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# Gingival Crevicular Fluid Levels of Trappin 2 In Individuals With And Without Chronic Periodontitis

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## Abstract

**Introduction:** Trappin-2 is a potent biologically active serine protease inhibitor with anti-inflammatory properties. The potential involvement of Trappin -2 in periodontitis remains undefined. As serine proteases and their inhibitors are most commonly found in mucosal fluids, their presence and levels can mirror periodontal status.

**Aim:** This study aims to investigate gingival crevicular fluid (GCF) levels of Trappin-2 in periodontally healthy individuals and patients with gingivitis or generalized chronic periodontitis (CP).

**Materials And Methods:** GCF samples were collected from 90 systemically healthy and non-smoking individuals with gingivitis and chronic generalized periodontitis and were grouped as follows (i) Group I: 30 patients who are periodontally healthy, (ii) Group II: 30 patients with gingivitis and (iii) Group III: 30 patients with chronic generalized periodontitis. Trappin-2 were analysed by enzyme-linked immunosorbent assay (ELISA).

**Results:** The mean Trappin 2 levels were significantly lower in periodontitis group (4676.66 ± 702.09 pg/ml) when compared to that of healthy and gingivitis group.

**Conclusion:** Reduced trappin-2 levels in individuals with periodontitis may denote potential protease–anti-protease imbalance, resulting in impaired host protective capacity and proteolytic tissue damage in periodontal disease.

**Keywords:** Bacteria, Neutrophil Biology, Periodontal Diseases, Bacterial Virulence, Inflammation

## Introduction

Periodontitis is a chronic inflammatory polymicrobial disease. The pathogenesis of periodontal disease results from interaction of periopathogens with host immune responses. There are various bacterial species associated with periodontitis and play a significant role in periodontal disease initiation and progression. Porphyromonas gingivalis is one among the major pathogens of chronic periodontitis[1]. A variety of virulence factors, including lipopolysaccharides, capsular material and fimbriae are implicated in the pathogenicity of P. gingivalis[2]. Among this, proteolytic enzymes secreted by P. gingivalis, cysteine proteases, referred to as gingipains are most important. These gingipains can degrade the host proteins and also, they account for higher proteolytic activity[3]. Although gingipains can directly degrade components of the connective tissue, they can inflict far more damage by encroaching functions of tightly regulated host proteases of coagulation, fibrinolysis, complement, receptor signalling, and kinin-release pathways[4]. Moreover, by inactivation of endogenous proteinase inhibitors gingipains can contribute to uncontrolled proteolytic

activity at the periodontitis sites infected with P. gingivalis[5].

During this periodontal tissue breakdown process, infiltration of large number of neutrophils have been implicated. Neutrophils can do a lot of collateral damage to the connective tissue by the release of protease enzymes, namely serine proteases, which includes neutrophil elastase (NE), proteinase 3 (PR 3) and cathepsin G[6]. These enzymes participate in inflammation and destruction of periodontal tissues. NE and PR3 are capable of increasing production of interleukin-8 and monocyte chemoattractant protein 1 in gingival fibroblasts[7] and NE degrades periodontal ligament[8].

So, in order to maintain homeostasis in tissues, the activity of neutrophil proteases has to be tightly regulated by blood plasma-derived and tissue-derived protease inhibitors[9].

Serine protease inhibitors play a critical role in host tissue homeostasis and the balance between proteases and their inhibitors contributes to maintenance of tissue integrity[10]. These protease inhibitors potentially target two kinds of proteases, host cell-derived proteases such as neutrophil elastase (NE), and pathogen-derived proteases such as the gingipains, and therefore may be important in controlling the extent of inflammatory tissue damage[11,12]. These protease inhibitors include secretory leukocyte protease inhibitor (SLPI), elastase-specific inhibitor (ELAFIN) and squamous cell carcinoma antigen (SCCA)[13]. SLPI inhibits mainly NE[14], elafin targets both NE and PR3[15].

