Effects of non-surgical periodontal therapy on procalcitonin levels of gingival crevicular fluid and serum in subjects with different periodontal conditions

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Abstract

Objectives: To analyze and correlate procalcitonin (PCT) levels in gingival crevicular fluid (GCF) and serum in periodontal health and disease. Further, to evaluate the effectiveness of non-surgical periodontal therapy (NSPT) on GCF and serum PCT levels of periodontitis patients.

Methodology: Thirty three patients, aged 30-39 years and sex matched, were recruited and divided into three groups of eleven (n=11) each based on clinical and radiographic parameters: Group I - Periodontal Health; Group II - Gingivitis; Group IIIa - Periodontitis; Group IIIb - After Treatment Periodontitis (includes group IIIa subjects 12 weeks after NSPT). Serum and GCF samples were analyzed for PCT using ELISA.

Results: PCT levels in GCF (0.19 ng/ml) and serum (3.25 ng/ml) were found to be highest in Group IIIa when compared to Group I GCF (0.01 ng/ml) and serum (0.06 ng/ml), whereas PCT GCF and serum levels in Group IIIb (0.11 ng/ml, 1.2 ng/ml) and Group II (0.07 ng/ml, 0.48 ng/ml) were intermediate between Group I and Group IIIa respectively.

Conclusion: The increase and decrease of PCT levels in both GCF and serum with periodontal inflammation and NSPT respectively indicates that PCT may be involved in the pathogenesis of periodontal disease, thereby highlighting the possible role of PCT linking periodontal disease and systemic conditions.

Keywords: Procalcitonin (PCT), Gingival Crevicular Fluid (GCF), Serum, Periodontitis, Non-surgical periodontal therapy (NSPT)

Introduction

In periodontal disease, a series of inflammatory mediators are released in saliva, gingival crevicular fluid (GCF) and blood following periodontal tissue destruction (Cekici *et al.*, 2014). These mediators reflect the extent of periodontal tissue breakdown and their evaluation is useful for the determination of the existing periodontal condition and development of disease in the future (Kinney *et al.*, 2014). One of the notable biomarkers is procalcitonin.

Procalcitonin (PCT) is a calcitonin precursor protein containing 116 amino acids with a molecular weight of 13kDa (Carrol *et al.*, 2002). It participates in calcium balance in the body under physiologic conditions (Maruna *et al.*, 2000). The major source of PCT is thyroid C cells, neuroendocrinal cells of lungs and the pancreas (Maruna *et al.*, 2000). It is an acute phase protein and its levels rise shortly after inflammation (Maruna *et al.*, 2000; Whicher *et al.*, 2001). Its rise might have a physiological role such as calcium metabolism, cytokine network modulation of nitric oxide synthesis and non-steroidal anti-inflammatory effects (Maruna *et al.*, 2000). PCT normally circulates at very low levels but rises dramatically

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in people with systemic infection, (Carrol *et al.*, 2002; Bassim *et al.*, 2008). Bacterial lipopolysaccharide (LPS) is found to be a potent inducer of PCT release into the systemic circulation which is not associated with an increase in calcitonin (Dandona *et al.*, 1994).

PCT is widely considered as a reliable diagnostic marker for several systemic infections such as sepsis, septic shock and meningitis (Assicot *et al.*, 1993; Brunkfrost *et al.*, 1998; Carrol *et al.*, 2002). Routine PCT analysis appears to be useful in the early recognition of systemic inflammation (Assicot *et al.*, 1993; Harbarth *et al.*, 2001). PCT contributes as a potentially harmful mediator of inflammation due to the fact that PCT acts as a vasodilator (like Calcitonin Gene Related Peptide), releases inflammatory mediators (i.e., tumor necrosis factor (TNF), interleukin (IL)-6 and coagulation proteins), and acute phase proteins (e.g. C-reactive protein) which participate in the mechanisms underlying periodontal inflammation (Dandona *et al.*, 1994; Muller *et al.*, 2001; Preas *et al.*, 2001; Whicher *et al.*, 2001; Simon *et al.*, 2004). Thus, the overall increase in PCT during infection and inflammation indicates that PCT is part of the immune response and has a proinflammatory role (Matwiyoff *et al.*, 2012).

