EAS Journal of Dentistry and Oral Medicine
Abbreviated Key Title: EAS J Dent Oral Med
ISSN: 2663-1849 (Print) & ISSN: 2663-7324 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-2 | Mar-Apr-2020 | DOI:10.36349/easjdom.2020.v02i02.002

Review Article

Role of matrix- Metalloproteinases in the Pathogenesis of Periodontal Disease

Dr. Saagar Bhargava* [1], Dr. Lavanya R[2], Dr. B. Shalini[3], Dr. Sharad K. Hiremath[3]

[1]Post-graduate, Dept. of Periodontology,Faculty of Dental Sciences,Ramaiah University of Applied Sciences, Bangalore, India
[2]Reader, Dept. of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bangalore, India
[3]Post-graduate, Dept. of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences, Bangalore, India

Article History
Received: 19.02.2020
Accepted: 02.03.2020
Published: 29.03.2020

Journal homepage:
https://www.easpublisher.com/easjdom

Quick Response Code

Abstract: Periodontal diseases are inflammatory disorders that give rise to tissue damage as a result of complex interaction between pathogenic microorganisms and host immune response. It is a term used to describe an inflammatory process initiated by plaque biofilms that lead to loss of periodontal attachment to the root surface and adjacent alveolar bone, which ultimately results in tooth loss. Though microorganisms are the initiating agents, the disease initiation and progression are influenced by the host response being modified by environmental and behavioural factors. The disease progression involves a network of interacting molecular pathways made of pro-inflammatory mediators like cytokines, growth factors, reactive oxygen species, matrix metalloproteinases (MMPs), and their inhibitors and regulators.

Keywords: fibrous dysplasia, maxilla, osteotomy, lesion, monostatic, symptoms.

INTRODUCTION:

Periodontal diseases are inflammatory disorders that give rise to tissue damage as a result of complex interaction between pathogenic microorganisms and host immune response. It is a term used to describe an inflammatory process initiated by plaque biofilms that lead to loss of periodontal attachment to the root surface and adjacent alveolar bone, which ultimately results in tooth loss (Alkalin *et al.,* 2005). Though microorganisms are the initiating agents, the disease initiation and progression are influenced by the host response being modified by environmental and behavioural factors (Lepilahti *et al.,* 2011). The disease progression involves a network of interacting molecular pathways made of pro-inflammatory mediators like cytokines, growth factors, reactive oxygen species, matrix metalloproteinases (MMPs), and their inhibitors and regulators (Birkedal-Hansen, 1993).

Type I collagen is the main component of extracellular matrix (ECM) in the periodontal tissues, which is considered as an important component determining the pathophysiology of periodontal disease (Konopka *et al.,* 2012). The breakdown of collagen is associated with tissue disintegration, tissue repair and remodelling during the course and treatment of periodontal disease. During early gingivitis, many of the collagen fibers in gingiva are broken down leading to inflammatory cell infiltration. Further progression to periodontitis is characterized by degradation of periodontal fibers along with supporting alveolar bone (Kinane *et al.,* 2003).

Pathogens in microbial dental plaque are capable of stimulating host cells to increase their MMP release, which is considered as one of the indirect mechanisms of tissue destruction occurring in periodontitis (Ozcaka *et al.,* 2011).

MMPs form the most important group of proteinases responsible for the degradation of matrix proteins during periodontitis. Any imbalance between MMPs and their inhibitors may trigger the degradation of ECM, basement membrane, and alveolar bone (Gursoy *et al.,* 2010). Matrix metalloproteinases are a large family of calcium dependent zinc containing endopeptidases with well-characterized structural and catalytic properties responsible for tissue remodeling and degradation of ECM including collagens, elastins, gelatin, matrix glycoprotein, and proteoglycans (Verma and Hansch, 2007). Since the discovery of first MMP in

***Corresponding Author:** Dr. Saagar Bhargava

1962, several MMPs have been identified and currently the family consists of 25 members with extensive physiological and pathological role. MMPs consist of a pro-domain, a catalytic domain, a hinge region, and a hemopexin domain. They are either secreted from the cell or anchored to the plasma membrane (Visse and Nagase, 2003). They are usually expressed as inactive zymogens, and the pro-domain must be dissociated from the catalytic one for its activation (Hannas *et al.*, 2007). Activation by cleavage of the pro-peptide may occur via autolysis, serine protease plasmin, or by other MMPs (Thomas *et al.*, 1999).

