Biochemistry in oral cavity

Anjana Goyal[1], Sumit Bhateja[2*], Reena Doomra[3], Aarushi Jain[4]

[1-3]HOD, [2]II Year Student, [1]Dept. of Biochemistry, [2]Dept. of Oral Medicine and Radiology, [3]Dept. of Pharmacology, [1-3]Manav Rachna Dental College, Faridabad, Haryana, India

***Corresponding Author: Sumit Bhateja**
Email: sumit.mrdc@mrei.ac.in

Abstract
Saliva is a unique and complex oral fluid both in sources and composition. More than 2300 proteins and peptides are present in human saliva. Saliva testing, a non-invasive alternative to serum testing may be an effective modality for diagnosis and prognosis prediction of various diseases, as well as monitoring post therapy status by measuring specific salivary macromolecules, examining proteomic and genomic targets such as enzymes, cytokines, growth factors, metalloproteinases, DNA transcripts etc.by employing highly sensitive new tools and technologies.

Keyword: Biomarkers, Oral cavity, Periodontitis.

Introduction
The hypotonic biofluid present in oral cavity, saliva, secreted by three major salivary glands (parotid, submandibular, sublingual), minor salivary glands along with gingivitis cervicular fluid, oral mucosa translate, secretions from nasal and pharyngeal mucosa, non-adherent bacteria, desquamated oral epithelial cells, keratin debris, blood cells and sometimes, food and medication residues, contains 99.5% water and 0.5% volume comprises of amino acids, histatins, cystatins, defensins, statherins, lysozyme, proline rich proteins, carbonic anhydrases, peroxidases, lactoferins, mucus, secretory immunoglobulins, lipids together with various ions like potassium, calcium, chlorides, sodium and phosphates. So far more than 2300 proteins and peptides have been found in human saliva. The main functions of saliva are lubrication, digestion, antimicrobial action, facilitation of tooth remineralization, taste sensation, buffering etc.

Another emerging significant use of saliva is helping in diagnosis of various diseases of oral cavity through the detection of biochemical markers which develop or occur in the salivary fluid during oral diseases.[1] One of the major reasons for it is its easy accessibility, simple easy and non-invasive collection with low cost and easy storage. But still the use of saliva as a diagnostic tool is yet to become mainstream because the levels of most analytes, though, found both in blood serum and saliva, are substantially diminished. For example, in normal adults IgA levels are 2.5 - 5 mg/ml in blood and 250 - 500 ug/ml in saliva.[2]

A lack of saliva or reduced amount of saliva in oral cavity results in a painful tongue and mucosa, problems with taste, swallowing, chewing and phonation, tooth decay and tooth loss and increased risk of infections.

Oral Tissue Decadence
Major biochemical changes in most widespread oral diseases like pulpitis, gingivitis, periodontitis, dental caries, pulpal and periapical diseases as well as oral cancer, which is the most cancer in male patients in India, and other chronic conditions occur due to tissue damage and collagen matrix degeneration through matrix metalloproteinases (MMPs), apoptosis, reactive oxygen species as well as antioxidants.

Infection induced MMPs cleave matrix component through hydrolysis and use zinc ions for their action, their inhibition is controlled by tissue inhibitors of metalloproteinases (TIMPS). MMPs also play an important role in regulation of cellular communication, molecular shedding and immune functions by processing bioactive molecules including cell surface receptors, cytokines, hormones, defensins, adhesion molecules and growth factors and releasing them from the cell surface.

Antioxidants like Vitamin C, Vitamin A, Vitamin E, thiol group containing substances etc. though prevent injury and damage of oral as well as other tissues but other antioxidants like catalase (CAT), glutathione S-transferase (GST) etc. also catalyze the oxidation of other molecules so they perform dual purpose in the body.