In a recent study, it was noted that patients with severe periodontitis have higher levels of salivary PCT than healthy controls (Hendek *et al.*, 2015). It was postulated that there was local PCT production which might have been stimulated and upregulated by endotoxin and release of proinflammatory cytokines at periodontal tissue injury sites (Dandona *et al.*, 1994; Bassim *et al.*, 2008). The author suggested that salivary PCT levels in patients with periodontal disease may have the potential to be a biomarker for periodontal tissue breakdown and its reduction with treatment could be used as an objective endpoint and therapeutic goal for guided intervention in patients with periodontitis (Bassim *et al.*, 2008; Hendek *et al.*, 2015).

Inflammatory constituents of GCF reflect the local tissue activity and are better indicators of the extent of severity of periodontitis than inflammatory constituents in saliva (Cekici *et al.*, 2014; Kinney *et al.*, 2014) and their observation in serum might throw more light on the PCT periodontal systemic continuum. To date there has only been one study reporting the detection of PCT levels in the GCF of periodontitis subjects, but this study did not estimate and correlate GCF PCT levels in periodontally healthy and gingivitis subjects (Giannopoulou *et al.*, 2012). Thus, the role of GCF PCT levels in periodontal inflammation is unclear. In this study, we aimed to measure PCT levels in GCF and serum in periodontal health and disease. Further, the impact of non-surgical periodontal therapy on GCF and serum PCT levels of periodontitis subjects was investigated.

Materials and methods

Experimental design

Thirty-three sex-matched subjects belonging to a common age group (30-39 years) with at least 20 teeth were enrolled in this study. Study approval was obtained from the Institutional Review Board and Ethical Committee (Ref. No. KCDS/984/2016-17) of Krishnadevaraya College of Dental Sciences and Hospital, affiliated with Rajiv Gandhi University of Health Sciences, Bengaluru, India. All participants were informed about the study procedures and written informed consent obtained in accordance with the Declaration of Helsinki. The subjects were excluded from the study if they had any of the following: any systemic diseases, such as diabetes mellitus or thyroid disease; any bone disease; any bacterial infection; immunologic disorders; hepatitis; any other bacterial oral infections; pregnant and lactating females; and former and current smokers. None of the participants had received antibiotics within the previous three months or treatment for periodontal disease within the six months before the study.

Study groups

Probing depth (PD), relative attachment loss (RAL) and bleeding on probing (BOP) were measured at six sites around each tooth. The subjects were categorized according to Classification of Periodontal Diseases and Conditions (American Academy of Periodontology and European Federation of Periodontology 2017) into three groups consisting of 11 subjects in each group as; i) Group I - periodontally healthy subjects; ii) Group II - gingivitis patients; iii) Group IIIa - Periodontitis patients; iv) Group IIIb comprised of the subjects from Group III, who had received non-surgical periodontal therapy (scaling and root planing).

Protocol for non-surgical periodontal therapy (NSPT)

Periodontitis patients in Group IIIb were subjected to non-surgical periodontal therapy that included removal of supragingival plaque and calculus and rectification or removal of overhanging restorations. Patients underwent thorough scaling and root planing procedures, in two sessions within seven days by means of hand and ultrasonic instrumentation under the influence of local anesthesia (2% lignocaine hydrochloride with 1: 80,000 adrenaline) with the session not lasting beyond an hour. Participating subjects were placed on a four week maintenance protocol to ensure there was effective plaque removal and inflammation control of gingival tissues. Complete periodontal therapy was carried out by a sole periodontist.

Examiner calibration for periodontal examination

Before recording probing depths, a calibration exercise for clinical parameters was performed on five patients before the actual study. The recording for clinical parameters was conducted at one week intervals. The order of patients was changed between the examinations. The periodontal probing depth estimation was judged to be reproducible if the intra examiner agreement was within ±1 mm between repeated measurements was at least 80%. The kappa value for intra-examiner agreement, between the two measurements was recorded to be 0.94.

