Review Article

The role of gingival gelatinases and salivary collagenases in the periodontal conditions

Adithya R. Pillai, R. Ponnu Lakshmi, V. Purushothaman*, K. Vinothkumar

ABSTRACT

This review focuses specifically on gingival gelatinases and salivary collagenases which are matrix metalloproteinases (MMPs) and their role in the periodontal conditions. A group of enzymes capable of degrading almost all extracellular matrix proteins, MMPs, contribute to both normal and pathological tissue remodeling. The expression of different MMPs has been reported in different stages of periodontal conditions. MMPs have been identified in pulpal and periapical inflammation and are strongly correlated with periodontal diseases since they are the major players in collagen breakdown during periodontal tissue destruction. The use of MMP inhibitors could help the prevention and treatment of many MMP-related oral diseases. This review summarizes the basic knowledge on periodontal diseases and the role of MMPs for the development of periodontal diseases.

KEY WORDS: Collagenases, Gelatinases, Matrix metalloproteinases, Periodontitis

WHAT IS PERIODONTAL DISEASE?

If your hands bleed when you washed them, you would be concerned. Yet, many people think it is normal if their gums bleed when they brush or floss. In a research study, researchers at the National Institutes of Health found that half of the populations over the world had bleeding gums (IDA report December 2017). Swollen and bleeding gums are early signs that your gums are infected with bacteria. If nothing is done, the infection can spread. It can destroy the structures that support your teeth in your jawbone. Eventually, your teeth can become so loose that they have to be extracted. "Peri" means around and "odontal" refers to teeth. Periodontal diseases are infections of the structures around the teeth. These include the gums, the cementum that covers the root, the periodontal ligament, and the alveolar bone. In the earliest stage of periodontal disease, gingivitis, the infection affects only the gums. In more severe forms of the disease, all of the supporting tissues are involved.

For many years, scientists have been trying to figure out what causes periodontal disease. It is now well accepted that bacteria in dental plaque are the major villains along with that some host immune mediators. Researchers also are learning more about how an infection in your gums can affect your overall health. In recent years, gum disease has been linked to other health problems.[1] This is a new and exciting area of research. Many questions remain. Studies have produced varying answers about how much of a connection exists between gum disease and other medical problems. More research is needed.

Researchers are studying possible connections between gum diseases.[1]

Atherosclerosis and Heart Disease

Gum disease may increase the risk of clogged arteries and heart disease. It also is believed to worsen existing heart disease.

Stroke

Gum disease may increase the risk of the type of stroke that is caused by blocked arteries.

Premature Births

A woman who has gum disease during pregnancy may be more likely to deliver her baby too early. The infant may be more likely to be of low birth weight.

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

Department of Central Research Laboratory, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. V. Purushothaman, Meenakshi Academy of Higher Education and Research, MAHER University, Chennai - 600 078, Tamil Nadu, India. E-mail: drvpurushothaman2001@yahoo.com

Received on: 08-11-2018; Revised on: 24-12-2018; Accepted on: 19-01-2019

Diabetes

Diabetic patients with periodontal disease may have more trouble controlling their blood sugar than diabetic patients with healthy gums.

Respiratory Disease

Bacteria involved in gum disease may cause lung infections or worsen existing lung conditions. This is particularly important for elderly adults in institutions such as nursing homes. In this group, bacteria from the mouth may reach the lungs and may cause severe pneumonia.

WHAT CAUSES PERIODONTAL DISEASE?

Periodontal disease is caused by bacteria in dental plaque. Plaque is the sticky substance that forms on your teeth soon after you have brushed. In an effort to get rid of the bacteria, the cells of your immune system release substances that inflame and damage the gums, periodontal ligament, or alveolar bone. This leads to swollen, bleeding gums, a sign of gingivitis (the earliest stage of periodontal disease). Damage from periodontal disease also can cause teeth to become loose. This is a sign of severe periodontitis (the advanced stage of disease).[1]