Trappin-2 is a potent biologically active serine protease inhibitor[16] and is a precursor peptide to elafin (matured form of trappin 2). It is expressed by epithelial cells of the gastrointestinal tract and lung epithelium, human epithelia of the tongue, palate, lingual tonsils, pharynx and gingiva. The role of trappin 2 has been documented in various chronic inflammatory disease[17,18]. Despite various studies on role of elafin in oral cavity, it is unknown if trappin 2 play a role in periodontal inflammation, even though trappin -2 has been shown to possess greater anti-bacterial activity than elafin[19]. So, its presence in gingival crevicular fluid may indicate the severity of inflammation at individual sites. Hence, this study aims to investigate GCF levels of trappin-2 in periodontally healthy individuals and patients with gingivitis or generalized chronic periodontitis (CP).

**Materials and Methods**

**1. Study population and clinical examination**

This Randomized controlled clinical trial included a total of 90 participants from department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore belonging to the age group of 18-60 years. Ethical clearance was obtained by the institutional ethical committee of Rajarajeswari dental college and hospital, Bangalore. Written informed consent was obtained from each participant after the purpose and procedure of the study were explained.

Individuals belonging to the age group of 18 to 60 years, systemically healthy patientsand patients who have not received any periodontal treatment for the last 6 months prior to the clinical examination were included in the study. Exclusion criteria included smokers, alcoholics, pregnant and lactating women, individuals with the history of Systemic diseases or immune deficiency and patients with a history of periodontal surgical treatment in the past 12 months on the involved sites.

All subjects underwent a full-mouth periodontal examination with a manual dental probe UNC-15 including the record of the following periodontal parameters: probing depth (PD), clinical attachment level (CAL) and papilla bleeding index (PBI)[20]. Parameters were recorded at four periodontal sites (distal, buccal, mesial, lingual) by a single trained examiner. The

cemento-enamel junction (CEJ) was used as a reference for measurement of CAL. Extent and severity of bone support and/or osseous lesions were evaluated radiographically in each patient.

Participants were classified into three groups based on their periodontal conditions according to criteria proposed by the 1999 International Workshop for Classification of Periodontal Diseases and Conditions[21].

a) PERIODONTALLY HEALTHY (H)

A total of 30 systemically and periodontally healthy individuals were included in the H control group. Individuals with periodontal health had no sites with PD > 3 mm and CAL > 2 mm, mean PBI of 0.17 at time of examination, and no detectable alveolar bone loss (ABL) (distance between CEJ and bone crest ≤3 mm at> 95% of proximal tooth sites).

b) GINGIVITIS (G)

Thirty patients were included in G group. They had varying degrees of gingival inflammation with mean PBI values > 2, but no CAL > 2 mm and no sites with ABL present in radiograph (distance between CEJ and bone crest ≤3 mm at > 95% of proximal tooth sites).

c) CHRONIC PERIODONTITIS (CP)

The CP group included 20 patients with severe generalized CP. These individuals had a minimum of four non-adjacent teeth with sites with CAL ≥5 mm and PD ≥6 mm, and ≥50% alveolar bone loss (ABL) in at least two quadrants. They also had mean PBI values > 2.9.

**2. Gingival crevicular fluid sampling**

Gingival crevicular fluid sampling was carried out 1 week after the periodontal examination among all participants. Gingival crevicular fluid samples were obtained from healthy sites (H) of each periodontally healthy individual, sites with gingivitis (G) and sites with periodontitis (CP). Before gingival crevicular fluid sampling, all sites were isolated with cotton rolls to avoid contamination with saliva and the tooth was gently air- dried. Samples of GCF were obtained from the sites by placing colour coded, calibrated, volumetric, micro capillary pipettes with 0-5µl range. Volumetric micropipettes were placed at the gingival crevice entrance, and the GCF samples were collected from each patient.

The pipettes contaminated with blood or saliva was discarded. The pipette with collected fluid was wrapped in a sterile aluminium foil to prevent oxidation, placed in a plastic vial and immediately stored at – $80^0$ C until analysis for trappin- 2 with commercially available ELISA kits.

**3. Quantification of trappin -2 with ELISA**

The gingival crevicular fluid levels of hT – 2 were measured (pg/mL) using the ELISA technique according to the manufacturer's guidelines. Briefly, 96 wells were added with 100 µL of sample or standard solution and the plates were incubated for 2.5 hours. After 4 washes with wash buffer (PBS), 100 µL of hT – 2 (human trappin – 2) specific antibody was added and incubated for 1 hour, followed by 4 washes with wash buffer. After this period, 100 µL of horse radish peroxidase (HRP) streptavidin was added to the wells and incubated for 45 minutes. After this period, the plates were washed and 100 µL of one step substrate reagent, tetramethyl benzidine (TMB) solution was added and incubated for 30 minutes, followed by the addition of 50 µL stop solution. After blocking the reaction with stop solution (H2SO4), the absorbance was measured at 450 nm using an ELISA reader. The results were expressed in pg/mL.