Procedure for GCF Collection

All subjects were assessed clinically for their periodontal status under illumination using a sterile graduated Williams's periodontal probe and mouth mirror. In each subject of Group IIIa and Group IIIb, the site with highest level of RAL and radiographic validation of bone loss was chosen for GCF collection and the selected site (only one site for each patient) was dried with a blast of air, supragingival plaque was removed without disturbing the marginal gingiva and isolated with cotton rolls to prevent saliva contamination. With standard paper strips (Periopaper; Oraflow Inc., Smithtown, NY, USA), GCF samples were collected by inserting a paper strip to a depth of approximately 2 mm into the sulcus/ pocket for 30 seconds (Wassall *et al*.2016). Paper strips were discarded if contaminated with blood. Collected GCF volume was quantified using a calibrated appliance (Periotron 8000; Proflow Inc., Amityville, NY, USA) and these interpretations was adjusted to an original volume (μL) by orientation to a standard curve generated using the Periotron readings of the fluid volume (μL) in the Periopaper strips. Following quantitative analysis, the strips were transferred to Eppendorf tubes containing 100 μL of buffered phosphate saline solution. To elute GCF from paper strips, the aliquots were centrifuged at 3000 rpm for 15 minutes, and then the strips were removed and the collected samples were refrigerated at -80°C until the assay was performed.

Procedure for serum collection

Patients were seated comfortably and by palpating the venipuncture area (ante-cubital fossa housing the cephalic veins and median cubital) a tourniquet was tied approximately 3 to 4 inches higher than the planned venipuncture area. A 20 gauge needle and 2 ml syringe was used to collect 2 ml of blood from the antecubital fossa which was immediately transferred to the red topped serum separator blood collection tube (SST). The blood samples were allowed to clot at normal room temperature for a minimum of 30 minutes, and then centrifuged at 3000 rpm for 15 minutes to separate serum and transferred to an Eppendorf tube which was stored at -80°C until the time of assay.

ELISA analysis of GCF and serum PCT

PCT levels in GCF and serum were evaluated using a standard ELISA kit (RayBio® Human Procalcitonin ELISA, Catalogue No: ELH-PROCALC, Assay sensitivity of <30 pg/ml) according to the manufacturer's instructions. This assay uses an antibody specific for human PCT coated on a 96-well plate. The samples were run in duplicate. Enzyme-substrate reactions were terminated by the addition of TMB substrate solution and color change was measured spectrophotometrically at a wavelength of 450 nm. PCT concentrations in GCF and serum were identified using the optical density values obtained with the known samples using standard curves.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS ver 18.0) software. Then the data accessed using Kolmogrov-Smirnov and Shapiro Wilk test. The null hypothesis for this test is that the data are normally distributed. Hence, tests for assessment of parametric data were applied for further calculations. To test the hypothesis of equality of means for three groups with respect to PCT GCF and serum concentrations, one way ANOVA test was carried out and p value <0.05 was considered to be statistically significant. Pearson's Correlation coefficient analysis was used to analyze the correlation between GCF and serum PCT concentrations and clinical parameters in Group I, Group II, Group IIIa and Group IIIb.

Results

The demographic parameters of the study population are represented in Table 1. There were no significant differences in age and gender distribution among the study population (p>0.05). The mean values of PCT GCF and serum levels are depicted in Table 2. The highest mean PCT concentration in GCF was obtained by Group IIIa (0.19 ng/ml) and least mean PCT concentration was obtained by Group I (0.01 ng/ml). The mean concentration of the Group IIIb (0.11 ng/ml) and Group II (0.07 ng/ml) were intermediate between Group I and Group IIIa. Similarly, the highest mean PCT concentration in serum was obtained for Group IIIa (3.25 ng/ml) and least mean PCT concentration was obtained for Group I (0.06 ng/ml). The mean concentration of the Group IIIb (1.2 ng/ml) and Group II (0.48 ng/ml) were intermediate between Group I and Group IIIa. There were statistically significant differences between the mean GCF and serum PCT levels studied by one way ANOVA test (p<0.001). These results show that there exists a positive correlation between PCT and presence of periodontal disease.

