Evaluation of deoxypyridinoline levels in gingival crevicular fluid and serum as alveolar bone loss biomarker in patients with periodontitis

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INTRODUCTION

Periodontitis is a chronic inflammatory disease of tooth-supporting tissues initiated by microorganisms in the dental plaque. The complex etiology of periodontitis includes a complex biofilm, behavioral factors, environmental, and genetic traits of the individuals. Although microbes in the biofilm have an established role in the initiation of periodontal disease, the host immunoinflammatory response to microbial onslaught plays a pivotal role in periodontal disease progression.\(^{11}\) If left untreated it continues with progressive bone destruction leading to tooth mobility and subsequent tooth loss.\(^{21}\)

Pathophysiology of periodontal disease is coordinated by a number of host-immuno inflammatory factors, either induced or intrinsic. Under normal physiologic conditions, balance between bone formation and bone resorption is well maintained. Inflammatory metabolic conditions such as osteoporosis, periodontal disease, and rheumatoid arthritis, are examples of disturbed balance between bone formation and bone resorption.\(^{22}\) Bone turnover is in concourse with various osseous metabolic and osteolytic diseases. The organic matrix of bone comprises 90% Type I collagen. Pyridinoline cross-links represent a class of collagen degrading products that include C, N-telopeptides, pyridinoline, and deoxypyridinoline (DPD). DPD is one among the above products found mostly in bones and formed by the reaction of side chains of one lysine and two hydroxylysine molecules. Following posttranslational modification of collagen molecules, cross-link molecules cannot

Key words:
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Abstract:
Background: Several components of gingival crevicular fluid (GCF) reflect the course and predictability of periodontal disease and provide a pointer toward disease status. Potential biomarkers deoxypyridinoline (DPD), a metallophosphoesterase would correctly determine the presence of osteoclast-mediated bone turnover activity and seems to hold great promise as a predictive marker to determine bone destruction and active phases in the disease progression. Aim: The aim of the current study is proposed to investigate the biologic plausibility for the levels of DPD as biomarker in chronic periodontitis patients. Materials and Methods: The present cross-sectional study comprised 15 periodontally healthy and 15 chronic periodontitis patients who were age and genders matched, recruited from the outpatient department of Periodontics. GCF and blood samples for DPD estimation were collected from all the patients and analyzed using enzyme-linked immunosorbent assay kit. The clinical parameters such as clinical attachment loss (CAL), probing pocket depth (PPD), modified gingival index, bleeding index, and plaque index were recorded. Results: GCF DPD levels were significantly higher in chronic periodontitis patients when compared to periodontally healthy group. There were no significant correlations found among GCF and serum DPD levels with increasing age, gender, disease severity, and increase in PPD and CAL in both the groups. Conclusion: Within the limitations of this study, increased GCF DPD levels in chronic periodontitis can gauge ongoing periodontal destruction.

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be reused during collagen synthesis, and hence are specific to bone resorption. Subsequent to osteoclastic bone resorption and collagen matrix degradation, these molecules are released into the circulation. DPD is considered to be a bone marker specific for resorption since it is formed during biosynthesis or collagen maturation and consequently appears only as a resorption product of the mature matrix.\(^6\)\(^-\)\(^8\)

Although routine diagnostic measures are easy and economical, such parameters are of limited use in the early diagnosis, and they represent an indicator of past devastation. Hence, to target the clear threat of the microbial there is a need for an innovative diagnostic test to correctly determine the current status of disease activity, predicting sites that are vulnerable for possible breakdown, and evaluate the response to periodontal interventions.\(^9\) This is where biomarkers assume a significant diagnostic function, monitoring the outcomes of the discovery in life sciences.\(^7\)

Most molecules of oral fluids include saliva, gingival crevicular fluid (GCF), and serum or plasma molecules in the bloodstream, have been investigated so far as an endeavor to provide a sensitive and accurate periodontal disease marker. GCF and saliva are non-invasive oral fluids easily obtained, with minimal or/no discomfort to the patient, and consist of both systemically derived and locally synthesized molecules.\(^7\)\(^,\)\(^8\)

The GCF DPD levels are likely to be better indicators for disease activity and their estimation might give useful information for the diagnosis of active periodontal disease as well as estimate progression of the disease. The present study is intended to explore the biologic plausibility of the levels of DPD in health and chronic periodontitis.

