Estimation and correlation of procalcitonin in saliva and serum of chronic periodontitis patients before and after nonsurgical periodontal therapy: An analytical comparative study

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Abstract:

Background: Procalcitonin (ProCT) is an emerging inflammatory biomarker in bacterial infections. Few studies have reported raising salivary ProCT in periodontitis patients. Hence, the study aims to analyze and correlate the changes in saliva and serum ProCT in periodontitis patients before and after nonsurgical periodontal therapy.

Materials and Methods: We have included 15 chronic periodontitis patients of mean age 41.8 ± 6.82 years who satisfy the inclusion criteria in the study. After saliva and serum collection, clinical parameters such as plaque index, gingival index, gingival bleeding index, probing pocket depth, and clinical attachment were recorded, and scaling and root debridement were performed. Reevaluation was done at 1- and 3-month interval. ProCT was estimated using the enzyme-linked immunosorbent assay.

Results: Salivary ProCT was significantly greater than its serum counterpart at baseline and 1 month after periodontal therapy (0.20 vs. 0.26, 0.13 vs. 0.14 ng/ml respectively). We noticed a significant reduction in salivary as well as serum ProCT (35% and 46%, respectively) 1 month after scaling and root debridement. A significant moderate positive correlation was found between paired observations of salivary and serum ProCT at baseline as well as after periodontal therapy ($r = 0.61$ and $0.7$). A further reduction of salivary ProCT was noticed 3 months after nonsurgical therapy (0.11 ng/ml).

Conclusions: Serum ProCT significantly decreases with periodontal treatment, indicating the impact of periodontal therapy on systemic inflammation. Since salivary ProCT is positively correlated with serum ProCT, we can consider it as an alternative biomarker to its serum counterpart.

Key words: Acute phase reactant, chronic periodontitis, procalcitonin, saliva, scaling and root debridement, serum

INTRODUCTION

Host response plays an important role in periodontal pathogenesis.[1] Release of acute-phase reactants like C-reactive proteins (CRP) occurs during the destructive phase of the disease.[2] High-sensitive CRP (hs-CRP) is the most widely investigated acute inflammatory marker in many systemic infections as well as periodontal disease.[3] However, it is a nonspecific inflammatory marker and is raised even in noninflammatory conditions. Procalcitonin (ProCT) is an emerging acute-phase reactant specifically elevated in bacterial infections.[3] ProCT is a precursor of the hormone calcitonin, which is involved in calcium homeostasis. It is composed of 116 amino acids and synthesized by the parafollicular cells (C cells) of the thyroid gland and neuroendocrine cells of the lungs where it is cleaved enzymatically and secreted as calcitonin.[4] Bacterial infection stimulates the secretion of uncleaved ProCT from the thyroid and many other tissues. In severe infection associated with systemic response, the blood levels of ProCT increase to 100 µg/L. An increase in serum ProCT is reported in many inflammatory conditions.

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such as bacterial meningitis, acute pancreatitis, upper respiratory tract infection, chronic obstructive pulmonary disease, urinary tract infection, arthritis, and endocarditis. ProCT is a useful indicator to find the degree of infection, predicting the prognosis and monitoring the response to treatment.

Previous studies have identified ProCT in saliva of patients with periodontal disease and other systemic infections. Saliva is a clinically informative biological fluid that has biomarkers such as enzymes, inflammatory mediators such as cytokines, prostaglandins, and acute-phase proteins like CRP. Compared to both gingival crevicular fluid (GCF) and serum, saliva collection is easy, simple, noninvasive, and does not require trained personnel.

Periodontal disease is linked with many systemic diseases such as diabetes mellitus, cardiovascular disease, respiratory diseases, and osteoporosis. The impact of periodontal disease and its treatment on serum ProCT levels need to be identified. Hence, the present study aims to estimate and correlate the level of salivary and serum ProCT before and after scaling and root debridement in chronic periodontitis patients.

**MATERIALS AND METHODS**

The present study was conducted in the Department of Periodontics, PMS College of Dental Science and Research, Trivandrum, from April 2016 to March 2017 after obtaining clearance from the Institutional ethics committee (IEC) NO: PMS/IEC/2015/07. The sample size was calculated using the formula $n = 2 \sigma^2 (Z_\alpha + Z_\beta)^2 \div \delta^2$. Type 1 error was kept as 0.05 and Type 2 error as 0.20. $\sigma^2$ (standard deviation) = 7.67 and $\delta$ (mean of difference) = 123 were obtained from a pilot study. Substituting the values, the minimum sample size required for the study was calculated as 15.

