ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 3, Issue – 3, May - 2020, Page No. : 434 - 440

Significance of cysteine protease inhibitor - Cystatin C in periodontal health and disease

[1]Dr. Jeneffar Roselin C, IInd year post-graduate, Dept of Periodontology, S.R.M Dental college, Ramapuram, Chennai – 89.

[2]Dr. Deepa Ponnaiyan, MDS, Reader, S.R.M Dental College, Ramapuram, Chennai –89.

[3]Dr. Harinath P, MDS, Professor, S.R.M Dental College, Ramapuram, Chennai – 89.

[4]Dr. Anupama Tadepalli, MDS, Reader, S.R.M Dental College, Ramapuram, Chennai – 89.

Corresponding author: Dr. Jeneffar Roselin C, IInd year post-graduate, Dept of Periodontology, S.R.M Dental college, Ramapuram, Chennai –89.

Citation of this Article: Dr. Jeneffar Roselin C, Dr. Deepa Ponnaiyan, Dr. Harinath P, Dr. Anupama Tadepalli, "Significance of cysteine protease inhibitor - Cystatin C in periodontal health and disease", IJDSIR- May - 2020, Vol. – 3, Issue -3, P. No. 434 – 440.

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Periodontal diseases are characterized by the inflammation and destruction of supporting tissues of the teeth. Periodontitis is initiated by bacterial accumulation between the gingiva and teeth, which triggers an inflammatory-immune response within the host. In susceptible individuals, the initial acute inflammatory response fails to resolve, and a dysregulated chronic inflammation ensues, which destroys the supporting connective tissues surrounding the teeth. Destructive process in periodontitis is caused by an imbalance of homeostasis between degradative enzymes such as the lysosomal cysteine proteinases, Cathepsins, and their inhibitors. Cystatin C (CSTC) is a physiologically active antimicrobial protein well recognized due to its inhibitory potential against lysosomal enzymes and cysteine proteinases, which are major contributors of periodontal disease. This review summarises the role of CSTC in periodontal health and disease.

Keywords: Cystatin C, gingival crevicular fluid, Saliva, serum, Biomarker, chronic periodontitis, gingivitis.

Introduction

Periodontitis is a chronic multifactorial disease characterized by an inflammation of the periodontal tissue, which is associated with intricate interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis,[1] resulting in irreversible loss of connective tissue attachment and supporting alveolar bone.[2] Substances produced by the subgingival bacterial flora and the tissue during inflammation and immune reactions affects the bone turnover by inhibition of bone formation by osteoblasts or differentiation and stimulation of osteoclasts, which originate from monocytic precursors of

the hematopoietic lineage by stimulation with the RANKL and macrophage colony-stimulating factor.[3]

Proteolytic processes are known to be critical in osteoclastic bone resorption. Degradation of bone matrix proteins is initiated by lysosomal proteinases and cysteine proteases secreted from osteoclasts into the bone resorption lacunae and then is continued and completed in the intracellular endosomal/lysosomal system.[4]

Cathepsin K (CSTK) a predominant effector in osteoclastic bone resorption is mainly derived from osteoclasts, hence its used as a well-known marker of osteoclast activity. It plays a key role in bone remodeling and cartilage breakdown, contributing to osteoclastic bone destruction in periodontal disease.[5] Destructive process in periodontitis is caused by an imbalance of the homeostasis between these degradative enzymes and their inhibitors, such as cystatins.

The cystatins are a family of cysteine protease inhibitors which is said to mainly inhibit protease enzymes belonging to peptidase families C1 (papain family) and C13 (legumain family). They could play a protective and regulatory role under inflammatory conditions.[6] Cystatin C (CSTC) belongs to the type - 2 cystatin gene (CST3) family. It is a potent inhibitor of lysosomal proteinases and is one of the most important extracellular inhibitors of cysteine proteases thereby preventing the breakdown of proteins outside the cell.

The levels of Cystatin C have been studied in saliva and GCF and observed that Cystatin C increases with periodontal disease progression and decreases after treatment. Similarly, increased concentrations of Cystatin S and Cystatin C were detected in whole saliva in both gingivitis and periodontitis subjects in comparison with healthy subjects.[7,8] In contrast to the above-mentioned studies, decreased levels of Cystatin C were reported in total saliva and gingival crevicular fluid (GCF) in patients with gingivitis. Additionally, Salivary cystatin C levels were highest in periodontal health when compared to periodontitis patients. Thus, the role of Cystatin C as a biomarker in periodontal diseases is controversial.[9,10] Further, Cystatin C protein expression studies in gingival tissue have revealed that expression of Cystatin C is higher in cyclosporine A - induced gingival overgrowth specimens when compared to healthy gingival tissues. In contrast, significantly lower expression of Cystatin C was seen in gingival tissue samples from the sites with greater probing depth.[11,12] Thus, based on the above literature evidence, the role of CSTC in periodontal health and disease is still controversial.