**MATERIALS AND METHODS**

**Study design**

This study was conducted from January 2018 to July 2018 in a population aged 30–60 years from outpatient department of Periodonitics after obtaining institutional ethical committee clearance. After the examination of the individuals clinically and radiographically, they were divided into two groups. All the patients recruited have been explained about study protocols and written informed consent was obtained. A thorough clinical examination with all relevant parameters was recorded, and comprehensive periodontal therapy was performed for all the recruited patients.

**Inclusion and exclusion criteria**

Group A (healthy group) – 15 systemically healthy individuals with clinically healthy periodontium with PPD ≤ 3 mm [Figure 1]. Group B (chronic periodontitis group) – 15 systemically healthy individuals having at least a total of 14 teeth, diagnosed with moderate-to-severe periodontitis with at least one site with a PPD ≥ 6 mm and CAL ≥ 3 mm in each quadrant and radiographic evidence of bone loss [Figure 2]. Patients with any known systemic diseases (hypertension and diabetes) which can alter the course of periodontal disease, history of any recent infections, smokers, patients with alcohol intake, pregnancy/lactation, patients on any analgesics and antibiotics within the past 3 months, patient with prior history of periodontal therapy within 6 months of the study, aggressive periodontitis were not included in the study.

**Clinical examination**

During clinical examination, following parameters were assessed: Plaque index (PI),\(^9\) modified gingival index (MGI),\(^10\) Saxton bleeding index,\(^11\) probing pocket depth (PPD)\(^12\) on each tooth from the gingival margin to the bottom of the sulcus/pocket and clinical attachment loss (CAL) as distance between a fixed point on the crown, such as cementoenamel junction and the base of the pocket using UNC 15 probe at six sites per tooth. Radiographic examination of the teeth to confirm the bone loss was done using full mouth intraoral periapical radiographs and orthopantomogram. Patients were recalled for subsequent appointments to avoid the GCF contamination with the blood associated during probing at inflamed sites.

**Site selection and gingival crevicular fluid collection**

Patients were asked to sit upright in the dental chair; the site with deepest probing depth was selected, isolated with rolls of cotton, and air-dried [Figure 3]. To avoid blockage and contamination of the microcapillary pipette, supragingival plaque was removed with a curette without touching the marginal gingiva. GCF was collected using calibrated 1–5 µl volumetric optimized black color-coded microcapillary pipette (Sigma Aldrich, USA [Catalog No. P0549]) by placing the tip at the entrance of gingival crevice (unstimulated) for 5–20 min. A standardized volume of 3 µl of GCF was collected [Figure 4].\(^13\)

In periodontally healthy individuals, adequate GCF was difficult to collect so; pooled samples were collected from multiple sites so that the minimum required amount (3 µl) was achieved from each patient. In cases of chronic periodontitis, collection of GCF was less cumbersome as the required quantity could be collected from the site with the highest PPD. Samples contaminated with blood or saliva and those with air bubbles were discarded, and the collected samples were stored at ~80°C till the time of assay.

**Blood collection**

The skin over the antecubital fossa was disinfected, and 3 ml of blood was extracted utilizing a 5 ml syringe with 20G needle at the venipuncture. The collected blood sample was allowed to coagulate at room temperature for approximately 30 min [Figure 5].