We selected 15 systemically healthy patients (9 females and 6 males) aged between 32 and 60 years (mean age of 41.8 ± 6.82) from the outpatient section of the department of periodontics. The main inclusion criterion was the presence of chronic generalized severe periodontitis according to the international workshop for a classification of periodontal disease in 1999. Other inclusion criterion included the presence of gingival inflammation, gingival index (GI) $\geq 2$, and probing pocket depth (PPD) ≥5 mm in >30% of sites. Patients who were currently under any medication and who have taken antibiotics or anti-inflammatory drugs for the past 3 months were excluded. Pregnant and lactating females and smokers were also excluded from the study. Written informed consent was obtained from those willing to participate in the study.

The detailed study plan is presented in Figure 1. Clinical examination included plaque index (PI), GI, gingival bleeding index (GBI), and full-mouth periodontal status evaluation of six sites per tooth, including PPD, clinical attachment loss (CAL), and recession/enlargement. A single calibrated examiner (LJ) performed a clinical examination for all the patients and one-stage, full-mouth periodontal scaling and root debridement. An intraoral periapical radiograph was taken in relation to the relevant sites for periodontal diagnosis.

Saliva was collected as described previously. Unstimulated whole expectorated saliva (2 ml) was collected from each subject after overnight fasting between 8 and 10 am. The patients were asked to swallow first and then allow the unstimulated saliva to pool at the bottom of the mouth. It was collected using a syringe and transferred to a sterile capped tube. For removing cell debris, saliva samples were centrifuged at 6000 revolutions/min (rpm) for 10 min at +4°C and the clear supernatant was transferred to the eppendorf tubes and stored at -80°C until analyzed.

One ml of blood was collected from the patient, which was immediately mixed with an anticoagulant. Samples were stored at 2°C to 8°C for up to 24 h. Samples were frozen at-20°C within 48 h and stored at -80°C. All samples were centrifuged before the analysis to ensure that they are free of fibrin or other particulate matter.

The supernatant was used for the estimation of ProCT using an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Shanghai, China) using the standard curve according to the manufacturer’s instructions. The sensitivity of the assay was 0.00249 ng/ml. The results were expressed in nanograms/milliliter.

Data were analyzed by the Kolmogorov–Smirnov test to study its normality. Data were then expressed as mean and standard deviation and analyzed using computer software (SPSS software version 22, IBM, Chicago, IL, USA). Repeated-measure analysis of variance with post hoc test (Tukey’s honestly significant difference test) was used for comparing salivary ProCT before and after treatment. Comparisons of mean serum ProCT before and after treatment were done using a paired t-test. The correlation between salivary and serum ProCT levels was evaluated using the Pearson correlation coefficient. For all statistical evaluations, the $P < 0.05$ was considered significant.

**RESULTS**

Table 1 presents the results of statistical analysis of clinical findings (PI, GI, GBI, PPD, and CAL) at baseline and 1 and 3 months after scaling and root debridement. All the parameters were reduced at 1-month recall visit compared to the baseline. There was a further reduction in all the parameters at 3-month
recall and all the intergroup comparisons were significant, as per post hoc analysis ($P < 0.05$).

Table 2 and Figure 2 depict salivary ProCT levels at baseline and 1 and 3 months after scaling and root debridement. The reduction in salivary ProCT level was found at 1 month after nonsurgical therapy (from $0.20 \pm 0.044$ to $0.13 \pm 0.012$ ng/ml). Further reevaluation after 3-month also demonstrated reduction ($0.11 \pm 0.006$ ng/ml) compared to baseline and 1-month recall values. All the intergroup comparisons were significant, as per the post hoc analysis ($P < 0.05$).

Serum ProCT levels at baseline and 1 month after scaling and root debridement were compared using paired t-test, and it was found to be significantly reduced (from $0.26 \pm 0.053$ to $0.14 \pm 0.01$ ng/ml). Table 2 and Figure 3 show the results.

Higher serum ProCT was noticed compared to salivary levels at baseline as well as 1 month after nonsurgical therapy. The reduction in serum ProCT levels was also higher compared to its salivary counterpart (46% vs. 35%).

The correlation of salivary and serum ProCT levels at the baseline is presented in Table 3 and Figure 4 and correlation with the same at 1-month recall is given in Table 3 and Figure 5. A moderate positive correlation was observed between salivary and serum ProCT levels at baseline and 1-month recall and it was statistically significant too.