You can prevent periodontal disease by practicing good oral hygiene and visiting your dentist regularly. Most people should see the dentist about once every 6 months. However, if you already have gum disease, you may need to visit more often. Daily brushing and flossing, when done correctly, can help to remove most of the plaque from your teeth. Professional cleanings by your dentist or dental hygienist will keep plaque under control in places that are harder for a toothbrush or floss to reach. If oral hygiene skips or you skip dental visits, plaque builds up on the teeth. Eventually, it spreads below the gum line. The bacteria are protected there because your toothbrush cannot reach them. If plaque is not removed, the bacteria will continue to multiply. Your gum inflammation may get worse.[2]

The buildup of plaque below the gumline causes the gums to become inflamed. As the gums swell, they detach from the tooth. This process forms a space, or "pocket," between the tooth and gum. Bacteria can grow rapidly in the pockets. This encourages further plaque buildup. If left untreated, periodontal disease may destroy the periodontal ligament and the alveolar bone, the structures that support your teeth. Another reason to remove plaque promptly is that overtime it becomes hardened or calcified and turns into calculus. This is commonly called tartar. Even more plaque attaches to calculus because it is a rougher surface than tooth enamel. It is also rougher than cementum, a layer that covers the tooth root. Calculus and plaque build up in layers. Using a tartar-control toothpaste may help slow the buildup of calculus around your teeth. However, it can't affect the tartar that already has formed below the gum line.[3]

RISKS AND PREVENTION

The bacteria in plaque are the main cause of periodontal disease. However, several other factors also can contribute. They include other diseases, medicines, and oral habits. These factors can increase your risk of gum disease or make it worse once the infection has set in.[4]

Genes

Some people are more likely than others to get periodontal disease due to their genes. However, your genes do not make gum disease inevitable. Even people who are highly prone to periodontal disease can prevent or control the disease with good oral care.

Smoking and Tobacco Use

Smoking increases the risk of periodontal disease. The longer you smoke, and the more you smoke, the higher the risk. If you have periodontal disease, smoking makes it more severe. Smoking is a major reason that some cases of periodontal disease are resistant to treatment. Smokers tend to collect more tartar on their teeth. They often develop deeper periodontal pockets once they have gum disease. They also are likely to lose more bone as the disease gets worse. Unlike many other factors that affect the health of your gums, the decision to smoke or not is under your control. Quitting smoking can play a major role in bringing periodontal disease under control.

Stress

Stress can make periodontal disease worse and harder to treat. Stress weakens your body's immune system. This makes it harder for your body to fight off infection, including periodontal disease.

Fluctuating Hormones

Whenever hormone levels go up and down in the body, changes can occur in the mouth. Puberty, pregnancy and sometimes menopause can temporarily increase the risk and severity of gum disease.[4]

Medicines

Several types of medicines can cause dry mouth or xerostomia. Examples include certain drugs for depression and high blood pressure. If you do not have enough saliva, plaque is more likely to form. This may lead to tooth decay (cavities).[4]

ROLE OF MMPs IN ORAL CONDITIONS

MMPs contribute to both normal and pathological tissue remodeling. Physiological roles for MMPs include cell

migration, tissue remodeling during organogenesis and growth, wound healing, angiogenesis, enamel formation, and antigen processing and presentation.[11] MMPs play a key role in the migration of normal and malignant cells and they act as regulatory molecules, by functioning in enzyme cascades, processing matrix proteins, and generating fragments with enhanced or reduced biological effects. MMPs are produced by tumor cells so that they can metastasize and force their way into the surrounding stroma, penetrating the basement membrane until they reach the blood vessels. MMPs are widely active on the biological processes. Collagenases have the ability to cleave interstitial collagen types I, II, and III, and they are capable of digesting other extracellular matrix (ECM) and non-ECM molecules. Gelatinases degrade the denaturated collagens, gelatin. Stromelysin 1 (MMP-3) and stromelysin 2 (MMP-10) have similar substrate specificities, but MMP-3 has a higher proteolytic efficiency. Besides digesting ECM molecules, MMP3 also activates pro-MMPs. Stromelysin 3, MMP-11, is also grouped as "other MMPs" since its sequence and substrate specificity differ from those of MMP3. Matrilysins are characterized by the lack of a hemopexin domain [5,6] Matrilysin 1 (MMP7) processes cell surface molecules such as pro-tumor necrosis factor-a, pro-a-defensin, and Fas-ligand. Matrilysin 2 (MMP-26) also degrades ECM components. Membrane-type MMPs (MT-MMPs) are divided into Type I transmembrane proteins (MMP-14, MMP-15, MMP-16, and MMP-24) and glycosylphosphatidylinositol-anchored protein MMP-17 and MMP-25. All MT-MMPs are capable of activating pro-MMP-2, except MT4-MMP. These enzymes can also digest ECM molecules. MT1-MMP is able to degrade collagen Types I, II, and III and also plays an important role in angiogenesis.[7-9]

