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BIOCHEMICAL MARKERS - A TOOL TO DETECT ORAL DISEASES

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ABSTRACT

Oral and systemic health are integral component. Most common dental diseases are dental caries, gingivitis, periodontitis, pulpitis as well as periapical pathosis. In recent times in India oral cancer is a disease of major concern. The key biochemical changes occur in these diseases are loss of collagen due to degradation. There are several ways by which extracellular matrix can be degraded. Most of these are directly or indirectly interlinked with the release of enzymes by host or pathogen, generation of reactive oxygen species, cytokine release or influence of inflammatory mediators and apoptotic proteins. The elevated levels of these molecules are an indicator of inflammation hence they can be used as biomarkers as well as medicinal target.

KEY WORD: Apoptosis, Dental caries, Matrix metalloproteinases,Oral cancer, Periodontitis, Reactive Oxygen Species.

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INTRODUCTION

Oral health is an essential as well as an integral component of systemic health throughout life. Dental diseases are not just a toothache; it can cause more serious problems, even can affect facial aesthetics as well as our ability to chew, speak or smile. The severity of dental diseases ranges from just a common cavity of tooth or small traumatic ulcer up to deadly oral cancer. The dental diseases of human include dental caries, pulpal and periapical inflammation, gingival as well as periodontal problems and many more other conditions. One of such condition in which India has the dubiously distinction among the world is cancer of the oral cavity. This disease secures first rank among all cancers in male patients in India.[1] In the study of medical sciences, the subject anatomy and pathology only deals with those body parts and tissue which can be assessed with the eye or eye aided by various microscope but when the examining matter extends to smaller particles the anatomy, physiology as well as pathology needs the help of physics and chemistry. Hence for understanding the most basic mechanisms behind oral diseases, detailed study through biochemical way can be approached.[2] The most widespread dental diseases such as gingivitis, periodontitis, dental caries, pulpal and periapical diseases as well as oral cancers are chronic conditions which needs interaction between the host and oral bacteria. For progression of these diseases major changes in biochemical constituents of connective tissue occurwhich manifest as degeneration and loss of collagen in connective tissue. Matrix metalloproteinases, apoptosis, reactive oxygen species as well as antioxidants play key role in this processes.[3]

Matrix metalloproteinases (MMPs)

Critical events of life such as embryogenesis, development, angiogenesis as well as wound healing depend upon controlled and coordinated synthesis, depletion and remodelling of extracellular matrix. This remodelling is conducted and controlled by various proteases. These are cysteine protease, aspartic protease, serine protease and metalloproteinases. Matrix metalloproteinases(MMPs) are also known as hydrolyze components of the extracellular matrix as they are having the ability to cleave matrix component. They are dependent on zinc ions for their activity. Till date 24 MMP genes have been identified in humans and 26 members have been listed by investigators. These are typed in 6 major groups namely collagenases, gelatinases, stromelysins, matrilysins, membrane type MMPs and other MMPs.[5-9] Commonly MMPs are activated extracellularly or at the cell surface, some of them can be activated intracellularly also.

Inhibition of MMP activity is specially controlled by endogenous tissue inhibitors of metalloproteinases(TIMPS). As per current studies TIMPS family consists of four members; these are TIMP-1, TIMP-2, TIMP-3 and TIMP-4. On the other hand by use of nonspecific endogenous inhibitor activity of MMPs can be controlled.[5-9]

Reactive oxygen species

In cellular metabolism chemical compounds which can generate free radicals are called pro oxidants on the other hand those compounds which scavenge these species, oppose their actions or reduce their formation are called antioxidants.[10,11] There should be a balance between pro oxidants and anti oxidants in normal cells. If the balance shift towards the pro oxidants due to more production of free oxygen radical or deficiency of antioxidants the oxidative stress occurs. It causes serious cell damage.[11] Free radical contains a single unpaired electron in their outer orbit so they are very highly reactive their life is very short. Due to this property they cause damage to cell structure such as protein, lipid, DNA etc. Free radicals may be two types. The species which derived from oxygen are called reactive oxygen species (ROS) on the other hand nitrogen derived species are called reactive nitrogen species (RNS).[10,12] The oxygen derived species include radicals such as superoxide, Hydroxyl , Hydroperoxyl, Alkoxyl, Aryloxyl, Arylperoxyl, Peroxyl, Acyloxyl , Acyloxyl , Acylperoxyl and non radicals such as singlet oxygen, Ozone,

Hypochlorous acid and Hydrogen peroxide.[10,13]

