cells from the oral cavity lining, however it is unknown how much of the total number of salivary epithelial cells is made up of crevicular or pocket epithelial cells. Additionally, the use of monoclonal antibodies to identify keratins may be useful in the diagnosis of tumours, odontogenic cysts, mouth cancer, and epithelial dysplasia.

There are no studies that show a correlation between the quantity or kind of epithelial cells or particular kinds of keratins in saliva and the advancement of periodontitis, despite the suggestion that phenotypic markers for junctional and oral sulcular epithelia may one day be utilised as markers of periodontal disease.[34]

9. Hormones

9.1. Cortisol

According to recent research, emotional stress may increase the chance of developing periodontitis. A possible explanation for this correlation is that emotional stress-induced high serum cortisol levels have a potent inhibitory influence on the immune system and inflammatory process. Salivary cortisol levels were shown to be higher in people with severe periodontitis.[35]

10. Inflammatory Cells

Each person has a different quantity of leukocytes in their saliva, and each person's cell counts change during the day. The gingival crevice is the main entry point for salivary leukocytes into the oral cavity. Klinkhammer established the orogranulocytic migratory rate (OMR) and standardised the collecting and counting of leukocytes in saliva.[36]

11. Bacteria

Certain bacterial species that inhabit the subgingival environment have been linked to the development of periodontal disease. It has been proposed that salivary components can serve as a substrate for oral plaque germs, allowing them to persist in saliva. It was demonstrated that Actinomyces viscous and oral Streptococus species could grow in saliva. Rosenberg et al. described the Oratest, an oral microbial rinse test. It was discovered in this study that Oratest is a straightforward technique for evaluating oral bacteria levels. The Oratest offers a credible measure of gingival inflammation, according to the authors of a companion study that found a correlation between test findings and plaque index and gingival index scores.[37]

12. Volatiles

Oral malodor is linked to volatile sulphur molecules, mainly hydrogen sulphide and methylmercaptan. It has been proposed that salivary volatiles may serve as both a diagnostic sign and a contributing component in periodontal disease. Estimating oral malodor based on saliva produced a significant connection with objective criteria in a study on self-estimation of oral malodor[37].

13. Markers of Periodontal Soft Tissue Inflammation

Proinflammatory cytokines are secreted from connective tissue fibroblasts, polymorphonuclear leukocytes, junctional epithelial cells, and macrophages. These cytokines include prostaglandin E2 (PGE2), interleukin (IL)-1beta, IL-6, and tumour necrosis factor-alpha. Alveolar bone and connective tissue collagen are degraded by enzymes produced by polymorphonuclear leukocytes and osteoclasts, including matrix metalloproteinase (MMP)-8, MMP-9, and MMP-13. According to studies, PGE2 increases capillary permeability and is a strong vasodilator, which causes redness and edema to manifest clinically. Moreover, it activates osteoclasts and fibroblasts to produce more MMPs.[38]

14. Markers in Saliva Via GCF

Biological molecular markers are extracted from the surrounding tissues as GCF passes through inflammatory periodontal tissues on its way to the sulcus, where they are then eluted into the whole saliva.

15. Markers of Alveolar Bone Loss

Many biomarkers linked to the bone formation, resorption, and turnover of bone. These mediators are linked to both systemic disorders and local bone metabolism.

15.1. MMPS

These are the host proteinases in charge of tissue remodelling as well as destruction. Gingival and periodontal ligament collagens are broken down by host cell-derived interstitial collagenases as periodontal deterioration progresses.

15.2. MMP-8

It is the most common MMP discovered in GCF and diseased periodontal tissue. Using a quick point-of-care microfluidic device, it was recently shown that patients with periodontal disease had significantly higher levels of MMP-8 in their saliva. Research is necessary to assess MMP-8 in order to monitor therapy interventions and forecast the likelihood that a disease will manifest in the future, either by itself or in combination with other molecular biomarkers.[39]

15.3. MMP-9

Produced by neutrophils, this additional collagenase component breaks down the collagen intercellular ground substance. Teng et al. discovered that patients with progressive attachment loss had mean MMP-9 levels that were two times higher. These findings suggest that MMP-9 may be used in the future to diagnose oral fluid, which could help with periodontal therapy monitoring.[40]

15.4. MMP-13

Another collagenolytic MMP with a remarkably broad substrate specificity is also known as MMP-13. In the future, MMP-13 might be helpful for measuring the effectiveness of treatment as well as for identifying and tracking the progression of periodontal disease.[41]

15.5. Telopeptide

Pyridinoline cross-links, such as the carboxyterminal telopeptide of type I collagen cross-linked with pyridinoline, have the specificity and sensitivity for bone resorption, making them a potentially useful diagnostic tool for periodontal disease. Pyridinoline cross-links have been investigated in a number of studies to see if they may identify bone resorption in periodontitis and in response to periodontal therapy. Thus far, interesting findings have been obtained from these investigations evaluating the function of GCF carboxyterminal telopeptide of type I collagen levels as a diagnostic marker of periodontal disease activity.[42]

15.6. Osteocalcin

Serum osteocalcin levels have been reported to be elevated at times of fast bone turnover, such as in multiple myeloma and osteoporosis, as well as during fracture repair. Consequently, research has looked into the connection between periodontal disease and GCF osteocalcin levels.[43]

