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Salivary Biomarkers – a diagnostic tool?

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ABSTRACT
Human saliva plays an important role in the health of the oral cavity and of the body as a whole. Salivary diagnostics is a dynamic and emerging field in the diagnosis of oral and systemic diseases. Saliva reflects the physiologic state of the body, including emotional, endocrinal, nutritional, and metabolic variations. The collection of saliva samples is non-invasive, safe, and inexpensive.The future of saliva-based techniques for early diagnosis of dental diseases, is promising and may offer a robust alternative for clinicians to use in the near future to make clinical decisions.

Keywords –Saliva, Diagnosis, Salivary Biomarkers,Oral Cancer

I. INTRODUCTION

Saliva is an accessible biofluid that contains elements derived from the mucosal layer surfaces, gingival crevices, and tooth surfaces of the mouth. Salivary secretion additionally contains microorganisms that colonize the mouth and other alternative exogenous substances that can probably offer an insight into the connection of the host with the oral atmosphere[1].

What was once deemed simply a digestive fluid is currently being considered a biological fluid capable of communicating an individual's current health standing. Continued efforts in this field could lead on to the establishment of clinically acceptable assays for the detection and observation of distinct disease states throughout the body. Here we review the use of saliva in molecular nosology and its potential future as a preferred mode of patient analysis.

NEED FOR A NEW BIOMARKER

WHAT IS A BIOMARKER?

According to Websters,Biomarker is defined as a distinctive biological or biologically derived indicator (such as a metabolite) of a process, event, or condition (such as aging, disease, or oil formation.

WHY DO WE NEED A BIOMARKER?

Currently, three major limitations have averted people from recognizing the complete potential of disease detection, and have seriously hindered the event of clinical diagnostics, namely:

1. Lack of definitive molecular biomarkers for specific diseases;
2. Lack of a simple and cheap sampling methodology with least amount of discomfort; and
3. Lack of an accurate, easy-to-use, and transportable platform to facilitate early disease detection.

Saliva, an oral fluid that contains an abundance of proteins and genetic molecules and is quickly accessible via a very non-invasive approach, has long been recognized as the potential answer to those limitations.[2]

In the sector of Periodontology, ancient clinical criteria are usually meagre for determining sites of active disease, for monitoring the response to medical care or for measuring the degree of susceptibility to future disease progression.

PERIODONTITIS AND SALIVA

Periodontitis is a chronic inflammatory disease that compromises the integrity of the tissues that support the teeth, that embrace the gingiva, periodontal ligament, dental cement, and alveolar bone, and are conjointly referred to as the periodontium [3,4].Currently, the diagnosing of periodontal disease needs rapidity, sensitivity, and specificity since determining the stage within which the patient is found is prime for a decent treatment; for this reason molecules are presently being sought that change once the person is healthy and once the person has the disease.

SALIVARY BIOMARKERS

The definition of biomarkers as established by the National Institute of Health (NIH) is as follows:biomarkers are the biological, biochemical, anthropometric, physiological, etc. characteristics, which are objectively measurable, capable of identifying physiological or pathological processes, or a pharmacological response or a therapeutic intervention [5]

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SPECIFIC MARKERS

1.Immunoglobulins (Ig)

Immunoglobulins (Ig) are vital specific defense factors of saliva. The predominant immunoglobulin in saliva is secretory immune globulin (sIgA), that springs from plasma cells within the secretory glands. Lesser quantity of immunoglobulin G and immunoglobulin M are found in saliva. IgA, IgG, and IgM influence the oral microbiota by meddling with the bacterial adherence or by inhibiting bacterial metabolism.[6] Specific immunoglobulins in saliva directed towards periodontal pathogens have additionally been examined for their diagnostic potential.

2.Salivary enzymes

Lysozyme is an antimicrobial protein with the flexibility to cleave chemical bonds within the bacterial cell wall. It can lyse some bacterial species by hydrolyzing glycosidic linkages within the cell wall peptidoglycan. It may additionally cause lysis of bacterial cells by interacting with monovalent anions and with proteases found in saliva. This combination ends up in destabilization of the cell membrane, most likely as a result of the activation and deregulation of endogenous bacterial autolysins. Patients with low levels of lysozyme in saliva are additionally vulnerable to plaque accumulation, that is taken into account a risk factor for periodontitis.[7]

Peroxidase is a salivary protein produced by acinar cells within the salivary glands. This protein removes noxious hydrogen peroxide produced by oral microorganisms and reduces acid production within the dental biofilm, thereby decreasing plaque accumulation and also the establishment of gingivitis and dental caries. Patients with periodontitis have incontestible high levels of this enzyme in saliva.[8]