**DISCUSSION**

ProCT is an inflammatory biomarker detected in biological fluids such as serum, saliva, and GCF. There are very few

![](image1.png)

**Figure 2:** Trendline of salivary procalcitonin (ProCT) from baseline to 1- and 3-month reevaluation, y is dependent variable and R2 is reliability

![](image2.png)

**Figure 3:** Trendline of serum procalcitonin (ProCT) from baseline to 1-month reevaluation, y is dependent variable and R2 is reliability

![](image3.png)

**Figure 4:** Correlation of salivary and serum procalcitonin (ProCT) levels at the baseline, y is dependent variable and R2 is reliability

![](image4.png)

**Figure 5:** Correlation of salivary and serum procalcitonin (ProCT) levels after 1 month, y is dependent variable and R2 is reliability

**Table 1: Mean values of clinical parameters at different timelines**

| Parameter | Observation | Mean±SD | F      | P       |
|-----------|-------------|---------|--------|---------|
| PI        | Baseline    | 2.31±0.17 | 750.23 | <0.0001*|
|           | 1 month     | 0.73±0.14 |        |         |
|           | 3 months    | 0.35±0.16 |        |         |
| GI        | Baseline    | 2.32±0.09 | 1188.59| <0.0001*|
|           | 1 month     | 0.7±0.16  |        |         |
|           | 3 months    | 0.26±0.13 |        |         |
| GBI       | Baseline    | 83.15±2.08| 5862.47| <0.0001*|
|           | 1 month     | 23.3±2.32 |        |         |
|           | 3 months    | 11.6±1.26 |        |         |
| PPD       | Baseline    | 6.26±0.17 | 286.01 | <0.0001*|
|           | 1 month     | 4.44±0.28 |        |         |
|           | 3 months    | 3.73±0.52 |        |         |
| CAL       | Baseline    | 6.22±0.12 | 896.7  | <0.0001*|
|           | 1 month     | 4.74±0.15 |        |         |
|           | 3 months    | 4.27±0.15 |        |         |

a, b, c – Means with different superscript within each parameter significantly differ from each other according to post hoc analysis. *$P<0.05$ is considered as statistically significant. PI – Plaque index; GI – Gingival index; GBI – Gingival bleeding index; PPD – Probing pocket depth; CAL – Clinical attachment level; SD – Standard deviation; F – Fisher in ANOVA test; P – probability

**Table 2: Statistical analysis of salivary and serum procalcitonin levels**

| Parameter       | Observation (n=15) | Mean (ng/ml)±SD | F      | P       |
|-----------------|-------------------|-----------------|--------|---------|
| Salivary procalcitonin | Baseline          | 0.20±0.044     | 37.68  | <0.05*  |
|                 | 1 month           | 0.13±0.012     |        |         |
|                 | 3 months          | 0.11±0.006     |        |         |
| Serum procalcitonin | Baseline          | 0.26±0.053     | 9.34   | <0.05*  |
|                 | 1 month           | 0.14±0.01      |        |         |

a, b, c – Means with different superscript within each parameter significantly differ from each other according to post hoc analysis. *$P<0.05$ is considered as statistically significant. SD – Standard deviation; ng/ml – nanogram per milliliter; F – Fisher in ANOVA test; P – probability
Table 3: Correlation between salivary and serum procalcitonin levels at baseline and at 1 month

| Parameter              | Groups(n=15)      | Mean (ng/ml) | r     | P    | Inference         |
|------------------------|-------------------|--------------|-------|------|-------------------|
| Procalcitonin at baseline |                  |              |       |      |                   |
| Saliva                 | 0.26              | 0.0607       | 0.017*|      | Moderate positive correlation |
| Serum                  | 0.26              |              |       |      |                   |
| Procalcitonin after 1 month |              |              |       |      |                   |
| Saliva                 | 0.13              | 0.691        | 0.004*|      | Moderate positive correlation |
| Serum                  | 0.14              |              |       |      |                   |

ng/ml - nanogram per milliliter, *P<0.05 is considered as statistically significant. ng/ml – nanogram/milliliter; r – Correlation coefficient; P – Probability, n – sample size

published reports of ProCT estimation in periodontitis patients. Salivary ProCT was significantly increased in gingivitis, chronic periodontitis, and generalized aggressive periodontitis compared to healthy individuals according to a previous report. Bassim et al. observed that severe periodontitis patients with diabetes mellitus demonstrated significantly higher salivary and serum ProCT values compared to periodontally healthy individuals. Hence, from most studies, it could be inferred that the level of ProCT is higher in periodontitis patients compared to healthy individuals both in serum as well as saliva. Hence, in our study, comparison of ProCT between healthy and periodontitis patients was not done, and we have included patients with generalized severe chronic periodontitis alone.

We could observe a statistically significant decrease in clinical parameters in 1- and 3-month recall visits after scaling and root debridement implicating the effectiveness of nonsurgical therapy. Correspondingly, we could observe a statistically significant decrease in salivary ProCT from baseline to both recall visits. Bassim et al. also observed a reduction in salivary ProCT levels 3 months after periodontal therapy, but it was not statistically significant. This may be due to heterogeneity in their patient characteristics where they have included periodontitis patients with varying severity, diabetic patients, and even smokers. Both smoking and poorly controlled diabetes are the common risk factors for periodontitis, and hence, it might have resulted in treatment resistance. On the contrary, in another recent study, we could find a significant reduction in serum ProCT after nonsurgical periodontal therapy in periodontitis patients who are smokers. To minimize these confounding factors, we have included systemically healthy nonsmokers with severe periodontitis alone in our study.