15.7. Osteopontin

It is a single-chain polypeptide with an around 32,600 molecular weight. Osteopontin is substantially concentrated in the bone matrix at the locations where osteoclasts adhere to the underlying mineral surface (i.e., the plasma membrane's clear zone attachment regions). According to periodontal research findings, GCF's osteopontin concentrations rose in direct proportion to the severity of the condition. Additionally, when nonsurgical periodontal therapy was administered, GCF's osteopontin levels were dramatically lowered.[44]

16. Systemic Markers

16.1. Reactive protein

The liver produces C-reactive protein in response to circulating cytokines from systemic and/or local inflammation, including periodontal disease, such as interleukin-1 and tumour necrosis factor-alpha. Saliva may be exposed to circulating C-reactive protein through the salivary glands or GCF. Elevated C-reactive protein levels have been linked to many inflammatory indicators, as well as aggressive and chronic periodontal disorders.Using a lab-on-a-chip technique, it has recently been demonstrated that C-reactive protein is detectable in saliva from individuals with periodontal disease.[45]

16.2. Oxidative stress marker

Oxidative stress can be caused by an overabundance of free radicals or by the antioxidant systems becoming less effective. It is characterised as the outcome of an imbalance between oxidant factors and protective antioxidant systems. In patients with periodontitis, oxidative stress is increased. The oxidised nucleoside 8 hydroxydeoxyguanosine (8-OHdG) is eliminated in body fluids along with DNA repair. It has been shown that the oxidative stress biomarker 8-OHdG can function as a biomarker in body fluids. Research has demonstrated that saliva is a biological product that is readily obtained and may be utilised to quantify 8-OHdG as an oxidative stress biomarker for periodontitis diagnosis and therapy monitoring.[46]

17. Emerging Salivary Biomarkers

There are strong arguments for using saliva as a diagnostic fluid to track the onset and progression of periodontal diseases. Over the past five years, the National Institute of Dental and Craniofacial Research has spearheaded a number of initiatives that have elevated the use of saliva for translational and clinical applications. Of particular relevance to periodontal diseases are the developing toolboxes of the salivary transcriptome and the salivary proteome for early disease detection, disease progression, and therapeutic monitoring.[47] With the help of these emerging technologies, we have demonstrated the use of salivary proteins and RNAs to detect Sjogren's syndrome and oral cancer. The stage is now prepared to employ these technologies for translational and clinical applications in periodontal diseases.[48]

17.1. Salivary proteome

Proteomics is the study of the expressed section of the genome, and the proteome is the genome's complement of proteins. The great therapeutic potential of bodily fluid proteomes as sources of disease indicators makes them valuable. Theoretically, a worldwide examination of the human salivary proteomes can offer a thorough range of dental and overall health. Moreover, salivary proteome profiling throughout complications may reveal early morbidity markers and track the development of the disease. The cataloguing of human saliva proteins and the investigation of their posttranslational modifications have advanced significantly. Hu et al. found 309 unique proteins in human entire saliva by combining "shotgun" proteomics techniques with two-dimensional gel electrophoresis mass spectrometry[46]. 1166 salivary proteins in all have been discovered; Denny et al. isolated 914 from the parotid fluid and 917 from the combined submandibular and sublingual fluids.[49]

17.2. Salivary transcriptome

The idea of the salivary transcriptome is still developing. Apart from the salivary proteome, it was also shown that salivary transcriptomes, or RNA molecules, exhibit exceptional stability in saliva. Among them were mRNA

molecules, which are used by cells to transmit the instructions that DNA carries for the synthesis of subsequent proteins. This finding opens up a new direction for salivary transcriptome diagnostics by presenting a second diagnostic alphabet in saliva. Li et al.'s discovery that RNA molecules prevalent in oral cancer tissues are also found in saliva led them to investigate the variety and complexity of RNA found in human saliva.[50] The capacity to use the salivary transcriptome down to the exon level has recently improved, allowing us to increase the diagnostic resolution of the transcriptome from 185 to 851 diagnostic units, or nearly seven times. This improved feature, which offers greater resolution to stratify the patient population, therapy responsiveness, and illness recurrences, strengthened the diagnostic alphabet's clinical value.[51] We have recently established highly clinically discriminatory panels of salivary proteins and mRNAs for the detection of Sjogren's disease and oral cancer using the salivary proteome and transcriptome as diagnostic toolboxes. This proteome-wide saliva tool is intended to be used to find markers for periodontal disease patients' early identification, disease progression, and therapy monitoring.[52]

18. Conclusion

Similar to blood, saliva has a large concentration of protein and nucleic acid molecules that indicate physiological state; however, salivary diagnostics, in contrast to other bodily fluids, provide a simple, affordable, secure, and noninvasive method for identifying diseases and have the potential to completely transform the diagnostics of the future.

The use of saliva-based oral fluid diagnostics to identify periodontal illnesses and predict the results of periodontal therapy appears promise, despite the obstacles still to be overcome. The fast developing discipline of salivary analysis has the potential to revolutionise how we track and diagnose illnesses. The basic elements of saliva, such as its composition, functions, biomarkers, samples, and analysis, were first covered. In order to create workable salivary analysis systems, we underlined the significance of salivary biomarkers in addition to sampling and analysis methods. Numerous potential salivary biomarkers have surfaced recently, offering new information on a range of illnesses that need for routine detection and observation.

19. Conflict of Interest

None.

20. Source of Funding

None.

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