There are several studies indicating that MMPs have a fundamental role in oral tissue development. MMPs are involved in the development of enamel and enamel fluorosis. They are also associated with remodeling of the organic matrix of dentin.[10] The activation of MMP-2 and MMP-9 has been shown to have a crucial role in dentin collagen breakdown in caries lesions. The inflammatory soft tissue breakdown has four recognized pathways: The plasminogen-dependent pathway, the phagocytic one, the osteoclastic pathway, and the MMP-dependent pathway. MMPs have been identified in both pulpal and periapical inflammation. They even more strongly correlate to periodontal diseases since MMPs are the major players in collagen breakdown during periodontal tissue destruction.

MMPs IN PERIODONTAL DISEASE

Collagen is the major extracellular component of gingiva. Periodontal disease is initiated by bacterial plaque, followed by inflammation, leading to the loss of periodontal attachment and bone destruction. It has been demonstrated that degradation of gingival tissue during active periodontitis is at least partly due to MMPs. They are expressed by inflammatory cells (monocytes, macrophages, lymphocytes, and polymorphonuclear cells) and resident cells (fibroblasts, epithelial cells, and endothelial cell).[12] Even though bone destruction is the hallmark of periodontal disease, the role and function of MMPs in periodontal bone loss are not very well described. However, basic research has gained insight into the MMP action in bone remodeling that most likely is applicable to periodontal bone destruction, too. Osteoclast express, along with cathepsin K, several MMPs, which together with preosteoblast cell and osteoblast-derived MMPs contribute to bone resorption.[8,13]

Mäkelä *et al*.[12] suggested that MMP-2 and MMP-9 could participate in tissue destruction in periodontitis. They located gelatinase production by various cells in the oral cavity and found that the amount of gelatinases increased during the periodontal disease, while conventional periodontal treatment effectively reduced the levels of these enzymes.[15] There is also strong *in vivo* evidence for a direct role of active neutrophil collagenase in the pathological destruction of periodontal connective tissue. For example, it was demonstrated in a longitudinal cohort study that collagenase activity derived from neutrophils and not from bacteria or other host cells. Moreover, a significant increase of active collagenase with time was observed only at sites with progressive periodontal destruction. It is currently known that gelatinases (MMP-2, -9) and all collagenases derived from various cellular sources and expressed in various molecular species are involved in periodontitis.[7,8,18,19] The production of collagenase, as well as the secretion of physiological activators and inhibitors of enzyme, is modulated by cytokines. Procollagenase produced endogenously (MMP-1), stored in the ECM of periosteal tissue, can be activated and the derived enzyme degrades collagenous matrix until inhibited by tissue inhibitors of metalloproteinases. A large amount of collagenous proteins (about 70% of the total amount of collagenous proteins) are degraded after procollagenase activation by plasmin in periosteum.[19]

Recent studies have indicated the importance of MMP-8 in periodontal destruction. It has been found that clinical improvement obtained by scaling and root planing and the periodontal maintenance therapy is associated with significant reductions in MMP-8 levels. This suggests that MMP-8 is a potential discloser of periodontal disease. The reduction of MMP-8 levels in gingival crevicular fluid (GCF) indicates that this enzyme may be useful as an indicator of current disease status and as a predictor

of future disease.[13,14] A chair-side test for MMP-8 was, therefore, developed.[14] While MMP inhibition is promising in the treatment of periodontal disease, further work that includes other approaches needs to be evaluated (reviewed by Reddy *et al*.).[9] It must be kept in mind that the mere presence of MMPs in GCF and/or periodontal tissue does not automatically mean increased activity of the enzymes in tissue. Therefore, a functional clinical research approach is preferable to disclose the exact role of MMPs in periodontitis.