Most MMPs are produced at low levels or not at all in resting-state adult tissues. However, their production is induced during various physiological and pathological events such as embryogenesis, ovulation, tooth eruption, arthritis, inflammatory conditions, and malignancy. MMP activation is regulated at multiple levels and mainly involves four mechanisms, namely positive and negative transcriptional control of MMP genes; by activation from latent state; by differences in substrate specificity and by modulation of serum inhibitors and tissue inhibitors of metalloproteinase (TIMP) (Birkedal-Hansen, 1993). Control of extracellular proteolysis by MMP is critically important for survival of organisms and this is performed by interaction with various inhibitors.

The main inhibitor is TIMP consisting of four families that inhibit MMP activity and restrict ECM breakdown (Verstappen and Von den Hoff, 2006). α-2 Macroglobulin, a plasma protein, is a powerful inhibitor of MMP in circulating fluids. Other inhibitors include tissue factor pathway inhibitor 2, C-terminal fragment of the pro-collagen C-terminal proteinase enhance protein, RECK, and membrane-bound β amyloid precursor protein (Visse and Nagase, 2003). This review discusses the types of MMPs and their physiological and pathological role with special emphasis on its association with periodontal diseases.

Pathophysiology of MMPs

Matrix metalloproteinases are a family of enzymes capable of modulating connective tissue components. The first member of the metalloproteinase family, collagenase, was discovered by Gross and Lapiere in 1962 in the tail of metamorphosing tadpole (Woesnner, 1991). They are multidomain enzymes containing a zinc ion, which are coordinated by three histidine residues in their active site. Most MMPs possess different primary structures but share common modules referred to as protein domains (Honibald *et al.*, 2012). MMPs contain several different functional domains, namely N-terminal signal peptide or prodomain that directs MMP synthesis inside the cell and is removed before they are secreted; the pro-domain that maintains the enzyme in an inactive state; a catalytic domain containing a conserved zinc-binding region and a conserved methionine, which determines substrate specificity of MMP; a hinge or linker domain connecting catalytic domain to hemopexin domain; and the hemopexin domain that binds TIMPs and certain substrates and participates in membrane activation and some proteolytic activities (Thomas *et al.*, 1999).

Matrix metalloproteinases are mostly produced in latent non-active forms, and activation through cysteine switch is required for the enzyme function (Sorsa *et al.*, 2004). Activation can occur either in extracellular or in intracellular space depending on the structure of MMP. The proenzyme form of MMP contains an unpaired cysteine sulfhydryl groups, which needs to be cleaved proteolytically or non-proteolytically for its activation. Proteolytic activation takes place by several proteolytic enzymes such as serine protease plasmin, bacteria proteases together with oxidative stress, and other MMPs (Nagase, 1997). Other activation mechanisms involve furin family proteinase and other cell surface MT-MMP (Thomas *et al.*, 1999). Nonproteolytic activation can be accomplished in vitro by SH-reactive agents, such as mercurial compounds, detergents, gold compounds, or by oxidation (Sorsa *et al.*, 2004).

To maintain balance, extracellular proteolysis through MMP needs to be controlled in a specific manner. The activity of MMPs can be inhibited by endogenous and exogenous inhibitors. Endogenous inhibitors originate from different human cells, while exogenous inhibitors are synthesized as therapeutic agents. TIMP accounts for the main endogenous inhibitors, while a-2 macroglobulin is a major inhibitor in tissue fluids. TIMPs are specific inhibitors that regulate MMP activity by forming high-affinity, non-covalent, irreversible 1:1 complex with the proteinases (Denhardt *et al.*, 1993). Other endogenous inhibitors play a minor role in MMP inhibition. Matrix metalloproteinases are classified into six groups based on the substrate specificity, sequence similarity, and domain organization and named as collagenases, stromelysins, matrilysins, membrane-type MMPs, and other types of MMPs (Verma and Hansch, 2007). Each class of MMPs contains group of enzymes denoted by a number and a descriptive name. These individual MMP types have specific physiological function and also vary in their pathological involvement. The details of the different types of MMPs are summarized in Table 1.