**Statistical Analysis**

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. One-way ANOVA test followed by Tukey's HSD Post hoc analysis was used to compare the mean Trappin 2 Levels

in GCF between 03 study groups. The level of significance [P-Value] was set at P<0.05.

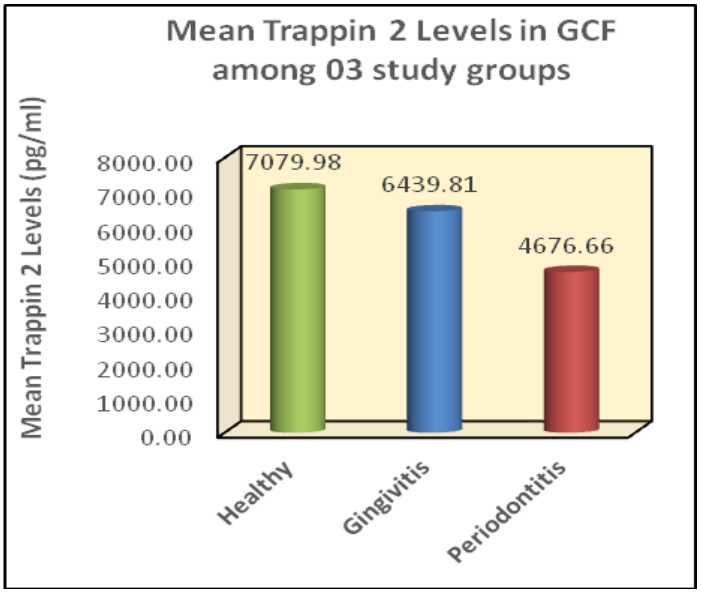
**Results**

The mean concentration of trappin - 2 levels in healthy group was 7079.98 ± 152.07 pg/ml, 6439.81 ± 363.99 pg/ml in gingivitis group and 4676.66 ± 702.09 pg/ml in periodontitis group. There was a significant difference in the mean Trappin 2 levels between three groups at P<0.001 (table 1 and figure 1). The level of trappin – 2 was found to be present in very high concentrations in periodontally healthy subjects. (*-Statistically Significant).

Table 1: Comparison of mean Trappin 2 (pg/ml) GCF levels between 03 groups using One-way ANOVA Test

| Groups | N | Mean | SD | Min | Max | P-Value |
|---|---|---|---|---|---|---|
| Healthy | 30 | 7079.98 | 152.07 | 6752.9 | 7518.4 | <0.001* |
| Gingivitis | 30 | 6439.81 | 363.99 | 5786.0 | 7133.6 | |
| Periodontitis | 30 | 4676.66 | 702.09 | 3154.2 | 5994.6 | |

Figure 1: Comparison of mean Trappin 2 levels [pg/ml] in GCF between the groups.




In periodontitis group, the levels of trappin -2 were significantly lower compared to other groups. Hence, we can infer that Healthy group has significantly higher mean Trappin 2 levels in GCF followed by Gingivitis and Periodontitis group.

**Discussion**

Periodontitis is strongly associated with a complex of microbial species within the biofilm, suggesting that multiple microorganisms contribute to the pathogenesis of chronic periodontal disease. These putative periodontal pathogens express a variety of proteolytic enzymes which may be involved in the processes of periodontal disease. The best characterized of these are gingipains from P. gingivalis, which can directly degrade many host proteins[3]. P. gingivalis can interfere with control mechanisms of proteolysis in the periodontal tissue utilizing the host inflammatory proteases elastase and proteinase 3 to facilitate local overall proteolytic activity[6]. In addition to having tissue destructive activity, neutrophil serine proteases have the ability to promote inflammation by processing proinflammatory cytokines and activating various inflammatory receptors[22]. Uncontrolled neutrophil protease activity results in enhanced local inflammation and tissue destruction providing peptides which are indispensable nutrients for asaccharolytic P. gingivalis[6]. Therefore, this present study investigates the level of an epithelial protease inhibitor, trappin-2 in GCF and its association with periodontal diseases.