SALIVARY MATRIX METALLOPROTEINASES (MMPs) IN PERIODONTITIS

Saliva is a slightly acidic and clear secretion of salivary glands in the oral cavity. Whole saliva is largely produced by the major salivary glands (parotid, submandibular, and sublingual), whereas minor salivary glands contribute 10% of the total.[16] Saliva is mainly composed of water with a small amount of organic and inorganic compounds; however, it also contains protein signatures and whole cells of oral microorganisms and the host. In addition, components of serum and blood, which leak into the oral cavity through gingival crevicular fluid or oral wounds, can be detected in saliva.[17] Studies investigating the use of saliva as a diagnostic fluid have a long history. This non-invasive approach is not limited only to the diagnosis of oral diseases because many systemic diseases, such as different types of cancers, cardiovascular diseases, immunologic syndromes, and hereditary deficiencies, can also be studied with the aid of salivary diagnostics.[20-22] In the context of periodontitis, an analysis of salivary proteins was first performed by Berg *et al*. 70 years ago.[23] Among MMPs, MMP-8 is that most commonly studied in salivary diagnostics of periodontitis. Neutrophil-derived MMP-8 is the main source of salivary MMP-8, and gingival fibroblasts also contribute MMP-8, but at a much lower proportion.[24]

Despite its potential value as a diagnostic marker, the use of salivary MMP-8 carries some limitations. One important limitation is smoking, which is a major risk factor for periodontal diseases and is significantly associated with progression of periodontal disease and tooth loss.[19] Lower levels of salivary MMP-8 have been found in smokers than in non-smokers or former smokers.[15,24,25] This lower level of salivary MMP-8 in smokers prevents the use of salivary MMP-8 as a reliable marker in patients with periodontitis who smoke. The impact of cigarette smoke on human vascular endothelial cells has been demonstrated as upregulated MMP genes and corresponding proteins.[26-33] In periodontology, the negative impact of smoking on the diagnostic utility of salivary MMP-8 can be circumvented using different antibodies and by combining enzymes with their inhibitors in terms of their ratios.[15] Salivary MMP-8 levels and the ratio of MMP-8/tissue inhibitors of MMP-1 were able successfully to differentiate periodontitis subjects from controls.[15]

COLLAGENASES AND GELATINASES

Collagenases and gelatinases are the main type of MMPs found in gingival subline and saliva. These are found in the clinical samples from the chronic periodontitis patients. There are research studies showing the activities and isolation of these MMPs. In healthy gingiva, salivary collagenases exist mainly in the latent form, while they appear to be activated in periodontitis.[34,35] Higher levels of MMP-8 are also detected in the saliva of subjects affected by periodontitis compared with healthy patients, whereas the levels of salivary MMP-1 are similar in both groups. There are no reports on collagenase-3 (MMP-13) in either saliva or GCF.[36-42]

CONCLUSIONS

MMPs are a family of proteolytic enzymes that are capable of degrading almost all ECM proteins. It has been demonstrated that MMP family members are involved in normal physiology and pathological events that occur in the oral environment. They have been identified in saliva, GCF, in enamel and dentin structures, and also in periodontal diseases and carious lesions. This review has shown the potential role of MMPs, especially gingival gelatinases and salivary collagenases in the prevention and maintenance of oral health. There is still much to learn. Further, investigations should elucidate the role of MMPs in the organization and the initiation of the disease and pathway. Since MMP inhibitors, such as chlorhexidine, doxycycline, and minocycline, have proved to be efficient adjuncts in periodontal therapy, and the use of some natural MMP inhibitors with less side effects in the treatment of gingivitis and periodontitis is definitely worthy of further research. The *in vitro* experiments may lay down the groundwork for focused *in vivo* animal experiments before clinical studies can be justified. In periodontitis, the present clinical evidence needs to be confirmed, preferably in prospective long-term clinical follow-up studies.

ACKNOWLEDGMENTS

The work was supported, in part, by the Meenakshi Academy of Higher Education and Research, Chennai, and Periodontitis Department of MADC, Chennai 600078.

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Source of support: Nil; Conflict of interest: None Declared