Cystatin C

CSTC was initially described as 'gamma-trace' in 1961 as a trace protein together with other ones in cerebrospinal fluid and in urine of patients with renal failure. Its amino acid sequence was first reported by Grubb and Löfberg.16 It is formerly been called in names like gamma trace, post-gamma-globulin, or neuroendocrine basic polypeptide. It is protein encoded by the CST3 gene and belongs to the type 2 cystatin gene family. In humans, all cells with a nucleus produces cystatin C as a chain of 120 amino acids. It is virtually found in all tissues and body fluids. CSTC is a potent inhibitor of lysosomal proteinases and is an important extracellular inhibitor of cysteine proteases. CSTC is mainly used as a biomarker of kidney function.Molecular biology of CST3 geneThe type 2 cystatin genes and pseudogenes where majorly found in the cystatin locus on the short arm of chromosome 20. The CST3 gene is located in the cystatin locus and comprises 3 exons, spanning 4.3 kilo-base pairs and it encodes the most of the extracellular inhibitor of cysteine proteases. CSTC is found in high concentrations in all biological fluids and is also expressed in mostly all organs of the body, hence acts as a housekeeping gene. The highest

levels are found in semen, followed by breastmilk, tears and saliva.Cystatin C is a non-glycosylated, basic protein with an isoelectric point at pH 9.3. The crystal structure of cystatin C is comprised by a short alpha helix and a long alpha helix that lies across a large antiparallel, five-stranded beta sheet. Like all the other type 2 cystatins, CSTC also has two disulfide bonds and around 50% of the molecules carries a hydroxylated proline. CSTC forms molecule pairs by exchanging the subdomains; in the paired state and each half is made up of a long alpha helix, one beta strand of one partner and four beta strands of the other partner.

Role of Cathepsin K in periodontal disease

Cathepsin K (CTSK) is a lysosomal cysteine protease that belongs to the peptidase C1 protein gene family. It is encoded by the CTSK gene and is defined by its high specificity for kinins.

Periodontitis is characterized by loss of hard and soft tissue changes such as attachment loss and destruction of the periodontal ligaments. After demineralization, the major proteolytic osteoclast enzyme cathepsin K degrades the type I collagen-rich organic bone matrix.[13]

Studies by Garg et al, 2009; Yamalik et al, 2011 states that GCF-cathepsin K values are higher in periodontitis than in healthy controls,[14,15] also scaling and root planing in patients with periodontitis lowers or even normalizes the cathepsin K values in GCF.[14] Statistically significant correlations have been found between GCF values of cathepsin K, RANKL and alveolar osteolysis.[15] Also, there was a positive correlation between cathepsin-K and RANKL levels, suggesting that both of them contribute to osteoclastic bone destruction in periodontal disease.[5]

CSTC in periodontal health and disease

Cystatin C is a cysteine protease inhibitor and an anti-inflammatory factor. It acts as a regulator of endogenous cysteine proteinases which are secreted or leaked from the lysosomes of dying or diseased cells. Exogenous CSTC downregulates cathepsin K, which as discussed before is one of the cysteine proteinases that is mainly expressed by osteoclasts and plays an important role in alveolar bone resorption.[20] Its level increases in patients with periodontitis, and it is known as a marker for the assessment of the activity of osteoclasts in periodontal disease.[14] Increase in the level of Cathepsin K leads to increased Cystatin C level as a marker to regulate the activity of this enzyme.[20]

Henskens et al.[7] found that CSTC levels were not present in GCF samples. He stated that increased concentrations of Cystatin C were detected in whole saliva in both gingivitis and periodontitis subjects when compared to healthy subjects. Similarly, in a study by Anuj Sharma et al.[21] the mean concentration of cystatin C in GCF and serum was higher in periodontitis group when compared to periodontally healthy subjects with intermediate concentration in gingivitis group. CSTC concentration in GCF and serum increased proportionally with the severity of periodontal disease and decreased after treatment, suggesting that CSTC increases with disease progression to prevent further degeneration of periodontium and decreases after treatment due to bone metabolic homeostasis.