Some evidences suggest a possible link between chronic periodontitis and many systemic diseases. Periodontitis is a potential source of infection and considered as an independent risk factor for cardiovascular diseases, cerebrovascular diseases, peripheral arterial disease, respiratory diseases, and low birth weight. Periodontitis results in excessive production of inflammatory mediators which can even reach the systemic circulation. Serum ProCT was significantly higher in arthritis patients with moderate and severe periodontitis compared to arthritis patients with no/mild periodontitis. However, it was not observed for salivary ProCT, salivary hsCRP, or serum hsCRP. In another recent study, it is reported that periodontitis independently contributes to elevated serum ProCT levels in chronic migraine patients. Elevation in serum ProCT level in periodontitis patients can be considered as a connecting link between periodontitis and systemic diseases.

In our study, we could observe a statistically significant reduction in serum ProCT level 1 month after scaling and root debridement, which was similar to a study conducted by Onder et al. where they have observed a reduction in serum ProCT 4 weeks after nonsurgical periodontal therapy. The statistically significant reduction in serum ProCT level along with successful periodontal therapy emphasizes the importance of periodontal therapy in maintaining not only oral health but also systemic health.

We observed higher ProCT in serum than saliva at baseline and 1 month after scaling and root debridement. Redman et al. reported similar results. But contradictory results are presented by Bassim et al. and they hypothesized that higher salivary ProCT may be due to the local production of ProCT by the gingival tissues in response to the bacterial infection. They have utilized different methods for salivary ELISA and serum ProCT estimation (KRYPTOR-TRACE technology), whereas our study and the study conducted by Redman et al. used ELISA for both saliva and serum analyses. Even though local secretion may contribute to the salivary component of ProCT, the serum-derived fraction of ProCT might be diluted in the saliva and salivary ProCT is the sum of locally produced and systemically derived components. However, the % of reduction was more in serum than saliva (46 vs. 35) again favoring local production of ProCT by gingival tissues.

In the present study, there was a moderately significant positive correlation between salivary and serum ProCT levels at baseline (r = 0.6067) and 1 month after scaling and root debridement (r = 0.691). This correlation strengthens the association of periodontitis with systemic disease. This shows the effectiveness of periodontal therapy and serum ProCT levels were also significantly decreased like salivary ProCT level. To the best of our knowledge, the correlation between salivary and serum ProCT has not been done so far. Oral fluid biomarkers have recently emerged as an adjunct to traditional methods for the early detection of periodontitis and an assessment of its progression, as well as monitoring the responses to periodontal treatments. Salivary estimation of ProCT can be considered as an alternative biomarker for serum ProCT which offers many advantages like ease of collection and handling.

CRP is the most popular acute-phase protein studied in inflammatory conditions. However, salivary ProCT estimation offers many advantages compared to CRP. ProCT levels return to normal soon after the acute-phase reaction compared to CRP, which takes a sufficiently long time for the same. ProCT is more stable than CRP in saliva for even up to 3 weeks at 4°C. Recently, the role of microbes other than bacteria such as viruses and fungi is extensively researched in periodontal pathogenesis. The change in ProCT level with the severity of disease as well as its response to treatment again points to the major role played by different strains of bacteria in periodontal disease.
One of the major limitations of our study was the characteristics of the selected samples. We have conducted the present study in a small patient group with nearly homogeneous characteristics. Future multicentric studies in large populations with heterogeneous characteristics should be done to generalize the results as well as to better understand the role of ProCT as a biomarker in periodontitis. ProCT can be used as a guide for antibiotic therapy in adult intensive care unit patients and patients with respiratory tract infections.\(^{[23]}\) Similarly, we can also conduct randomized controlled clinical trials in periodontitis patients utilizing ProCT to determine the need of antibiotics along with our conventional therapy. Thus, we can eliminate the unnecessary antibiotic use, thereby lowering the cost of treatment and reducing the problems associated with drug resistance.

**CONCLUSION**

In the present study, we have evaluated salivary and serum ProCT in systemically healthy periodontitis patients before and after nonsurgical therapy. We have found a significant reduction in saliva and serum ProCT 1 month after nonsurgical periodontal therapy. The impact of periodontal therapy in systemic circulation is evident from these observations. Moreover, serum ProCT significantly correlates with salivary levels at baseline as well as after periodontal therapy. Hence, salivary ProCT is found to be an excellent alternative biomarker to its serum counterpart. Large epidemiological studies should be done in future for identifying salivary ProCT in patients as a potential biomarker in the diagnosis and management of periodontitis. Future studies can also utilize ProCT as an objective endpoint and therapeutic goal after periodontal therapy.

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**Conflicts of interest**

There are no conflicts of interest.

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