Role of MMPs in Periodontal Disease:

Inflammatory destruction of periodontal attachment apparatus is the hallmark of periodontal disease, and degradation of type I collagen seen in periodontal tissues is a key step in periodontal attachment loss. Degradation action on collagen fibers is performed by MMPs released by the resident cells of PDL that responds to the inflammatory stimuli. The most common type of MMPs related to tissue

destruction belongs to collagenases family and includes mainly MMP-8 and MMP-13, with significant contribution from MMP-9 and MMP-14. Other MMPs are found to play a minor role in periodontal tissue destruction.

Collagenases:

Matrix metalloproteinase -1, 8, 13, and 18 belong to this group. Majority of the studies performed on the role of this group of MMPs have studied the effects of subtype 8 and 13 in periodontitis. MMP-8 has the unique ability to break down the type I and III collagen which is critical for periodontal destruction (Rai *et al.,* 2008). Neutrophils are the major cellular sources of MMP-8, and a large persistent neutrophil influx is seen in periodontal diseases (Ozcaka *et al.,* 2011). Increased salivary MMP-8 levels were found in periodontitis and gingivitis patients with significant correlation with clinical parameters, which could be attributed to the ability of MMP-8 to degrade type I and III collagen necessary for periodontal destruction (Rai *et al.,* 2008). Romanelli et al (1999) determined the neutrophil collagenase activity in GCF of patients with periodontitis and concluded that collagenase activity was positively associated with the severity of periodontal disease and MMP-8 accounted for most of the activity. The predominance of MMP-8 in GCF is correlated with increased number of polymorphonuclear neutrophils (PMNs) recruited as a part of the inflammatory response, which suggests that neutrophil hyperresponsiveness may contribute to tissue destruction in periodontal diseases. In comparison, MMP-1 and MMP-13 may be associated with tissue remodelling in chronic wounds and thus could be used as potential markers of repair in diseased periodontal tissues (Romanelli *et al.,* 1999).

Another study by Marcaccini et al (2010) demonstrated increased levels of MMP-8 in patients with chronic periodontitis than in controls with decreased levels 3 months after therapy and moderate correlation with clinical parameters. Kinane et al (2003) found significantly higher MMP-8 levels in chronic periodontitis, and scaling and root planing significantly reduce their levels 4 weeks after therapy although the levels did not reduce to normal values. Also, there was no correlation between MMP-8 levels and clinical parameters. The study findings suggested that short-term non-surgical therapy resulted in an improvement in clinical signs of inflammation, but that inflammatory and destruction process in periodontal tissues were not entirely eliminated. Ozcaka et al (2011) demonstrated significantly higher serum concentration of MMP-8 in smoker healthy controls than in non-smokers. No significant difference existed between chronic periodontitis group and healthy controls and also between smokers and non-smokers of the diseased group. Increased MMP-8 expression is associated with remodeling of ECM and basement membrane components including collagen destruction in periodontal tissues.

Marcaccini et al (2009) reported increased plasma levels of MMP-8 and MMP-9 in patients with chronic periodontitis and emphasized the importance of periodontal therapy to avoid elevated levels, which are associated with many systemic disorders. Increased salivary MMP-8 concentration in periodontitis subjects than in controls could be used as valuable marker for the detection of periodontitis (Gursoy *et al.,* 2010). Hernandez et al (2010) compared MMP-8 levels in GCF from untreated active and inactive sites from patients with progressive periodontitis and found high MMP-8 levels and a strong MPO-/MMP-8-positive correlation in active and inactive sites at baseline. After treatment, decreased levels were noted except in active sites, where MMP-8 differences were not significant. The data support a role for MPO/MMP-8 interaction in progression episodes of periodontal supporting tissue destruction and potential of MPO and MMP-8 as biomarkers of treatment outcome. The lack of differences in MMP-8 levels after treatment of active sites might be interpreted as poor host response, representing sites at risk for further loss of periodontal support. Additionally, the MPO/MMP-8 association could reflect the persistence of MMP-8 activation and consequently the need for further treatment and follow-up. Costa et al (2010) measured salivary MMP-8 levels in CP patients with type II diabetes and found significantly increased levels in diseased groups than the controls, which could be related to collagen breakdown observed during periodontal destruction.