Table 1. Demographic data of the study population

Variables	Group I	Group II	Group III a and b	'p' value
Age (years)	33.0±2.36	33.0±2.04	34.6±2.29	0.15*
Gender (male/female)	(6/5)	(4/7)	(6/5)	0.616†

Group I - Periodontally healthy
Group II - Gingivitis subjects
Group IIIa - Periodontitis subjects
Group IIIb - After treatment periodontitis subjects
The values represent age in years and gender in numerals
* Non-significant 'p' value using one way ANOVA test
†Non-significant 'p' value using Chi-square test

Table 2. Descriptive analysis of PCT in GCF and serum among the study groups

Variable	No of samples	Mean± SD (GCF) (ng/ml)	Mean± SD (Serum) (ng/ml)	Minimum		Maximum		'p' value
				GCF	Serum	GCF	Serum	
Group I	11	0.01+0.004	0.06+0.03	0.009	0.03	0.01	0.08	*<0.001**
Group II	11	0.07+0.01	0.48+0.07	0.06	0.43	0.07	0.53	
Group IIIa	11	0.19+0.03	3.25+0.56	0.17	2.87	0.22	3.63	
Group IIIb	11	0.11+0.03	1.2+0.43	0.08	0.90	0.13	1.49	

Group I - Periodontally healthy subjects
Group II - Gingivitis subjects
Group IIIa - Periodontitis subjects
Group IIIb - After treatment periodontitis subjects
GCF - Gingival crevicular fluid. The values represent average mean and standard deviation (SD) of PCT GCF and serum levels expressed in nanogram per millimeter (ng/ml). *Statistically significant difference between the study groups using one way ANOVA test ($p < 0.001$)

The pair-wise comparison of study population with clinical parameters such as plaque index (PI), modified gingival index (MGI), bleeding on probing (BOP%), probing depth (PD) and relative attachment level (RAL) has been tabulated in Table 3. Group IIIa showed the highest mean PI, MGI, BOP%, PD and RAL scores such as 2.67, 2.4, 83.54%, 7.09 mm and 9.63 mm respectively. The lowest score was obtained by Group I (0.49, 0.53 and 16.09% respectively) whereas scores of Group II (1.54, 1.96 and 65.27% respectively) and Group IIIb (0.73, 0.79, 24.74%, 4.81 mm and 7.63 mm respectively) were lesser than Group IIIa but higher than Group I. One way ANOVA test showed that there were statistically significant difference ($p<0.001$) between the periodontal clinical parameters among the study groups.

Correlation of GCF and Serum PCT concentration

Pearson correlation coefficient test was performed to check for correlation between the GCF and serum PCT concentrations with the periodontal clinical parameters in all the study groups (Table 4). The results show that there exists a strong positive correlation between PCT GCF and serum levels with the periodontal clinical parameters PI, MGI, BOP%, PD and RAL of all the study groups. This suggests that PCT levels in both GCF and serum are directly proportionate to each other.

To summarize, PCT was detected in GCF and serum samples of all the subjects participated in the study. The quantified results confirmed the hypothesis that GCF and serum PCT concentrations rise with the increasing grade of inflammation during periodontal diseases and their levels reduce after NSPT.

Discussion

This study demonstrates that periodontal inflammation is associated with an increase in PCT GCF levels as shown by the rise in PCT GCF levels from Group I (0.01 ng/ml) to Group II (0.07 ng/ml) and Group IIIa (0.19 ng/ml). Since, PCT is produced primarily by C cells of thyroid and periodontal tissues do not contain these cells, the upregulation of PCT levels in GCF is somewhat puzzling and is a matter of speculation (Bassim *et al.*, 2008; Hendek *et al.*, 2015). First, it may have been derived from serum as GCF is considered to be serum exudate (Cekici *et al.*, 2014). Second, pathogenic gram negative bacteria in periodontal infection which generates endotoxin would have caused the enhancement of PCT levels (Bassim *et al.*, 2008). Third, its expression might have been upregulated by the release of proinflammatory cytokines such as TNF-alpha and IL-6 at the periodontal tissue injury sites (Russwurm *et al.*, 1999; Matwiyoff *et al.*, 2012; Hendek *et al.*, 2015).