Free radicals are generated by compounds called as pro-oxidants, antioxidants decrease the number and action of free radicals. Free radicals arising from free oxygen radicals like superoxide, hydroxyl, acyloxyl etc. can cause serious cell damage due to their single unpaired electron and high reactivity because of it and excess of them can cause oxidative stress i.e. number of pro-oxidants exceeds number of antioxidants and creates an imbalance in the body. Nowadays, even non-radicals which cause radical changes in extracellular and intracellular environment are included in reactive oxygen species (ROS). These reactive species are produced exogenously in smoking, radiation, ultrasound etc. as well as endogenously in cell metabolism, phagocytosis or 'respiratory burst'.

When bacterial pathogens enter host polymorphonuclear leukocytes (PMN) are the first defense cells to be made and then macrophages and other defense cells are produced. Enzymatic complexes like NADPH oxidase increase ROS production and catalyze oxygen and free radical production by PMN via 'respiratory burst' during phagocytosis, which kills bacteria as well as surrounding cells and matrices. Non-radicals species like

HOCl are also generated by azurophilic enzyme myeloperoxidase (MPO) during phagocytosis degranulation.

Cytokines also contribute to connective tissue degradation. They increase MMP activation and also act as substrates for their action.

Thus, these substances when increase in amount in blood serum and saliva impair the healing responses of the body and enhance the dental disease progression and also act as biochemical markers, their measure having significant diagnostic consequence in detection of disorders at an early stage.

Biomarkers

Biochemical markers or biomarkers are substances released in the body by body cells or tumor cells in response to any malignant or destructive process occurring in the body. These are wide-spectrum bio-macromolecules synthesized in excess concentration by a wide variety of neoplastic cells and can either be normal endogenous products that are formed at a greater rate in cancer cells or the products of the newly switched on genes that remained quiescent in the normal cells.

An ideal biomarker should be highly sensitive and specific and should not give false high or low predicting values and should be accurate in differentiating between healthy individuals and patients. It should be able to differentiate between neoplastic and non-neoplastic conditions and detect occurrence at an early stage, should be easily assayable and should indicate all changes occurring patient during treatment.

Biomarkers can be epithelial, mesenchymal, muscle, neural, endothelial, melanocytic, lymphoid, neuro-endocrine, meta-static tumor, minor salivary gland tumor markers, Ewings tumor markers and primitive neuroectodermal tumor markers. They are also classified on the basis of their use in dentistry as markers for odontogenic cysts and tumors, oral cancer, TMJ diseases, bone activity, TB, auto antibodies as markers, micronutrients as markers and miscellaneous markers.

These biomarkers are detected through isolation by Immunohistochemistry (IHC), Polymerized Chain Reaction (PCR) and enzyme-linked immuno-sorbent assay (ELISA) techniques. Once the patient is positive for a particular marker the biomarker can be used as a monitoring tool for the effectiveness of the therapy. The biomarker tests are mainly to help diagnose the condition, monitor the disease process, stage the disease, assess the prognosis, guide and monitor the treatment and determine the recurrence.

Oral Biochemistry in Dental Caries

Dental caries a multifactorial infectious disease in which there are complex interactions among acid-producing bacteria, fermentable carbohydrates and many host factors.

Salivary Bacteria

Earlier research has given a lot of information about connection between dental caries and salivary bacteria.

1. Bacteria associated with dental caries are mutants streptococci[3] in which Streptococcus mutants (S. mutans) and Streptococcus sobrinus (S. sobrinus) are most prevalent. S. mutans synthesizes extracellular polysaccharides from sucrose which fosters its firm attachment to teeth and promotes tight cell clustering, fermentation of carbohydrates to acids and tolerance to low pH.[4]
2. Lactobacilli have also been implied to be an important contributor in progression of dental caries due to its highly acidogenic and aciduric nature.[5]
3. Non-mutans like S. oralis, S. sanguinis etc.contribute to the caries growth as well.[6] Also, other oral bacteria like Actinomyces sp. Promotes root surface caries and Candida indicates increased carious risk.