Gursoy et al (2013) in their study demonstrated increased levels of salivary MMP-8 in subjects with generalized periodontitis than in normal controls and suggested that MMP-8 was the only marker capable of differentiating subjects with severe alveolar bone loss from those with slight bone loss. Statistically significant increase in MMP-8 expression was noted in periodontal tissue specimens obtained from patients with periodontal disease and diabetes, periodontal disease alone, and in healthy controls, and increased expression in periodontitis with diabetes group could be due to hypercholesterolemia, which releases pro-inflammatory cytokines causing overexpression of MMP-8 (Hardy *et al.,* 2012). Yakob et al (2012) investigated the GCF MMP-8 levels in relation to the presence of specific periodontal pathogens and found significantly increased levels in patients with Treponema denticola or Treponema forsythia and concluded that these organisms induce a cascade of host response with increased MMP-8 levels in GCF. In addition to its pathological role in periodontal tissue destruction, physiological levels of MMP-8 can also exert anti-inflammatory effects by processing some anti-inflammatory cytokines and chemokines. The findings of high levels of MMP-8 in

GCF, saliva, and affected tissues in patients with chronic periodontitis have indicated its potential role as a biomarker. This has led to the development of chair-side point-of-care diagnostics for detection of chronic periodontitis. Monoclonal antibodies have been developed in the form of dip stick test, which allows for rapid detection of MMP-8 thereby helping in differentiating healthy and gingivitis sites from periodontitis sites, and reduction in GCF MMP-8 levels can be observed after successful periodontal therapy (Mantyla et al 2003). Lepilahti et al (2011) found that MMP-8 levels in oral rinse samples were higher in subjects with strongest periodontal inflammatory burden than in subjects with less inflammatory changes. The authors concluded that oral rinse sample analysis of MMP-8 could be clinically useful in rough screening to identify individuals who are at risk to develop periodontitis or in analyzing the individual level of host response. Simultaneous analysis of MMP-8 and TIMP-1 could be beneficial. Oral rinse samples analysis may be useful in defining the optimum period between periodontal maintenance visits after active therapy. Also, during the active phase, it would be possible to monitor.

Gelatinases:

Matrix metalloproteinase-2 and MMP-9 belong to this family of metalloproteinases. MMP-2 is secreted by gingival fibroblasts, and MMP-9 is mainly secreted by PMLs and they degrade collagen type IV present in gingival tissues (Ingman *et al.,* 1994). Rai et al (2008) showed that crevicular MMP-9 levels were higher in periodontitis than in healthy controls, while the reverse was true for MMP-2 and the levels highly correlated with clinical attachment loss and bleeding on probing. Similar findings were also seen in several other studies (Makela *et al.,* 1994; Korostoff *et al.,* 2000; Maeso *et al.,* 2007). Ozcaka et al (2011) demonstrated that smokers with chronic periodontitis and smoking controls exhibited significantly higher serum concentration of MMP-9 than non-smoker counterpart. Increased salivary and serum concentrations of MMP-2 and MMP-9 and decrease after periodontal therapy were demonstrated in several other studies implicating their role in periodontal diseases (Marcaccini *et al.,* 2009, 2010; Gursoy *et al.,* 2013). MMP-2 and MMP-9 were suggested to participate in tissue destruction and periodontitis (Makela *et al.,* 1994).

Membrane-type MMPs:

Scarce literature data are available regarding the role of MT1-MMPs (membrane-type-1 MMPs) in periodontal diseases with conflicting results. Studies performed to assess the MT1-MMP levels in chronic periodontitis group did not reveal any statistically significant increase in their levels when compared to normal controls (Hernandez *et al.,* 2010). Gursoy et al (2010) also did not find any differences in salivary MMP-14 levels between diseased group and controls. It was hypothesized that overexpression of MMP-14 in early phases of disease may be due to its function as an activator of several other MMPs and its weak expression in periodontitis could be through a regulatory mechanism for controlling the periodontal disease progression (Gursoy *et al.,* 2010).