**Serum preparation**

The sample was then centrifuged to extract the serum portion at 3000 rpm for 10 min. Thus, the separated serum component was transferred to a plastic vial and stored at ~80°C till the assay time [Figure 6].\(^14\)

**Test principle**

The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of human DPD in samples [Figure 7]. The contents of the kit and the samples were brought to room temperature. A 50 µl standard was added to the standard wells. A 40 µl of diluted sample and 10 µl of DPD monoclonal antibody were added to wells. Consequently, 50 µl of biotin combined with streptavidin HRP was added to wells to
Figure 1: Clinical photograph of periodontally healthy patient

Figure 2: Clinical photograph of chronic periodontitis patient

Figure 3: Site with deepest probing depth for gingival crevicular fluid collection

Figure 4: Gingival crevicular fluid collection at deepest probing depth site

Figure 5: Collection of blood from the antecubital fossa

Figure 6: Centrifuge used in the study

Figure 7: Enzyme-linked immunosorbent assay kit used in the study

Figure 8: Enzyme-linked immunosorbent assay microplates after addition of samples
form an immune complex [Figure 8]. The plate was covered with a sealer and incubated for 60 min at 37°C. The later sealer was removed and the plate was washed five times with wash buffer for 30 s to 1 min. The plate was blotted onto paper towels. A 50 µl substrate with chromogen A and B were added to individual wells one after the other. The plate was shielded with a new sealer and incubated for 10 min at 37°C in the dark. A 50 µl stop solution was added to individual wells, and the blue color changed into yellow immediately. The optical density values of the individual wells were determined immediately using a microplate Elisa reader [Figure 9] which is set to 450 nm within 30 min following the addition of stop solution. The concentration of DPD was expressed as nanomole per microliter.

**STATISTICAL ANALYSIS**

A statistical software SPSS version 20.0 (IBM company, USA) was used for the statistical analysis. Shapiro–Wilk test for normality was done and data were found to show normal distribution. Hence student’s t test was used for comparison and analysis. Correlation of HDPD levels in serum and GCF with age, MGI, PI, BI, PPD, and CAL was done using the Pearson’s correlation coefficient test. The data were presented as mean ± standard deviation.

**RESULTS**

The demographics, clinical parameters, serum, and GCF DPD levels are summarized in Table 1.

The mean age of the periodontally healthy and chronic periodontitis patients were 41.113 ± 6.54 and 41.2 ± 7.84 years, respectively. There were eight males (53.3%) and seven females (46.7%) in A Group (control), and seven males (46.7%) and eight females (53.3%) in B Group (case). The mean age in A and B Groups was statistically nonsignificant between the groups.
The intergroup comparison of clinical parameters such as mean MGI, Saxton BI, PI, PPD, and CAL scores revealed a statistically significant high score for Group B [Table 1 and Graph 1].

**Deoxypyridinoline concentration**

DPD concentrations were detectable in serum and GCF levels of all patients both in Group A and B [Graph 2]. The maximum DPD levels in serum of Group A patients was 1.05 nmol/L and 1.128 nmol/L in Group B patients, respectively [Graph 3]. In Group A, the mean DPD serum concentration was 0.37 ± 0.42 nmol/L and 0.52 ± 0.42 nmol/L in Group B. Intergroup comparison of mean serum DPD levels between Group A and Group B reveals no statistical difference [Table 2]. The mean DPD GCF concentration in Group A patients was 4.71 ± 1.84 nmol/L and 11.5 ± 3.73 nmol/L in Group B. While the maximum DPD levels in GCF of healthy individuals was 6.03 nmol/L and 18.36 nmol/L in Group B. The intergroup comparison of GCF DPD levels between Group A and Group B reveals no statistically significant difference [Table 2].

**Correlation between serum and gingival crevicular fluid deoxypyridinoline levels and clinical parameters**

On Pearson correlation analysis, serum DPD and GCF DPD concentration, age and gender revealed no statistically significant correlation for Group A and Group B [Table 3]. The Pearson correlation analysis between serum DPD and GCF DPD concentration with PPD, CAL, MGI, BI, and PI showed no statistically significant correlation in Group B [Graph 4].

**DISCUSSION**

Periodontal disease is a chronic microbial infection that triggers inflammation, affects the immune system resulting in alveolar bone destruction. Documentaion of the crosstalk between the immune and bone systems is strengthened. Immune response cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukins (ILs), and interferon impair osteoclasts activity and differentiation resulting in bone resorption.$^{[5,14,15]}$

To the best of our knowledge, the present study is believed to be first of its kind to evaluate the concentrations of DPD in serum and GCF in periodontal disease and health and to assess the impact of periodontal damage on DPD levels.