There are 10^8– 10^9 CFU/ml microbes in saliva[7] and this microbial community serves as a biomarker for the disease or caries status in the oral cavity.

Salivary Flow Rate

Another biomarker which can be used as a diagnostic tool is salivary flow rate. Low salivary flow rate is a risk factor for caries incidence. Saliva clearance takes less time than bacterial cell division, thus, bacteria cannot survive in the oral cavity unless they bind to the teeth or oral mucosa. When unstimulated salivary flow rate is less than 0.30 ml/min and stimulated salivary flow rate is less than 0.70 ml/min it is considered a potential caries risk factor.

Oral PH and Salivary Buffering Capacity

Caries-active individuals have faster acid production, lower oral pH and decreased salivary buffering capacity than caries-free individuals and this causes reduced plaque acid neutralization and decreased remineralization of early enamel lesions.[8]

Salivary Proteins

Salivary proteins like mucins (MUCT) decreases S. mutans levels and decrease caries risk. Lower levels alpha defensins HNPI-3 may increase caries susceptibility in children.[9] Statherins and Cystatins levels when low may be risk indicators for caries. Salivary secretory IgA (SIgA) antibodies also have a relationship with dental caries and the literature is equally divided for and against an anticaries role of SIgA.

Oral Biochemistry in Periodontitis

Periodontitis is a chronic, multifactorial oral condition comprising of a group of inflammatory conditions affecting the supporting structures of the dentition. Salivary biomarkers are effective and efficient tools to measure disease activity and risk for periodontitis.

Immunoglobulin

IgA, IgG and I'm present in saliva inhibit bacterial metabolism and adherence. Immunoglobulin levels in healthy and treated patients is higher to periodontal

pathogens Porphyromonas gingivalis and Treponema denticola than compared to control subjects.[10]

Salivary Enzymes

They are produced from PMNs, oral epithelial cells, salivary glands, oral microbes etc. Patients with low levels of lysozyme are often more susceptible to plaque formation which is a risk factor for periodontal disease.[11] Peroxidase whereas is present in high levels in saliva of patients with periodontal disease.

Salivary Lons

High calcium concentration is essentially detected in periodontitis patients.

Salivary Proteins

The following salivary proteins are altered in amount in saliva during inflammatory diseases.

1. Mucins (MG1 and MG2) produced by submandibular and sublingual salivary glands provide lubrication, protection against dehydration, viscoelasticity in secretions etc. A decreased concentration of MG2 in saliva increases colonization of the periodontopathogen A.actinomycetemcomitans.[12]
2. Lactoferinspresent in saliva prevents bacterial proliferation by sequester ingredients iron from the environment and depriving bacteria of it. It is seen in high concentration in the saliva of periodontal disease patients.
3. Histatins neutralize endotoxin lipopolysaccharide present in bacteria, thus, is an antimicrobial agent and it also inhibits both host and bacterial enzymes involved in destruction of periodontium.
4. Fibronectins promotes adhesion and colonization of certain bacteria while inhibiting others. They are also involved in wound healing and tissue repair.
5. Cystatins (cysteine proteinases) are proteolytic enzymes that have collagenolytic activity and cause tissue damage[13] by modulating enzyme activity in periodontium. These are high in amount in saliva of periodontitis patients.
6. Platelet Activating Factor (PAF) is a phospholipid mediator of inflammation and is increased in concentration in periodontal inflammation.
7. Proline levels are elevated in some patients due to bacterial metabolism or degradation of proline-rich salivary proteins. But it was not so in all the cases so, it was concluded that levels of amino acids don't have diagnostic value in periodontal diseases.