However, another study by Oyarzun et al (2010) found significantly increased MT1-MMP levels in periodontitis-affected gingival tissues than in healthy gingiva. The findings supported the possibility that MT1-MMP expression is induced in the periodontium under inflammatory conditions and probably plays a role in periodontal tissue destruction. They proposed that this metalloproteinase might modify the inflammatory response through the modulation of TNF-a within these tissues. In addition to its role in collagen remodeling, MT1-MMP also activates other MMPs like MMP-13 and MMP-2, which may amplify the proteolytic potential for tissue turnover. MMP-14 might also play a role in cell signaling and regulation of inflammatory response. Kim et al (2011) also found significantly increased levels of MMP-14 in diseased tissue of patients with chronic periodontitis than in healthy controls (Table 2).

Inhibitors of MMPs

Matrix metalloproteinases are countered by TIMPs, which inhibit the formers activity and thereby restrict ECM breakdown. The balance between MMPs and TIMPs plays an important role in maintaining the inhibitory activity of healthy tissues, and any imbalance between them is involved in the progression of periodontal diseases. Increased MMP and decreased TIMP levels are responsible for initiation of collagen degradation from connective tissue and alveolar bone. TIMPs are endogenous inhibitors that bind MMPs in a 1:1 stoichiometry and are composed of four types named TIMP 1–4. Their expression is regulated during development and tissue remodeling. Gursoy et al (2010) found reduced salivary TIMP levels in periodontitis group than in controls. These findings could be related to periodontal attachment loss.

Marcaccini et al (2010) did not find any differences in TIMP-1 levels between healthy controls and patients with chronic periodontitis, but detected significantly elevated TIMP-2 levels in the diseased group at baseline which decreased 3 months after therapy. The study also found a difference in MMP-8, MMP-9/TIMP ratios between controls and diseased group, and the resultant imbalance could be due to increased levels of MMP-8 and MMP-9. Lepilahti et al (2011) also found increased MMP-8/TIMP-1 ratios in subjects with increasing inflammatory burden. Oyarzun et al (2010) found significantly increased TIMP-2 levels in periodontal-affected gingival tissue than in healthy gingiva. The study also reported an imbalance in MT1-MMP/TIMP-2 ratio in diseased tissue. MT1-MMP may

activate pro-MMP-2 in the presence of a small amount of TIMP-2, but at higher levels, its activity is inhibited. Kim et al (2011) also found a statistically significant difference in TIMP-2 levels between chronic periodontitis and healthy controls.

Therapeutic Considerations

Targeted therapy aimed at inhibition of MMP activities could be used as potential therapeutic strategy toward the management of periodontitis. This along with routine clinical therapy such as scaling and root planing would prove beneficial in improving disease prognosis. Both macromolecular inhibitors (natural TIMPs and monoclonal antibodies) and small molecules (synthetic and natural products) have been considered as potential therapies for diseases in which excess MMP activity is seen (Whittaker *et al.,* 1999).

The first disease target was rheumatoid arthritis with broadened application in cancer therapy which is at various stages of clinical trials (Amalinei *et al.,* 2010). MMPIs used in treatment of periodontal diseases are modified tetracyclines which are approved by FDI. Tetracyclines are antibiotics that also inhibit the breakdown of connective tissue. Inhibitors have been obtained through chemical modification of the tetracycline family of molecules, where it has been possible to separate the antibiotic and protease inhibitory activities (Golub *et al.,* 1987). Chemically modified antibiotics without antibiotic activities have several potential advantages in the absence of gastrointestinal side effects or toxicity and higher plasma concentrations for prolonged time periods (Acharya *et al.,* 2004). Doxycycline hyclate, a low-dose tetracycline analogue deprived of anti-microbial activity, is used for treatment of periodontal diseases and acts via the inhibition of MMP-8 and MMP-13 protease mechanisms. The therapeutic effect of this drug is primarily due to the modulation of the host response because the low-dose formulations have lost their anti-microbial activity (Ashley, 1999). Tetracyclines are found to inhibit MMP activity by cationic binding proteins. The use of this drug along with standard mechanical therapy of scaling and root planing has gained widespread acceptance. MMPs are almost completely inhibited by chelating agents such as EDTA, EGTA, or 1,10 phenanthroline. Different MMPs are also found to be inhibited by chlorhexidine. A study by Gendron et al (1999) showed that the chlorhexidine directly inhibits MMP-2, MMP-8, and MMP-9 which could act via a chelating mechanism. Other inhibitors such as dichloromethylene bisphosphonate (clodronate) inhibit MMP-1 and MMP-8 acting as a cationic chelator (Teronen *et al.,* 1997).