Table 3. Descriptive statistics showing mean values of periodontal clinical parameters among the study population

Variable	Group I	Group II	Group IIIa	Group IIIb	'p' value
PI	0.49±0.14 †	1.54±0.18 †	2.67±0.40 †	0.73±0.15 †	***<0.001****
MGI	0.53±0.22 ‡	1.96±0.44 ‡	2.40±0.24 ‡	0.79±0.09 ‡	***<0.001****
BOP%	16.09±3.50§	65.27±4.75§	83.54±3.20§	24.74±3.40§	***<0.001****
PPD			7.09±0.94	4.81±0.87	***<0.001****
RAL			9.63±1.28	7.63±1.28	***<0.001****

Group I - Periodontally healthy subjects
Group II - Gingivitis subjects
Group IIIa - Periodontitis subjects
Group IIIb - After treatment periodontitis subjects
GCF - Gingival crevicular fluid. PI, plaque index; MGI - modified gingival index; BOP - bleeding on probing; PD - probing depth; RAL - Relative attachment level. The values represent average mean ± standard deviation and are expressed as percentages for BOP and in millimeters for PD and CAL. * Significant difference among the groups using one way ANOVA test (p <0.001). †Significant difference in pairwise comparison of PI among the study groups using one way ANOVA test (p <0.001). ‡Significant difference in pairwise comparison of MGI among the study groups using one way ANOVA test (p <0.001). §Significant difference in pairwise comparison of BOP% among the study groups using one way ANOVA test (p <0.001).

Table 4. Pearson Correlation test to compare GCF and serum PCT, PI, MGI, BOP, PD, RAL among the study population

Group	Correlation between	PCT Serum (ng/ml)	Plaque Index	Bleeding On Probing Index (%)	Modified Gingival Index	Probing Depth (mm)	Relative Attachment Level (mm)
Group I	PCT GCF (ng/ml)	0.88*	0.62*	0.78*	0.68*	.	.
	PCT Serum (ng/ml)		0.63*	0.79*	0.69*	.	.
Group II	PCT GCF (ng/ml)	0.89*	0.72*	0.78*	0.74*	.	.
	PCT Serum (ng/ml)		0.77*	0.79*	0.75*	.	.
Group IIIa	PCT GCF (ng/ml)	0.84*	0.76*	0.75*	0.74*	0.79*	0.81*
	PCT Serum (ng/ml)		0.79*	0.79*	0.81*	0.83*	0.86*
Group IIIb	PCT GCF (ng/ml)	0.83*	0.74*	0.82*	0.76*	0.76*	0.81*
	PCT Serum (ng/ml)		0.81*	0.85*	0.79*	0.81*	0.82*

Group I - Periodontally healthy subjects
Group II - Gingivitis subjects
Group IIIa - Periodontitis subjects
Group IIIb - After treatment periodontitis subjects
GCF - Gingival crevicular fluid. PI, plaque index; MGI - modified gingival index; BOP - bleeding on probing; PD - probing depth; RAL - Relative attachment level. The values represent correlation (r) of GCF and serum levels of PCT along with periodontal clinical parameters among the study groups. * Significant correlation between periodontal clinical parameters and biochemical parameters (PCT levels) using Pearson correlation test (p <0.001).

A rise in PCT levels in serum was noted when comparing Group I (0.06 ng/ml) to Group II (0.4 ng/ml) and Group IIIa (3.25 ng/ml) subjects. This rise in serum PCT levels with increasing grade of periodontal inflammation demonstrates the potential influence of chronic periodontal inflammation on the systemic continuum which could be due to various reasons. First, bacterial lipopolysaccharides, enhanced due to periodontal infection, act on peripheral mononuclear cells where in turn PCT mRNA expression occurs and stimulates PCT secretion systemically (Muller *et al.*, 2001; Balog *et al.*, 2002). Secondly, migration and adherence of the monocytic cells with the cells such as adipocytes enhances the sustained PCT release (Matwiyoff *et al.*, 2012). Thirdly, PCT expression is elevated by other proinflammatory cytokines which depicts their increased levels in circulation (Russwurm *et al.*, 1999; Matwiyoff *et al.*, 2012). Thus, the raise in GCF and serum PCT levels is of great importance, as it potentially sustains and augments the inflammatory response. Further, it implies a positive correlation between PCT levels in GCF and serum with periodontal inflammation. These observations support our initial hypothesis and demonstrate that there is a correlation between GCF and serum PCT levels in chronic periodontitis subjects.