Growth Factors

Epidermal and vascular endothelial growth factors. EGF is involved in wound healing thus, increased in concentration in periodontal patients. Vascular Endothelial Growth Factor or vasculotropin or vascular permeability factor is a multifunctional angiogenic cytokine important in inflammation and wound healing.[13]

Epithelial Keratins

Detection of epithelial Keratins by monoclonal antibodies may have diagnostic value in oral cancer, tumours etc.[15] but no relation between periodontitis and number or type of epithelial cells have been made.

Hormones

Cortisol, emotional stress increases risk of periodontitis. Salivary cortisol levels were high in severe periodontitis patients. But still the diagnosis of disease severity based on cortisol levels is premature and additional studies are required.[13]

Inflammatory Cells

Increased inflammatory cells are seen in periodontal diseases and they can be counted using the Orogranulocytic Migratory Rate (OMR) and used as a laboratory test.[16]

Bacteria

There is presence of periodontopathic microorganisms A. Actinomycetemcomitans, P. Gingivalis, Prevotella intermediate and T. Denticola in saliva of periodontitis patients[14] and these use saliva itself as a nutrient source for their growth. An oral microbial rinse test (oratest) can be utilized to know microbial levels, gingival index score and plaque index and an estimation of inflammation can be done.

Volatiles

Salivary volatiles like methylmercapton or hydrogen sulphide from oral malador in oral cavity and it is increased in amount in periodontal patients.

Oral Biochemistry During Alveolar Bone Resorption and Soft Tissue Inflammation

The alterations in the following substances levels are seen during alveolar bone metabolism in saliva and GCF.

MMP

These are members of collagenase family and control bone remodeling and their levels are elevated during inflammation of alveolar region. MMP-8 is the most prevalent MMP. Another MMP, Gelatinase (MMP-9) produced by neutrophils, increases to twice its concentration in patients with progressive loss of tooth attachment,[17] thus its future can be of great importance in disease diagnosis. MMP-13, Collagenase-3 also increases in inflammation.

Telopeptide

Pyridinoline cross-linked carboxyterminal telopeptide of type 1 collagen isanimportantbiomarker in periodontitis.[18] Its levels gets increased during the disease and indicates tissue destruction.

Osteocalcin

Its concentrationgets increased during fracture repair, multiple myeloma and osteoporosis in the body and similar case is seen in periodontitis.

Osteopontin

It is a polypeptide whose concentration in saliva gets elevated significantly as alveolar bone resorption progresses.

Cytokines like prostaglandin E2 (PGE2), interleukin (IL) –beta, IL-6, tumor necrosis factor – alpha (TNF-alpha), MMP-8, MMP-9, MMP-13 etc. are produced from PMNs, fibroblasts, macrophages etc. and they cause tissue damage, redness and edema[19]. Free radicals damage nucleoside like 8-hydroxydeoxyguanosine (8-OHdG) and it gets excreted in saliva.

Oral Biochemistry in Oral Cancer

Oral cancer causes the 6th most common cancer related death and accounts for 30-40% cancers in India. Its prognosis till date is poor because symptoms appear only at later stage.[20] Thus, chemical changes in serum and saliva can be very helpful in detection, development and course of tumor. These changes are called tumor markers. More than 100 potential tumor markers have been reported.

Three major markers which occur as elevated serum levels are levels of beta 2-microglobulin, total silicon acid (TSA) and lipid-bound sialic acid (LBSA) in oral cancer patients.[21]

Beta-microglobulin is part of Major Histocompatibility Chain (MHC) I having rapid turnover and low molecular weight. Its serum levels may be increased because since beta 2-microglobulin is a part of cell membrane so accelerated cell division in carcinoma leads to elevated levels or may be due to increased active synthesis by tumor cells and increased cell breakdown. There is decrease in body's immune functions in oral cancer because of reduced production and phagocytosis activity of monocytes and higher Beta-microglobulin which causes increased destruction of MHC I particles thus elevating the levels of cytokines: IL-6, IL-10 which increase neoplasm production.