CONCLUSION

To summarize, this review provides information regarding the role of MMPs in periodontal diseases. Several studies especially in the past decade have shown significant alterations in MMP in chronic periodontitis especially MMP-2, MMP-8, and MMP-9 with minor contribution from MMP-13 to MMP-14. The discovery of point-of-care chair-side diagnostics using MMP-8 has helped in gross screening of periodontitis and in maintenance therapy. Detection of MMP levels may be useful as a biomarker to detect periodontitis and a tool to assess prognostic follow up. The application of targeted therapy toward MMP may serve as a useful adjunct to periodontal diagnosis and advanced Periodontal medicine

REFERENCES:

1. Acharya, M. R., Venitz, J., Figg, W. D., & Sparreboom, A. (2004). Chemically modified tetracyclines as inhibitors of matrix metalloproteinases. *Drug Resistance Updates*, *7*(3), 195-208.
2. Akalin, F. A., Toklu, E., & Renda, N. (2005). Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. *Journal of clinical periodontology*, *32*(3), 238-243.
3. Amalinei, C., Caruntu, I.D., Ginsca, S.E., & Balan, R.A. (2010). Matrix metalloproteinase involvement in pathological condition. *Romanian Journal of Morphology and Embryology*, 52, 215–228.
4. Ashley, R.A. (1999). Clinical trials of a matrix metalloproteinase inhibitor in human periodontal disease. SDD clinical research team. *Annals of New York Academy of Sciences*, 878, 335–346.
5. Birkedal-Hansen, H. (1993). Role of matrix metalloproteinases in human periodontal diseases. *Journal of Periodontology*, 64, PP: 474–484.
6. Caron, C., Xue, J., Sun, X., Simmer, J. P., & Bartlett, J. D. (2001). Gelatinase A (MMP-2) in developing tooth tissues and amelogenin hydrolysis. *Journal of dental research*, *80*(7), 1660-1664.
7. Costa, P. P., Trevisan, G. L., Macedo, G. O., Palioto, D. B., Souza, S. L., Grisi, M. F., ... & Taba Jr, M. (2010). Salivary interleukin-6, matrix metalloproteinase-8, and osteoprotegerin in patients with periodontitis and diabetes. *Journal of periodontology*, *81*(3), 384-391.
8. Denhardt, D. T., Feng, B. O., Edwards, D. R., Cocuzzi, E. T., & Malyankar, U. M. (1993). Tissue inhibitor of metalloproteinases (TIMP, aka EPA): structure, control of expression and biological functions. *Pharmacology & therapeutics*, *59*(3), 329-341.
9. Folgueras, A. R., Pendas, A. M., Sanchez, L. M., & Lopez-Otin, C. (2004). Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *International Journal of Developmental Biology*, *48*(5-6), 411-424.