Decrease in the level of CSTC in periodontitis patients after non-surgical periodontal therapy was further confirmed by studies conducted by Graziani et al.[22,23] stating that the serum CSTC levels reduced significantly from baseline to 180 days after non-surgical periodontal therapy.

In contrast the above-mentioned studies, a study by Evren Ulker et al.[9] conducted in children's aging from 11 to 16 years, stated that both the salivary and GCF cystatin C levels were higher in periodontally healthy children's than children with gingivitis.

Similarly, a study by Skaleric et al.[12] reported that CSTC concentrations were significantly lower in gingival tissue samples taken from the sites with greater probing depth in subjects with inflamed gingiva when compared to healthy subjects. Also, in a study by Aditishrikrishnadhage et al.[10] Salivary cystatin C levels were highest in periodontal healthy subjects when compared to periodontitis patients and salivary CSTC levels were slightly greater in smokers when compared to non-smokers with chronic periodontitis. Patients with aggressive periodontitis exhibited remarkably low levels of salivary cystatin C with a negative correlation to all the clinical parameters.

Considering the above-mentioned studies, the CSTC levels in serum, GCF, saliva and tissue samples in various levels of periodontal disease and health are still controversial. Hence the role of CSTC, whether a protective or destructive marker in the pathogenesis of periodontal disease still remains questionable.

Discussion

Periodontitis is a multifactorial disease associated with a specific group of indigenous oral bacteria mainly the gram-negative bacteria. Inflammation and bone loss being the hallmarks of periodontal diseases are primarily associated with proteolytic actions carried out by enzymes such as cathepsins derived from both host and microbes. (1)(2)

Cathepsin K, a cysteine protease mainly derived from osteoclasts play a major role in the destruction of periodontium by osteoclastic activity. In health under normal physiologic conditions, the proteolytic activity of these enzymes such be tightly controlled at several levels. Disturbance in the regulation CSTK leads to uncontrolled release of proteinases that aggravates the chronic inflammatory condition, thereby resulting in autolysis and tissue destruction observed in periodontitis. (5)

Cystatin C, an inhibitor of cysteine protease regulates proteolytic enzymes such as cathepsin K, B, H and L in periodontal disease. CSTC plays a m major role in tissue remodeling by inhibiting this collagen degrading cathepsins and down regulating the protease activity. It was initially used as a marker of renal function to assess the GFR. Later its role as a marker of inflammation was studied in patients with liver disease, thyroid disorders, cardiovascular diseases and neurovascular diseases like Alzheimer's disease.

The role of Cystatin C in the pathogenesis of periodontal diseases has been widely studied by many authors by assessing their values in bodily fluids such as serum, GCF, saliva and tissue sample during periodontal health and at various levels of periodontal disease.

During the period of 1993 to 1996, Henskens et al. did a group of studies on subjects on with healthy periodontium and periodontitis subjects to evaluate the source of CSTC other than plasma and its level in the GCF and saliva. Results stated that the salivary levels of CSTC was higher in patients with chronic periodontitis when compared with healthy subjects and also that cystatin C could not be detected in GCF samples.(7)(24)(25) Furthermore, it was also noted that the salivary CSTC levels in chronic periodontitis subjects reduced after non-surgical periodontal treatment.

In 2010, Graziani F et al. (22)(23) studied the serum levels of CSTC in subjects with CP before and after non-surgical and surgical periodontal treatment and the results stated that there was a significant decline in the serum levels of CSTC following periodontal treatment. The reason behind the reduction in serum CSTC levels after non-surgical periodontal therapy was considered as the systemic acute-phase inflammatory response that was triggered by the inflammatory markers from periodontal disease which

further leads to increased levels of acute phase proteins that affect function of renal system.

In 2012, Anuj Sharma et al. [21] carried out a study to explore the GCF and serum cystatin C concentration with severity of periodontal disease on a total of 30 subjects and results stated that the concentration of CSTC increased from periodontal health to chronic periodontitis and subsequently decreased after non-surgical periodontal therapy in both GCF and serum samples.

Unlike the study by Graziani F et al, this study indicates that during the transformation from periodontal health to disease, the production of CSTC increases in local periodontal tissue as a protective marker and transfers over to the serum leading to elevation of serum CSTC and after non-surgical periodontal therapy, local inflammatory components subside owing to reduction in CSTC levels in periodontal tissues and so in serum. This study also states that the use of filter paper strip for collection of GCF and immunoblotting technique used for CSTC detection in Henskens et al. study can be the reason for not detecting CSTC in GCF samples and they have overcome these by using microcapillary pipettes for GCF collection and detection of CSTC by using sensitive ELISA kits.