In recent year the term reactive oxygen species include non radicals; as not being radicals also they cause radical transformation in extracellular & intracellular environment. Reactive oxygen species originate from exogenous as well as endogenous source. Exogenous source include UV light, ultrasound, smoking, exhaust fumes, radiation, infection, heavy exercise as well as therapeutic drugs. On the other hand it also originates as a by product during cell metabolism in the process of glycolysis. It may also be generated by phagocytes and other cells of connective tissue via respiratory burst.[10,14,15] Reactive species causes tissue destruction mainly of protein damage, lipid peroxidation as well as DNA damage. Protein damage is mainly by fragmentation and polymerization reaction of various protein molecule to protein radicals and protein bound ROS whereas DNA damage include strand breaks, base pair mutations, insertions, deletations, nicking as well as sequence amplification.[10,15,16,17]

Antioxidants

Antioxidants are classified in several ways. Depending upon the mode of action they are either preventing or scavenging. Preventing antioxidants again can be divided into enzymes and metal ion sequestrators. Enzymes include superoxide dismutase, catalase, glutathione peroxidise etc on the other hand metal ion sequestrators are albumin, lactoferrin, transferring, ceruloplasmin etc whereas scavengers are ascorbate, carotenoids, Vitamine-E etc. Otherwise depending upon the ability to prevent injury they can be classified as antioxidants which are chain breaking or scavenging such as vitamin E (α-tocopherol), vitamin C (ascorbic acid), vitamin A (β-carotene), urate; substance which contain thiol groups, preventative antioxidants, which function largely proteins by nature like albumin, transferrin, lactoferrin, ceruloplasmin, ascorbic acid and glutathione and antioxidants which function by catalyzing the oxidation of other molecules such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST) and glutathione reductase (GR). Again according to their location they are intracellular, extracellular and membrane associated.Depending upon solubility they can also be classified as water soluble and lipid soluble. [10,14,18,19]

Biochemical basis of dental caries, pulpal & periapical pathosis

Dental caries is an irreversible infectious diseases caused by certain cariogenic bacteria of the oral cavity. The main causative organisms are S.Mutans, S.Sobrinus and Lactobacillus species. They degrade sugar component and produce acids, mainly lactic acid. These acids diffuse through dental calcified tissues; as a consequence at that site local pH drops below 5.5, which dissolute mineral crystals and causes cavitation. [5,20-22]

Dentin contains more organic material and water than enamel so it acts as a better substrate for degradation, which may be due to bacteria or host proteinases. 90% of dentin organic matrix is constitutes by mainly type-I collagen; sometime type- III and type-V have also been identified. Another 10% of dentin organic matrix consists of phosphorylated proteins called dentinphosphosialoprotein (DSPP).It is a transient protein and is cleaved immediately after secretion and form dentin sialoprotein (DSP) & dentin phosphoprotein (DPP).In general collagens are degraded by collagenases (MMP 1,8,13) and form ¾ th to ¼ th peptides. These peptides further degraded by gilatinases (MMP-2 & 9).[5,23-26]

In dentin the small leucine –rich proteoglycans such as decorin, biglycan, fibromodulin, lumical and osteoadherin have been identified. They are highly susceptible to degradation by MMP-3 and bacterial enzymes. It was also found that several growth factors such as transforming growth factor beta, fibroblast growth factor-2 & insulin like growth factor 1 and 2 are present in dentin. As per researchers; due to dental caries, matrix degradation take place and these growth factors get released and stimulates odontoblasts to form reactionary dentin.[5,27,28] It has been thought that bacterial collagenases was the culprit for the

destruction of organic matrix but later it was found that host derived proteolytic enzymes such as MMPs which is located both in dentin and saliva have more important role to degrade organic matrix of dentin.

It has been suggested that gingival crevecular fluid (GCF) is the major source of salivary MMPs. [5,29,30,31] Another group of MMPs are present in dentin, predentin or odontoblasts. These are collagenase MMP-1, the gelatinases MMP-2 and MMP-9, stromelysin-1 (MMP-3), the MMP-2 activator MT1-MMP, and enamelysin (MMP-20).[5, 32-39] Whether MMPs are derived from saliva or dentin,in dencal caries MMPs are activated by bacterial proteinases. Low pH and heat may also cause MMP activation. When dental caries lesions are active, the bacteria produce acids; as a result an acidic environment gets created by decreasing the pH. This acidic pH activates host derived pro MMPs from both dentin and saliva by altering the conformation of the propeptide & induce the cysteine switch which is an essential step in the activation process.[5,40] Though the acidic pH is very essential for activation of MMPs yet it cannot degrade organic matrix in this pH. Followed by the pH drop the salivary buffer system neutralizes the pH and there is a momentary increase in the pH at the spot of demineralised dentin. This allows activated MMPs to degrade the organic matrix[5, 41]