The results of the present study are in accordance with previous studies. For example, Bassim *et al.* (2008) showed higher salivary PCT levels in chronic periodontitis subjects with Type II diabetes but serum PCT levels were not correlated with periodontal parameters or glycemic control.

Similarly, Zhang *et al.* (2013) studied PCT plasma levels in aggressive periodontitis patients and healthy subjects but failed to compare and correlate PCT levels in other periodontal disease status. Hendek *et al.* (2015) demonstrated higher PCT levels in saliva of patients with periodontal disease compared to periodontally healthy controls. However, these results were preliminary and were not supported with additional studies performed in different body fluids such as GCF and serum samples. Giannopoulou *et al.* (2012) studied GCF PCT levels in patients with previous history of periodontitis but failed to compare and correlate PCT levels of healthy and gingivitis subjects.

Not withstanding the above findings, there are conflicting reports which differ from our study. Yousefimanesh *et al.* (2015) showed no significant increase in salivary PCT levels compared with control group and concluded that PCT concentration is not affected by periodontal disease progression. Redman *et al.* (2016), who studied salivary and serum PCT levels in arthritis patients with periodontitis, found no significant increase in their levels. Since, these studies were conducted on other body fluids such as saliva and serum in systemically compromised patients and the impact of intervention therapy was not evaluated, the role of PCT in the pathogenesis of periodontal diseases was unclear.

Our study might differ from previous studies due to some plausible factors involved in the research such as: i) involvement of difference in the type of population (ethnicity) and selection of younger age group where PCT levels are not significantly higher in response to periodontitis; ii) difference in the analytical method of PCT; iii) PCT being estimated in salivary samples which is more diluted, not site specific and reflects less of the overall disease activity; iv) different case definitions used for periodontitis; v) another potential variable could be difference in the sample storage (Bassim *et al.*, 2008; Giannopoulou *et al.*, 2012; Zhang *et al.*, 2013; Hendek *et al.*, 2015; Yousefimanesh *et al.*, 2015; Redman *et al.*, 2016).

After 12 weeks of non-surgical periodontal therapy there was significant reduction of PCT levels. This could be attributed to resolution of microbial inflammatory load which was primarily responsible for rise in inflammatory PCT levels (Russwurm *et al.*, 1999; Matwiyoff *et al.*, 2012). These results are consistent with a previous study by Giannopoulou *et al.* (2012) who reported that PCT GCF levels were elevated in periodontitis and their levels fell following non-surgical periodontal therapy and steadily increased in the consecutive months. This supports the notion of an active role of PCT in periodontal inflammation and further confirms the relationship between local and serum PCT levels with periodontal inflammation (Bassim *et al.*, 2008; Giannopoulou *et al.*, 2012; Zhang *et al.*, 2013; Hendek *et al.*, 2015). Thus, our study suggests that serum/GCF PCT levels might be useful in monitoring the recurrence of periodontitis.

The results from this study are preliminary and have some limitations. First, the study is cross-sectional in design, thus additional longitudinal randomized controlled trials with larger sample size are necessary to confirm PCT levels and its alterations in GCF and serum (Giannopoulou *et al.*, 2012; Zhang *et al.*, 2013; Hendek *et al.*, 2015; Yousefimanesh *et al.*, 2015; Redman *et al.*, 2016). Second, since different samples give different results, it is recommended to study their relationship in the future research through gingival tissue samples (Yousefimanesh *et al.*, 2015). Third, although several biomarkers and inflammatory cytokines have been identified in GCF, PCT alone or in combination with other biomarkers may give a deeper insight on its role (Vijayan *et al.*, 2017). Despite intensive research, a number of uncertainties still exist concerning the physiological and inflammatory role of PCT (Russwurm *et al.*, 1999; Matriyoff *et al.*, 2012; Kim *et al.*, 2018). Thus, further studies need to be carried out to obtain more detailed understanding of the role of PCT in periodontitis and verify these conjectures.

Conclusion

Within the limitations of this study we summarize that with a greater extent of periodontal inflammation there was a substantial increase in GCF and serum levels, and with NSPT there was significant reduction in PCT levels both locally and systemically. This suggests that PCT could act as a proinflammatory biomarker of periodontal inflammation.

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