In the present study, the mean concentration of DPD levels were found to have significantly higher value in Group B (11.5 nmol/L) than in Group A (4.21 nmol/L). These results are in accordance with the value disclosed by Dharmayanti in which the periodontitis group showed significantly higher GCF DPD levels than the healthy group. The present study results are in agreement with other studies that evaluated different bone markers such as I-carboxy telopeptide pyridinoline (ICTP), osteopontin, and osteocalcin.$^{[16,17]}$

In chronic periodontitis, initial events are activated by lipopolysaccharides. Activated macrophages and monocytes respond to them by releasing cytokines such as TNFα, IL-1, and receptor activator of nuclear factor-kappa B ligand, which mediate osteoclastogenesis and bone breakdown at active periodontal destruction sites. Bone-specific markers, such as ICP and DPD are released into the surrounding area and transported to the sulcus or pocket through GCF and serves

### Table 1: Intergroup comparison of demographic data, clinical parameters, and Deoxyypyridinoline concentration in gingival crevicular fluid and serum

| Group, mean±SD | P  |
|----------------|----|
| Control        |    |
| Age (years)    | 41.1±6.54 | 40.7±5.8 | 0.853 (NS) |
| Sex (%)        |    |
| Female         | 8 (46.7) | 7 (53.3) |    |
| Male           | 7 (53.3) | 8 (46.7) |    |
| Overall PPD (mm) | 2.03±0.4 | 6.75±0.41 | ≤0.001 (S) |
| Overall CAL (mm) | 0%   | 7.0±0.38 | ≤0.001 (S) |
| MGI            | 0.68±0.16 | 2.41±0.52 | ≤0.001 (S) |
| PI             | 0.62±0.2 | 1.76±0.35 | ≤0.001 (S) |
| BI             | 0.5±0.2 | 1.42±0.31 | ≤0.001 (S) |
| Serum DPD (nmol/L) | 0.37±0.42 | 0.52±0.42 | 0.234 (NS) |
| GCF DPD (nmol/L) | 4.71±1.84 | 11.5±3.73 | ≤0.001 (S) |

NS – Statistically nonsignificant (P>0.05); S – Statistically significant (P<0.05); GCF – Gingival crevicular fluid; PPD – Periodontal probing depth; CAL – Clinical attachment loss; MGI – Modified gingival index; PI – Plaque index; BI – Bleeding index; DPD – Deoxypyridinoline concentration; SD – Standard deviation

### Table 2: Details of Deoxypyridinoline concentration in Group A and B

| Group | Mean±SD | Minimum-Maximum |
|-------|---------|-----------------|
| A     | Serum (nmol/L) | 0.37±0.42 | 0.022-1.05 |
|       | GCF (nmol/L)  | 4.71±1.84 | 1.25-6.03 |
| B     | Serum (nmol/L) | 0.52±0.42 | 0.52-1.128 |
|       | GCF (nmol/L)  | 11.5±3.73 | 7.5-18.36 |

DPD – Deoxypyridinoline (DPD) concentration; GCF – Gingival crevicular fluid; nmol/L – nanomole per liter; SD – Standard deviation

### Table 3: Correlations between serum and gingival crevicular fluid, deoxypyridinoline concentration with age and gender in the Groups A and B using Pearson correlation

| With age |
|----------|
| Group  |
|        |
| A      |
| Correlation coefficient | -0.208 | 0.106 |
| P      | 0.45 (NS) | 0.70 (NS) |
| B      |
| Correlation coefficient | 0.311 | 0.654 |
| P      | 0.26 (NS) | 0.008 (NS) |

S – Statistically significant (P<0.05); NS – Statistically nonsignificant (P>0.05); DPD – Deoxypyridinoline concentration; GCF – Gingival crevicular fluid

| With gender |
|-------------|
| Group  |
|        |
| A      |
| Serum DPD | 0.47±0.46 | 0.28±0.38 | 0.17 (NS) |
| GCF DPD | 5.44±1.61 | 4.07±1.9 | 0.16 (NS) |
| B      |
| Serum DPD | 0.54±0.41 | 0.49±0.47 | 0.17 (NS) |
| GCF DPD | 12.04±4.83 | 11.8±2.74 | 0.91 (NS) |