There are several ways by which free radicals interact with several cellular components and disturb their integrity as well as functions. Lipid peroxidation (LP) is one of these routes. In lipid peroxidation there is oxidative degeneration of lipids. In this process free radicals take up electrons from lipid of cell membrane and as a consequence damage of cells happens. This process proceeds by a free radical chain reaction mechanism which forms a reactive aldehyde known as malondialdehyde (MDA). MDA is an electrophile species which cause toxic stress in cells and form advanced glycation as an end product. . [42,43] To maintain the immunological balance of oral cavity it is essential to maintain normal salivary levels of oxidants and antioxidants. As dental caries has got a strong immunological basis, it is hypothesized that some or other way MDA is interlinked with dental caries. A study done by Gargi Sarode et al has shown that salivary MDA is elevated in patients with dental caries compared to control.[42] Studies have shown that high level of MMP-8 is present in pulpal and periapical pathosis. After root canal treatment the level of MMP-8 goes down whereas in persistent inflammation the level remains high. According to researchers the activity of superoxide dismutase and catalase is high in reversible pulpitis whereas they get decreased in irreversible pulpitis. [3,7,42-47]

Biochemical basis of Periodontitis

Periodontitis is the inflammation of gingival tissue which is associated with the loss of periodontal ligament attachment and bony support. Plaque is appeared as essential for initiation and development of periodontal disease. It has been also recognised that the host response to the pathogen contribute a major role in connective tissue breakdown and bone loss. At present researchers came to this conclusion that the host derived enzymes, MMPs as well as cytokines and prostanoids which alter the osteoclastic activity is most responsible for tissue destruction in the periodontium.[4] In disease susceptible individuals, inflammation extends apically and laterally towards deep connective tissue and bone as endogenous and exogenous factors starts collagen degradation. Further when defence cells are recruited and activated at the site of inflammation, macrophages produces large quantities of prostaglandin E2, interlukins, tumor necrosis factor alpha (TNF-alpha) and MMPs.[3,4,48] Initially it was assumed that collagenases in periodontal disease originates from microbes but researchers found that mammalian collagenases (MMP-1,8,13) cleave collagen at a single point, so only two fragments can be obtained but due to attack of microbial proteolytic enzymes various short peptides are produced, as they cleave collagen at multiple sites.[4,49] In periodontitis two fragments of collagen was noted; so it was concluded that in periodontitis, collagenases comes from host cells rather than microbes. Previously it was thought that MMP-8 was related to neutrophil only but as per present study normal and

diseased periodontium (sulcular epithelium, fibroblasts, endothelial cells, macrophages and plasma cells) can be induced to express MMP-8.[50] In study Makela et al have shown that higher level of MMP-9 & MMP-2 gelatinases are present in periodontitis than normal periodontium.[50]

When the inflammation goes apically the level of prostaglandins, interlukins and (TNF-alpha) become high. This stimulates osteoclasts to resorb alveolar bone. It is known that MMPs plays vital role in bone remodelling.MMP-2,9,13,14 are very essential in it. MMP-14 which can be seen in a ruffled border of osteoclasts contributes to the interaction between osteoclast and matrix. This matrix metalloproteinase is also important in normal bone homeostasis. Again MMP-14 along with MMP-9 plays the key role in cell migration, due to which MMPs are called very critical for osteoclast access to the resorption site. It has been noticed that MMp-13 is present in resorption lacunae and plays a vital role to remove collagen remnants left over by osteoclasts.[48,51] Reactive oxygen species(ROS) also plays an essential role in periodontitis. As Robert Koch postulated criteria to establish causal relationship between an organism and disease, same way Halliwell also proposed four criteria.The criteria says[10,14]

1. ROS or the oxidative damage caused must be present at the site of injury.
2. The time course of ROS formation or the oxidative damage caused should occur before or at the same time as tissue injury.
3. Direct application of ROS over a relevant time course to tissues at concentrations found in vivo should reproduce damage similar to that observed in the diseased tissue.
4. Removing or inhibiting ROS formation should decrease tissue damage to an extent related to their antioxidant action in vivo.