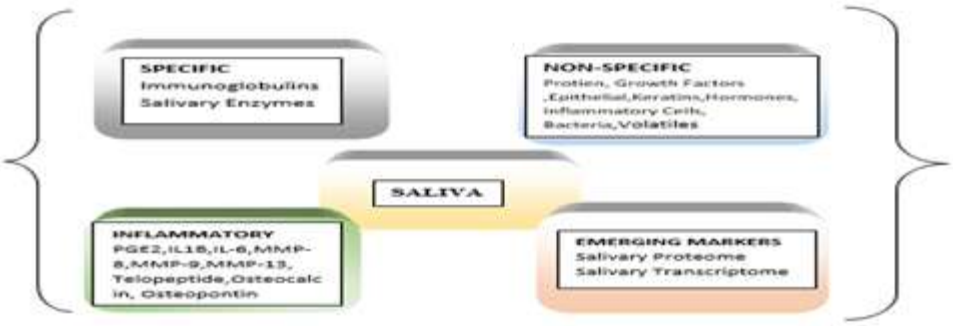
3.Salivary ions

Calcium (Ca) is the ion that has been most intensely studied as a possible marker for periodontitis in saliva. however, the importance of the secretion Ca concentration in relationship to progression of periodontitis isn't outlined. Considering the distribution of Ca, this ion seems to carry restricted promise as a marker for periodontitis.[9]

NON-SPECIFIC MARKERS

1.Protiens

Other proteins: A variety of studies have examined the correlation between nonenzymatic, non-immunoglobulin proteins in saliva and periodontitis.



Mucins are glycoproteins produced by submandibular and sublingual salivary glands and various minor secretion glands. The physiological functions of the mucins (MG1 and MG2) are cyto-protection, lubrication, protection against dehydration, and maintenance of viscoelasticity in secretions. The mucin, MG2, affects the aggregation and adherence of bacteria and is thought to act with A. actinomycetemcomitans, and a reduced concentration of MG2 in saliva might increase colonization with this periodontopathogen.

Lactoferrin is an iron-binding glycoprotein produced by salivary glands, that inhibits microbic growth by sequestering iron from the environment, therefore depriving bacteria of this essential part. Lactoferrin is powerfully upregulated in mucosal secretions throughout gingival inflammation and is detected at a high concentration in saliva of patients with periodontitis compared with healthy patients.[10]

Histatin is a salivary supermolecule with antimicrobial properties and is secreted from salivary gland and submandibular glands. It neutralizes the endotoxic lipopolysaccharides situated within the membrane of gram-negative bacteria. Histatin is additionally an inhibitor of host and bacterial enzymes concerned in the destruction of the periodontium. Additional to its antimicrobial activities, histatin is involved in the inhibition of the release of histamine from mast cells, influencing their role in oral inflammation.[11]

Fibronectin is a glycoprotein that promotes selective adhesion and colonisation of

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certain bacterial species whereas inhibiting others. It mediates adhesion between cells and is additionally concerned in chemotaxis, migration, inflammation, and wound healing and tissue repair.

Cystatins (cysteine proteinases) are proteolytic enzymes originated from morbific bacterium, inflammatory cells, osteoclasts, and fibroblasts. These enzymes have collagenolytic activity, which can cause tissue destruction. Cystatins are physiological inhibitors of cysteine proteinases, and will operate by modulating protein activity within the periodontium.[12]

Platelet activating issue (PAF) may be a potent phospholipid mediator of inflammation. Garito et al. found apositive correlational statistics between PAF and periodontic inflammation.[13]

Amino acidsMany studies have examined the degree of free amino acids in saliva in respect to periodontal standing. It seems that in some patients, elevated levels of certain amino acids, particularly proline, is also detected.[14,15].These amino acids most likely appear in whole saliva as a results of bacterial metabolism or degradation of secretion proteins rich in amino acid.

2.Growth factors

Epidermal growth factor (EGF) is concerned in oral wound healing and functions with hormone-like properties to stimulate epithelial cells. In humans, the parotid gland is the major source of EGF.

Vascular endothelial growth factor (VEGF), also called vascular permeability factor or vasculotropin, maybe a multifunctional angiogenic protein necessary in inflammation and wound healing. This protein was found to be an element of whole saliva. Higher levels of VEGF were detected in whole saliva from periodontitis patients.[16]

3.Epithelial keratins

Epithelial cells from the lining of the oral cavity are found in saliva, however the contribution of crevicular or pocket epithelial cells to the overall variety of salivary epithelial cells isn't acknowledged.[17]Moreover, detection of keratins by monoclonal antibodies could have diagnostic worth within the detection of epithelial dysplasia, carcinoma, odontogenic cysts, and tumors.[18]

4.Hormones

Cortisol: Recent studies have denoted that emotional stress may be a risk factor for periodontal disease.One mechanism projected to account for the connection is that elevated serum corticosteroid levels related to emotional stress exert a robust repressive result on the inflammatory process and reaction.[19] The presence of corticosteroid in saliva has been recognized for over forty years.[20] It are often argued that additionally to changes within the host response (ie, smoking, poor oral hygiene, and poor compliance), stress conjointly results in behavioural changes, that may have a major result on the periodontium..[21]