A study by Mohammad Hassan Najafi Neshli et al. [26] stated that the level of cystatin C in the female group was significantly higher in periodontitis patients, whereas in the male group there was no significant difference and suggested that the lower periodontal destruction in female group could be due to the higher level of cystatin C.

In contrary to the above-mentioned studies, Skaleric U et al. [12] in his study on 22 patients with varying degrees of periodontal disease stated that there was decreased levels of Cystatin C present in the inflamed human gingiva of patients with CP when compared to that of healthy gingiva.

Similarly, Evren Ulker et al. [9] stated that in total saliva and GCF, the levels of cystatin-C were higher in periodontally healthy children when compared to that of children with gingivitis.

Recently in a study by Aditishrikrishnadhage et al. [10] in 2014**,** conducted to assess the severity of periodontal disease based on cystatin C levels in saliva, highest levels of salivary cystatin C levels was found in periodontal health when compared to patients with CP and aggressive periodontitis. It was also stated that the lower salivary cystatin C levels in periodontitis could be due to tissue pathology attributable to the persistence of unopposed proteolysis.

Based on the studies mentioned above, the role of cystatin C in the pathogenesis of periodontal disease is still conflicting and lacks a clear perspective on whether it is protective marker or destructive marker in periodontal disease. Studies state that recombinant human CSTC also inhibits cysteine proteinases like papain with same efficiency as that of natural CSTC, [27] thus, in future paving a way for novel therapies in periodontal disease.

Conclusion

Based on the above literature evidence, it has been proved that Cystatin C has a significant role in periodontal health and disease. The concentration of Cystatin C in GCF, saliva, serum and tissue samples increased proportionally with the severity of periodontal disease and subsequently reduced after non-surgical periodontal treatment in most of the studies. Still, some studies provide conflicting data stating that it CSTC levels were higher in healthy periodontium in comparison with periodontal disease. Studies have also proposed that an enzyme called peptidyldiazomethane, that mimicks a part of the proposed substrate like-binding region of Cystatin C, portrays an irreversible inhibition of papain and Streptococcal cysteine proteinase and also appears to have a potential to

act as an antimicrobial drug. The role of Cystatin C as an inhibitory biomarker of osteoclastic activity can be explored in future as a potential therapeutic target in treatment of periodontal disease. Further, longitudinal studies involving larger population, meta analyses, genetic studies exploring the gene polymorphisms of CST3 gene are needed to better understand and confirm its role in the pathogenesis of periodontal diseases.

References

1. Taubman MA, Kawai T, Han X. The new concept of periodontal disease pathogenesis requires new and novel therapeutic strategies. Journal of clinical periodontology. 2007 May;34(5):367-9.
2. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. Journal of clinical periodontology. 2018 Jun;45:S149-61.
3. Lee ZH, Kim HH. Signal transduction by receptor activator of nuclear factor kappa B in osteoclasts. Biochemical and biophysical research communications. 2003 May 30;305(2):211-4.
4. Sasaki T, Ueno-Matsuda E. Cystein-proteinase localization in osteoclasts: an immunocytochemical study. Cell and tissue research. 1993 Jan 1;271(1):177-9.
5. Mogi M, Otogoto J. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. Archives of oral biology. 2007 Sep 1;52(9):894-8.
6. Saklatvala J, Nagase H, Salvesen G, Abrahamson M, Alvarez-Fernandez M, Nathanson CM. Cystatins. InBiochemical Society Symposia 2003 Sep 1 (Vol. 70, pp. 179-199). Portland Press.
7. Henskens YM, Veerman EC, Mantel MS, Van der Velden U, Nieuw Amerongen AV. Cystatins S and C in human whole saliva and in glandular salivas in periodontal health and disease. Journal of dental research. 1994 Oct;73(10):1606-14.
8. Henskens YM, Van den Keijbus PA, Veerman EC, Van der Weijden GA, Timmerman MF, Snoek CM, Van der Velden U, Amerongen AN. Protein composition of whole and parotid saliva in healthy and periodontitis subjects: Determination of cystatins, albumin, amylase and IgA. Journal of periodontal research. 1996 Jan;31(1):57-65
9. Ülker AE, Tulunoglu Ö, Özmeric N, Can M, Demirtas S. The evaluation of cystatin C, IL1β, and TNF α levels in total saliva and gingival crevicular fluid from 11 to 16 year old children. Journal of periodontology. 2008 May 1;79(5):854-60.
10. Aditishrikrishnadhage, Savitha N. Salivary Cystatin C- A dynamic biomarker of periodontal disease. International Journal of Medicine and Pharmaceutical Sciences. 2014 Apr;4(2):55-62.
11. Tsai CH, Yang SF, Huang FM, Chang YC. The upregulation of cystatin C in human gingival fibroblasts stimulated with cyclosporine A. Journal of periodontal research. 2009 Aug;44(4):459-64.
12. Skalerič U, Babnik J, Čurin V, Lah T, Turk V. Immunochemical quantitation of cysteine proteinase inhibitor cystatin C in inflamed human gingiva. Archives of oral biology. 1989 Jan 1;34(4):301-5.
13. Hernandez M, Dutzan N, García-Sesnich J, Abusleme L, Dezerega A, Silva N, Gonzalez FE, Vernal R, Sorsa T, Gamonal J. Host-pathogen interactions in progressive chronic periodontitis. Journal of dental research. 2011 Oct;90(10):1164-70.
14. Garg G, Pradeep AR, Thorat MK. Effect of nonsurgical periodontal therapy on crevicular fluid levels of Cathepsin K in periodontitis. Archives of oral biology. 2009 Nov 1;54(11):1046-51.