S – Statistically significant (P<0.05); NS – Statistically nonsignificant (P>0.05); DPD – Deoxypyridinoline concentration; GCF – Gingival crevicular fluid; SD – Standard deviation
as potential biomarkers for periodontal disease. Thus, locally DPD levels were found to be elevated in GCF of periodontitis patients.\[16,18,19\]

The mean serum concentration of DPD in Group A was 0.37 nmol/L and 0.52 nmol/L in Group B. There was no statistically significant difference found between the groups. This indicates that GCF is a localized site-specific potent inflammatory exudate fluid that seeps out into the sulcus or periodontal pockets and a more reliable method to detect the DPD levels when compared to serum which is a diluted fluid present throughout the body.

In the present study, DPD was also found in GCF and serum of healthy patients. Physiological bone metabolism could be the reason, as the bone is a dynamic tissue which undergoes constant remodeling and bone turnover throughout the life span.\[20\]

In the present study, age and gender have not shown a statistically significant correlation with DPD levels in both groups. The probable reason might be that the patients recruited in the study were systemically healthy and did not exhibit any bony or collagen related inflammatory changes elsewhere which would have influenced DPD levels. The modest sample size of thirty patients could be another reason for the possible lack of association between disease and serum levels. Further studies with a larger sample size can possibly throw more light on the influence of chronic periodontitis on DPD levels.

In the present study, an increase in disease severity as measured by PPD and CAL did not correlate with the DPD levels both in GCF and serum in the chronic periodontitis group. This is in contrast to the observations of Dharmayanti Augustin\[17\] (2012) who reported that GCF DPD levels correlated positively with the severity of periodontal disease. It has been generally accepted that increase in pocket depth and a concomitant increase in the total amount of biomarker in the disease.\[13\] However, it has also been proposed earlier (1984)\[21\] that disease activity is not a continuous process but consists of phases of active disease state and a remission state. If one had collected a sample in a site at the state of remission which otherwise would have been active earlier, the levels would not be higher. In spite of the conflicting opinion, it suffices to say that in the present study, the DPD levels did not correlate with the increase in PPD/CAL and this aspect needs further investigation with more samples of patients having varying degrees of active and inactive sites.

The other probable reason for no correlation in the present study might be because of the modest sample size, nature of the disease progression, and site-specific sample. Further, it also can be explained based on the fact that GCF collection in periodontitis sites may not yield similar volumes of GCF, due to the difference in the rate of GCF production and flow based on the inflammatory status of the periodontium. Further, no intervention was done to evaluate DPD levels before and after the treatment to determine whether decrease in PPD and CAL would eventually decrease DPD levels.\[13,21\]

It is pertinent here to recall the observations of Loos and Tjoa who evaluated almost 100 components as markers of GCF with regards to their potential utility to detect a case of periodontitis. They argued that the identification of single diagnostic marker for all forms of periodontal disease appears to be misleading.\[22\] There are some advantages of these marker systems over conventional diagnosis, and few of them may be capable of detecting or predicting periodontal disease activity. However, there are drawbacks such as the choice of the most appropriate biomarker and its cost-effectiveness.\[23\]

As it is difficult to diagnose the early periodontitis in patients with the absence of clinically detectable attachment loss, the estimation of GCF DPD levels could be helpful in diagnosing the early periodontal tissue destruction.\[24\]

**CONCLUSION**

The following conclusions are drawn from the present study:

1. GCF DPD levels in chronic periodontitis patients were significantly higher when compared to the periodontally healthy group
2. Increase in periodontal disease severity, i.e., PPD and CAL did not show a significant correlation with the GCF and serum DPD levels in the chronic periodontitis group.

By combining the results in the present study and those of earlier studies, it might be concluded that further cross-sectional and interventional studies with a larger sample size are required to evaluate the GCF DPD levels in chronic periodontitis and gauge it as a potential biomarker for alveolar bone loss.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts
will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**
There are no conflicts of interest.

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