Trappin-2 is considered mostly as a mucosal and locally produced defence molecule, contributing to mucosal immunity and integrity[18]. Intestinal and tracheal epithelial expression of trappin-2 was shown to be critical to maintenance of tissue homeostasis by playing a protective role against destructive bacterial proteinases[19]. Results also support a protective role of trappin-2 in oral mucosa as individuals with periodontal health showed significantly higher concentrations of trappin-2 in their saliva according to a study by Afacan et al (2007)[16]. It can be speculated that higher levels of trappin-2 in saliva may help to keep

bacterial numbers lower in the oral cavity as trappin-2 was also shown to inhibit growth of several non-oral Gram-positive and Gram-negative bacterial pathogens[19]. Although to date there are no studies for trappin-2 levels in GCF, few earlier studies investigated the levels of its mature secreted form, namely elafin.

In the present study, the levels of trappin-2 decreased with severity of periodontal disease. The present findings are also in support of earlier studies demonstrating association between reduced trappin-2 levels and other chronic inflammatory diseases. Kretschmar et al. showed that patients with advanced forms of periodontitis exhibited higher P. gingivalis in their plaque, with lower elafin levels[13].

Assuming the twofold interaction between serine protease inhibitors and neutrophil elastase, two contributing factors might be involved in the reduced amount of protease inhibitors in periodontally involved groups. Bacterial infection with its biofilm leads to the release of proinflammatory cytokines, which results in vast recruitment of potent neutrophils and neutrophils in a primed state are hyper functional[23] and contribute to excessive neutrophil elastase in sites of periodontal disease. Thus, the two contributors, host-derived neutrophil elastase and P. gingivalis-derived gingipains, might be involved in the reduction of host protease inhibitors. This reflects the various degrees of reduction of host protease inhibitors between the diseased group with and without infection[16]. In addition to its antiprotease activity, recombinant trappin - 2 possesses anti-inflammatory activity by inhibiting nuclear factor-kappa B, leading in turn to reduced IL-1$\beta$ expression[24,25].

Also, studies report that new concepts of drug delivery are designed with the use of protease inhibitors in the field of medicine[26]. Future studies will have to demonstrate the therapeutic potential for selective inhibitors of neutrophil serine proteases to treat inflammatory diseases especially when it comes to its application in dentistry**.**

**Conclusion**

In conclusion, higher levels of trappin-2 from periodontally healthy individuals could be ascribed to its direct antimicrobial and anti-inflammatory activities. Decreased trappin-2 levels in chronic periodontitis may denote protease–anti-protease imbalance, resulting in impaired host protective capacity and thereby proteolytic tissue damage. Additional studies are necessary to investigate the contribution of this molecule in the etiology of periodontal disease and its therapeutic potential.

**References**


1. Lopez N J. Occurrence of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia in progressive adult periodontitis. J Periodontol 2000; 71: 948–954.
2. Sundqvist G. Pathogenicity and virulence of black pigmented gram-negative anaerobes. FEMS Immunol Med Microbiol 1993; 6: 125–137.
3. Imamura T, Potempa J, Tanase S, Travis J. Activation of blood coagulation factor X by arginine – specific cysteine proteinases (gingipain-Rs) from Porphyromonas gingivalis. J Biol Chem 1997; 272:16062–16067.
4. Potempa J. and Pike R.N. Corruption of innate immunity by bacterial proteases. J. Innate Immun. 2009; 1, 70–87.
5. Travis J. and Potempa J. Bacterial proteinases as targets for the development of second - generation antibiotics. Biochem. Biophys. Acta 2000; 1477, 35–50.
6. Kantyka T, Latendorf T, Wiedow O, Bartels J, Glaser R, Dubin G, Schroder J M, Potempa J, Hoffert U M. Elafin is specifically inactivated by RgpB from

Porphyromonas gingivalis by distinct proteolytic cleavage. Biol Chem 2009; Vol. 390, pp. 1313–1320.