10. Gendron, R., Grenier, D., Sorsa, T., & Mayrand, D. (1999). Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. *Clin. Diagn. Lab. Immunol.*, *6*(3), 437-439.
11. Golub, L. M., McNamara, T. F., D'angelo, G., Greenwald, R. A., & Ramamurthy, N. S. (1987). A non-antibacterial chemically-modified tetracycline inhibits mammalian collagenase activity. *Journal of dental research*, *66*(8), 1310-1314.
12. Gursoy, U. K., Könönen, E., Huumonen, S., Tervahartiala, T., Pussinen, P. J., Suominen, A. L., & Sorsa, T. (2013). Salivary type I collagen degradation end-products and related matrix metalloproteinases in periodontitis. *Journal of clinical periodontology*, *40*(1), 18-25.
13. Gursoy, U. K., Könönen, E., Pradhan-Palikhe, P., Tervahartiala, T., Pussinen, P. J., Suominen-Taipale, L., & Sorsa, T. (2010). Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *Journal of clinical periodontology*, *37*(6), 487-493.
14. Gusman, H., Santana, R. B., & Zehnder, M. (2002). Matrix metalloproteinase levels and gelatinolytic activity in clinically healthy and inflamed human dental pulps. *European journal of oral sciences*, *110*(5), 353-357.
15. Hannas, A. R., Pereira, J. C., Granjeiro, J. M., & Tjäderhane, L. (2007). The role of matrix metalloproteinases in the oral environment. *Acta Odontologica Scandinavica*, *65*(1), 1-13.
16. Hardy, D. C., Ross, J. H., Schuyler, C. A., Leite, R. S., Slate, E. H., & Huang, Y. (2012). Matrix metalloproteinase-8 expression in periodontal tissues surgically removed from diabetic and non-diabetic patients with periodontal disease. *Journal of clinical periodontology*, *39*(3), 249-255.
17. Hernández Ríos, M., Sorsa, T., Obregón, F., Tervahartiala, T., Valenzuela, M. A., Pozo, P., ... & Gamonal, J. (2009). Proteolytic roles of matrix metalloproteinase (MMP)-13 during progression of chronic periodontitis: initial evidence for MMP-13/MMP-9 activation cascade. *Journal of clinical periodontology*, *36*(12), 1011-1017.
18. Hernandez, M., Gamonal, J., & Tervahartiala, T. et al (2010). Associations between MMP-8 & -14 and myeloperoxidase in gingival crevicular fluid from subjects with progressive chronic periodontitis- A longitudinal study. *Journal of Periodontology*, *81*, PP: 1644–1652.
19. Honibald, E. N., Mathew, S., Padmanaban, J., Sundaram, E., & Ramamoorthy, R. D. (2012). Perioceutics: Matrix metalloproteinase inhibitors as an adjunctive therapy for inflammatory periodontal disease. *Journal of pharmacy & bioallied sciences*, *4*(Suppl 2), S417.
20. Huo, N., Ichikawa, Y., Kamiyama, M., Ishikawa, T., Hamaguchi, Y., Hasegawa, S., ... & Shimada, H. (2002). MMP-7 (matrilysin) accelerated growth of human umbilical vein endothelial cells. *Cancer letters*, *177*(1), 95-100.
21. Impola, U., Uitto, V. J., Hietanen, J., Hakkinen, L., Zhang, L., Larjava, H., ... & Saarialho-Kere, U. (2004). Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *202*(1), 14-22.
22. Ingman, T., Sorsa,, T., Michaelis, J., & Konttinen, Y.T . (1994). Matrix metalloproteinases-1, -3 and -8 in adult periodontitis in situ: an immunohistochemical study. *Ann NY Acad Sci 732: 459– 461.*
23. Johansson, N., Airola, K., Grenman, R., Kariniemi, A. L., Saarialho-Kere, U., & Kähäri, V. M. (1997). Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. *The American journal of pathology*, *151*(2), 499–508.
24. Kim, J.B., Jung, M.H., Cho, J-Y., Park, J-W., Sun, J-Y., & Lee, J-M. (2011). The influence of type -2 diabetes on the expression of inflammatory mediators and tissue inhibitor of metalloproteinase-2 in human chronic periodontitis. *Journal of Periodontal Implant Sciences, 41*, 109–116.
25. Kinane, D. F., Darby, I. B., Said, S., Luoto, H., Sorsa, T., Tikanoja, S., & Mäntylä, P. (2003). Changes in gingival crevicular fluid matrix metalloproteinase-8 levels during periodontal treatment and maintenance. *Journal of periodontal research*, *38*(4), 400-404.
26. Konopka, Ł., Pietrzak, A., & Brzezińska-Błaszczyk, E. (2012). Effect of scaling and root planing on interleukin-1β, interleukin-8 and MMP-8 levels in gingival crevicular fluid from chronic periodontitis patients. *Journal of periodontal research*, *47*(6), 681-688.
27. Korostoff, J. M., Wang, J. F., Sarment, D. P., Stewart, J. C., Feldman, R. S., & Billings, P. C. (2000). Analysis of in situ protease activity in chronic adult periodontitis patients: Expression of activated MMP-2 and a 40 kDa serine protease. *Journal of periodontology*, *71*(3), 353-360.
28. Kusukawa, J., Sasaguri, Y., Morimatsu, M., & Kameyama, T. (1995). Expression of matrix metalloproteinase-3 in stage I and II squamous cell carcinoma of the oral cavity. *Journal of oral and maxillofacial surgery*, *53*(5), 530-534.