Bacteria:Specific species of bacteria colonizing the subgingival setting are involved within the pathologic process of periodontitis.[22] De jong et al., stated that microorganisms from supragingival plaque were grown on salivary agar.The authors concluded that the supragingival microflora may utilize saliva as a complete nutrient source.[23]

Volatiles: Volatile sulfur compounds, primarily hydrogen sulfide and methylmercaptan, are related to oral malodour. Salivary volatiles have been suggested as potential diagnostic markers and causative factors in periodontitis.[24]

Markers of periodontal soft tissue inflammation: Proinflammatory cytokines, like prostaglandin E2 (PGE2), interleukin (IL)-1beta, IL-6, and tumor necrosis factor-alpha are released from cells of the junctional epithelial tissue, connective tissue fibroblasts, macrophages, and polymorphonuclear leukocytes. Enzymes, like matrix metalloproteinase (MMP)-8, MMP-9, and MMP-13, produced by polymorphonuclear leukocytes and osteoclasts, all result in the degradation of connective tissue collagen and alveolar bone.[25]

Markers of alveolar bone loss: Many different biomarkers related to bone formation, resorption, and turnover, like alkaline phosphatase, osteocalcin, osteonectin, and collagen telopeptidases, are evaluated in GCF and saliva.[26]

INFLAMMATORY CELLS

Matrix metalloproteinases (MMP):They are host proteinases accountable for each tissue degradation and remodelling throughout progressive periodontal breakdown, gingival and periodontal ligament collagens are cleaved by host cell–derived interstitial collagenases.

MMP-8:It is the foremost prevalent MMP found in diseased periodontal tissue and GCF.MMP-8, either alone or in conjunction with different molecular biomarkers, to predict the chance of future disease prevalence and to observe treatment interventions.[27]

Gelatinase (MMP-9): Another member of the collagenase family, is produced by neutrophils and degrades collagen intercellular ground substance.[28]

Collagenase-3: Conjointly remarked as MMP-13, is another collagenolytic MMP with an exceptionally wide substrate specificity.[29]

Telopeptide: Carboxyterminal telopeptide of type I collagen has been shown to be a promising predictor of each future alveolar bone and attachment loss.Salivary carboxyterminal telopeptide of type I collagen as a predictor of periodontal tissue destruction, disease activity and response to therapy in periodontal patients.[30]

Osteocalcin: Elevated serum osteocalcin levels are found in periods of rapid bone turnover, like in osteoporosis and multiple myeloma and through fracture repair. When a combination of the biochemical markers osteocalcin, collagenase, prostaglandin E2, alpha-2 macroglobulin, elastase, and alkaline phosphatase was evaluated, increased diagnostic sensitivity and specificity values.[31]

Osteopontin:Periodontal studies indicated that osteopontin concentrations in GCF increased proportionately with the progression of disease; and once nonsurgical periodontal treatment was provided, the osteopontin levels in GCF were considerably reduced.[32]

Systemic markers:C-reactive protein is produced by the liver and is stimulated by circulating cytokines, like tumor necrosis factor-alpha and interleukin-1, from local and/or systemic inflammation like periodontal inflammation.C-reactive protein are related to chronic and aggressive periodontal diseases and with different inflammatory biomarkers.[33]

II. DISCUSSION

Interest in saliva as a diagnostic medium is on an all time surge because of its several advantages over different diagnostic biofluids. Each saliva and blood serum contain similar proteins and ribonucleic acid, that is why saliva is taken into account a "mirror to the body."[34]Saliva is readily available which makes the collection method fairly simple, even when multiple samples are required. Its assortment is non-invasive, that makes the procedure more acceptable to patients and also more conducive to a stress-free appointment. Several hazards related to blood assortment don't apply to saliva. There's no potential for cross-contamination among patients when used improperly and present a danger to health care personnel. Saliva is additionally easier to handle because it doesn't clot. As salivary testing becomes more mainstream, the prices may drop below those presently incurred for urine/blood sampling. However, because of the individuality of the technique, the analysis these days continues to be quite overpriced.Because of the straightforward and non-invasive technique of assortment, salivary diagnostic tests seem to carry promise for the long run.

Novel technologies like lab-on-a-chip and microfluidic devices have the potential to manage complicated oral fluids, like complex and GCF, and to produce a determination of a patient's periodontal disease-risk profile, current disease activity, and response to therapeutic interventions. This approach ought to accelerate clinical decision-making and observation of episodic disease progression in a chronic infectious disease like periodontitis.[35]

III. CONCLUSION

The determination of biomarkers in saliva is changing into a crucial a part of laboratory diagnostics and therefore the prediction of periodontal and other diseases. Biomarkers in saliva can also serve to determine the context of periodontal disease with an altered state of the body or with chronic diseases. Saliva conjointly contains a series of markers, which can predict the risk of some systemic disease development.Although challenges stay ahead, the utilization of saliva-based oral fluid diagnostics appear promising for future application to diagnose periodontal diseases and to foretell periodontal treatment outcomes.

Conflict of interest – No conflict of interest

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