7. Uehara, A., Muramoto, K., Takada, H. and Sugawara, S. Neutrophil serine proteinases activate human nonepithelial cells to produce inflammatory cytokines through protease-activated receptor 2. J Immunol 2003; 170: 5690–5696.
8. Ujiie Y, Oida S, Gomi K, Arai T, Fukae, M. Neutrophil elastase is involved in the initial destruction of human periodontal ligament. J Periodontal Res 2007; 42: 325–330.
9. Laugisch O, Schacht M, Guentsch A, Kantyka T, Sroka A, Stennicke H R, Pfister W, Sculean A, Potempa J, Eick S. Periodontal pathogens affect the level of protease inhibitors in gingival crevicular fluid. Molecular Oral Microbiology 2012; 27; 45–56.
10. Magert HJ, Drogemuller K, Raghunath M. Serine proteinase inhibitors in the skin: role in homeostasis and disease. Curr Protein Pept Sci 2005; 6: 241 - 54.
11. Into T, Inomata M, Kanno Y, Matsuyama T, Machigashira M, Izumi Y. Arginine-specific gingipains from Porphyromonas gingivalis deprive protective functions of secretory leukocyte protease inhibitor in periodontal tissue. Clin Exp Immunol 2006; 145: 545 - 54.
12. Williams SE, Brown TI, Roghanian A, Sallenave JM. SLPI and elafin: one glove, many fingers. Clin Sci (Lond) 2006; 110: 21 - 35
13. Kretschmar S, Yin L, Roberts F, et al. Protease inhibitor levels in periodontal health and disease. J Periodontal Res. 2012; 47:228– 235.
14. Fritz H, Schiessler H, Gieger R., Ohlsson K, Hochstrasser K. Naturally occurring low molecular weight inhibitors of neutral proteinases from PMN granulocytes and of kallikreins. Agents Actions 1978; 8: 57–64.
15. Zani ML, Nobar SM, Lacour SA. Kinetics of the inhibition of neutrophil proteinases by recombinant elafin and pre-elafin (trappin-2) expressed in Pichia pastoris. Eur J Biochem 2004; 271: 2370–2378.
16. Afacan B, Ozturk VO, Emingil G, Kose T, Bostanci N. Alarm anti-protease trappin-2 negatively correlates with pro inflammatory cytokines in patients with periodontitis. J Periodontol. 2018; 89:58–66.
17. Verrier T, Solhonne B, Sallenave JM, Garcia-Verdugo I. The WAP protein Trappin-2/Elafin: A handyman in the regulation of inflammatory and immune responses. Int J Biochem Cell Biol. 2012; 44:1377–1380.
18. Sallenave JM. Secretory leukocyte protease inhibitor and elafin/trappin-2: Versatile mucosal antimicrobials and regulators of immunity. Am J Respir Cell Mol Biol. 2010; 42:635–643.
19. Bellemare A, Vernoux N, Morin S, Gagné SM, Bourbonnais Y. Structural and anti-microbial properties of human pre-elafin/trappin2 and derived peptides against Pseudomonas aeruginosa. BMC Microbiol. 2010; 10:253
20. Loe H. The gingival index, plaque index and the retention index systems. J Periodontol. 1967; 38:610–616. Suppl. 6.
21. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999; 4:1–6
22. Wiedow O, Meyer-Hoffert U. Neutrophil serine proteases: potential key regulators of cell signalling during inflammation. J. Intern. Med. 2005; 257, 319–32
23. Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil-mediated tissue injury in periodontal disease pathogenesis: findings from localized aggressive periodontitis. J Periodontol 2003; 74:66–75.

24. Tremblay GM, Vachon E, Larouche C, Bourbonnais Y. Inhibition of human neutrophil elastase-induced acute lung injury in hamsters by recombinant human pre-elafin (trappin-2). Chest. 2002; 121:582– 588
25. Vachon E, Bourbonnais Y, Bingle CD, Rowe SJ, Janelle MF, Tremblay GM. Anti-inflammatory effect of pre-elafin in lipopolysaccharide-induced acute lung inflammation. Biol Chem. 2002; 383:1249–1256.
26. Grano V, Tasco G, Casadio R et al. Reduction of active elastase concentration by means of immobilized inhibitors: a novel therapeutic approach. Biotechnol Prog 2004; 20: 968–7