There are several adverse effects of ROS on periodontal tissue. They causes degradation of ground substances .There is direct and indirect collagenolysis; or collagenolysis may be due to oxidation of proteases.ROS activate nuclear factor kappa-light-chain-enhancer of activated B cells(NF-κB).This results stimulation of proinflammatory cytokines.On the other hand ROS causes superoxide release and PGE-2 production via lipid peroxidation.Both of them are linked with bone resorption. [10,14] Smoking causes oxidative stress in the body due to imbalance of reactive oxygen species and antioxidants. On the other hand smoking is a known risk factor for periodontitis. These relationships highlight the role of oxidative stress in periodontitis. Myeloperoxidase, which is important for the generation of hypochlorous acid as well as other ROS, is released extracellularly or into the phagosome during phagocytosis or activation of neutrophils[10,52]

Biochemical basis of Oral cancer

Oral carcinogenesis is a very complex and multistage process. First a normal cell undergoes genetic changes, later the altered cell obtained the ability to invade and metastasis. For invasion first there should be a breach in basement membrane and later invasion into connective tissue. This process depends upon the interaction of cells and extracellular matrix and matrix degrading enzymes among which MMPs are very essential. Invasion and metastasis is a multistep process which consist of interaction between cells, extracellular matrix and matrix degrading enzymes among which MMPs are very essential. MMPS are very important for these processes. Studies have shown that for breakdown of basal membrane matrilysin & gelatinases (type-IV colagen) and for destruction of type-I collagen in ECM for invasion, collagenases plays very crucial role. MMPs can be secreted by tumour cells themselves, interstitial cells around tumour, interactions between tumour cells and interstitial cell to promote invasion. Along with tumour progression MMPs plays vital role in growth, cell migration as well as angiogenesis. It has been shown that expression of MMP-2(m-RNA) was associated with lymph node metastasis and expression of MMP-9 m-RNA with tumor progression, lymph node metastasis as well as survival rate. It has also been shown that MMP-2 & -9 are associated with tumor environment.[4,9,49,52-55]

According to investigators there is increased expression of MMP-3,MMP-14,MMP-11,MMP-8,MMP-1,MMP-2 in head and neck squamous cell carcinoma. MMPs are very important for tumor angiogenesis as they regulate endothelial cell attachment, its proliferation, migration and growth.MMP-1,-2,-9,-19 & MT1-MMP can be produced by endothelial cells. It has also been shown that angiogenetic inhibitory factor,endostatin can inhibits invivo invasive capacity of tongue carcinoma by inhibiting activation of MMP-2,9&13.[4,55,56,57] Reactive oxygen species also plays an important role in development of cancer. Due to attack of free radicals DNA damage occurs which includes modification of bases. Tumor cells also overproduce reactive oxygen species. Along with tumor cells tumor associated macrophages secrets tumor necrosis factor-alpha, which again induce cellular oxidative stress. Oxygen radicals also increase blood supply to tumor cells via Interlukin-8 and vascular endothelial growth factor. In reverse, if deficiency of antioxidants occurs it also leads to carcinogenesis. Various studies have shown that; various antioxidants, such as vit-A, vit-C & vit-E are used in treatment of various potentially malignant lesions and conditions. They inhibit tumor development by cytokines. They also disregulate oncogene such as mutant p-53 & H-ras as well as they also stimulate cancer suppressor genes such as wild type p-53. [11,58,59] Betel nut is a well known carcinogen for oral cancer. Under alkaline condition betelnut releases various free radicals such as superoxide radicals, hydroxyl anions, hydrogen peroxide. All these causes cell denaturation .[60]

Apoptosis and cell death in Dental diseases

Programmed cell death is called apoptosis. It is well regulated suicidal programme in which cells activate certain enzymes to dissolute their own nuclear component and various proteins of nucleus and cytoplasm. Disturbance of apoptosis may lead to various diseases which includes oral cancer as well as other diseases of oral cavity. [61] Though activation of protease is not identical with apoptosis yet proteolytic damage of biological active molecule caused by proteases trigger cell death. During proteolysis of several biological molecule MMPs up regulate or down regulate apoptosis depending upon relative concentration, tissue specificity as well as spatiotemporal balance between MMPs and TIMPs.[3,62-64]

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. In inflammation of oral tissue along with proteolytic enzymes cytokines are also elevated,hence they can be used as biomarkers for diagnosing the extent of disease as well as useful targets of medication. Using monoclonal antibody against MMP-8 it is possible to diagnose periodontitis within 5 minutes. This test is also very sensitive, specific, rapid and practical. The MMP-8 level is also very essential to monitor the effect of doxycycline as an adjunctive treatment. [65]

In recent an in vitro study done in rat has shown that MMPs inhibition by several synthetic inhibitors reduce dental caries progression under fissure which points us that knowledge of biochemical basis of dental diseases is not only helping us in diagnosis or treatment, but it also opening up new horizon of research and invention day by day to make our world more prosper.[5,66]

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