15. Yamalik N, Günday S, Kilinc K, Karabulut E, Berker E, Tözüm TF. Analysis of cathepsin-K levels in biologic fluids from healthy or diseased natural teeth and dental implants. International Journal of Oral & Maxillofacial Implants. 2011 Oct 1;26(5).
16. Grubb A, Löfberg H. Human gamma-trace, a basic microprotein: amino acid sequence and presence in the adenohypophysis. Proceedings of the National Academy of Sciences. 1982 May 1;79(9):3024-7.
17. Harmoinen A, Ylinen E, Ala-Houhala M, Janas M, Kaila M, Kouri T. Reference intervals for cystatin C in pre-and full-term infants and children. Pediatric Nephrology. 2000 Oct 1;15(1-2):105-8.
18. Bruno RM, Gross JL. Prognostic factors in Brazilian diabetic patients starting dialysis: a 3.6-year follow-up study. Journal of Diabetes and its Complications. 2000 Sep 1;14(5):266-71.
19. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. Diabetes. 2003 Apr 1;52(4):1036-40.
20. Schick C, Pemberton PA, Shi GP, Kamachi Y, Çataltepe S, Bartuski AJ, Gornstein ER, Brömme D, Chapman HA, Silverman GA. Cross-class inhibition of the cysteine proteinases cathepsins K, L, and S by the serpin squamous cell carcinoma antigen 1: a kinetic analysis. Biochemistry. 1998 Apr 14;37(15):5258-66.
21. Sharma A, Pradeep AR, Raghavendra NM, Arjun P, Kathariya R. Gingival crevicular fluid and serum cystatin c levels in periodontal health and disease. Disease markers. 2012 Jan 1;32(2):101-7
22. Graziani F, Cei S, Tonetti M, Paolantonio M, Serio R, Sammartino G, Gabriele M, D'Aiuto F. Systemic inflammation following non-surgical and surgical periodontal therapy. Journal of clinical periodontology. 2010 Sep;37(9):848-54.
23. Graziani F, Cei S, La Ferla F, Vano M, Gabriele M, Tonetti M. Effects of non-surgical periodontal therapy on the glomerular filtration rate of the kidney: an exploratory trial. Journal of clinical periodontology. 2010 Jul;37(7):638-43.
24. Henskens YM, Van der Velden U, Veerman EC, Amerongen AN. Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis periodonitis. Journal of periodontal research. 1993 Jan;28(1):43-8.
25. Henskens YM. Velden U van der, Veerman ECI, Nieuw Amerongen A van. Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis or periodontitis. J Periodont Res. 1993;28:43-8.
26. Neshli N, Hassan M, Taheri M, Rafatpanah H, Moeintaghavi A, Mokhtari MR, Mohammadzade Rezae M, Farazi F. Evaluation of the Relationship between Cystatin C Level in Whole Saliva and Chronic Periodontitis. Journal of Mashhad Dental School. 2013;37(3):185-200.
27. Abrahamson M, Mason RW, Hansson H, Buttle DJ, Grubb A, Ohlsson K. Human cystatin C. Role of the N-terminal segment in the inhibition of human cysteine proteinases and in its inactivation by leucocyte elastase. Biochemical Journal. 1991 Feb 1;273(3):621-6.