Sialic acids are part of carbohydrate components of cell membrane glycolipids and glycoproteins and influences cell differentiation, development and interaction. Thus, in tumor conditions as the cell number increases the serum levels of Sialic acids and LBSAs due to atypical glycosylation of cell membrane also increases. Also there is de novo synthesis of sialylated sequence due to tumor.

These 3 biomarkers can help in early detection and prognosis of oral cancer as they have good specificity, sensitivity and efficiency.

The major histological changes occurring in the oral cavity during carcinogenic are:

1. Cell proliferation
2. Insensitivity to inhibitory growth signals
3. Evasion of apoptosis and activation of anti-apoptotic genes
4. Sustained angiogenesis
5. Unlimited replication potential
6. Invasion and metastasis ability
7. Genomic instability
8. Proto oncogene mutation

First the saliva sample after collection undergoes centrifugation to remove solid debris from it like desquamated oral epithelial cells, keratin debris, blood cells etc. and then it is stored in a frozen state until further analysis.

The tests utilized to identify the biomarkers are as follows:

1. Non-organic compound biomarkers–Flame photometry, atomic absorption and spectrophotometry
2. Peptide or protein biomarkers –High performance liquid chromatography (HPLC), enzyme-linked immuno-sorbent assay (ELISA), radio – immunoassay, 2D gel electrophoresis (2DE) followed by mass spectrometry, 2DE and reverse phase liquid chromatography followed by LC-tandem Ms, Matrix – assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS)
3. DNA, mRNA or microRNA biomarkers – Polymerase chain reaction (PCR), Quantitative PCR, Microarrays followed by qPCR.
4. Metabolic biomarkers–Capillary electrophoresis TOF MS, HPLC with quadruple/TOF MS
5. Miscellaneous biomarkers –HPLC, Colorimeter assays.

The salivary biomarkers found in the salivary sample in OSSC patients are as follows:

1. IAP – Apoptosis inhibitor
2. SCC - squamous cell carcinoma associated antigen
3. CEA – Carcinogenic embryonic carcinogen
4. CA19-9 – Carcino-antigen
5. CA128, CA125, Cyfra 21-1 –Serum tumour marker and intermediate filament protein
6. TPS – Tissue Polypeptide Specific Antigen
7. RNS, 8-OHdG –Reactive nitrogen species DNA damage marker
8. LDH –Lactate dehydrogenase –marker of tissue breakdown
9. IgG – Immunoglobulin
10. Sec IgA –Mucosal immunoglobulin
11. IGF – Growth Factor
12. MMP-2 and MMP-11 – Metalloproteinases
13. LOH – Loss of heterozygosity –loss of specific chromosomal regions
14. DNA Hypermethylation –Gene inactivation

RNA Biomarkers

1. IL8 and IL 1B –Chemokine – mediator of inflammatory response
2. DUSP1 – Cell proliferation regulator
3. HA3 –Oncogene
4. OAZ1 – Polyamine synthesis regulator
5. S100P –Calcium binding protein, cell cycle and differentiation regulator
6. SAT – Polyamine metabolism.

Some challenges are still faced in using saliva as a potential diagnostic tool which are:

1. A lack of standardisation of conditions and methods of saliva sample, collection, processing and storage
2. Variability in the levels of potential OSSC salivary biomarkers in both non-cancerous individuals and OSSC patients, suggest unknown confounding factors.
3. The need for further validation of OSSC salivary biomarkers.

Conclusion

The main aim of gaining knowledge regarding disease activity is to diagnose the danger before significant destruction, make an early treatment as well as measurement of treatment results. Till date blood has been the major gold standard for detection of disease markers and diagnosis of diseases and drugs. However, saliva offers a non-invasive alternative to serum as a biological fluid for diagnostic purposes, detection of hormonal levels and its functional value has been re-evaluated due to its important roles in biochemical pathways. It is almost predicatable that in near future the whole spectrum of salivary screening would be evolved.

Conflict of Interest: None.

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