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Plasma and crevicular fluid osteopontin levels in periodontal health and disease

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Background and Objective: The level of osteopontin in gingival crevicular fluid has been found to correlate with clinical measures of periodontal disease. The present study was designed to assess the relationship between clinical parameters and osteopontin levels of the gingival crevicular fluid from inflamed gingivae, periodontitis sites and after treatment of periodontitis sites, and to correlate them to the osteopontin levels of the plasma.

Material and Methods: Thirty, gender-matched subjects were divided into three groups – healthy, gingivitis and chronic periodontitis – based on modified gingival index scores and clinical attachment loss. The fourth group consisted of 10 subjects in the periodontitis group, 6–8 wk after initial therapy. Plasma and gingival crevicular fluid samples were collected and quantified for osteopontin using an enzyme immunoassay.

Results: The highest mean gingival crevicular fluid and plasma osteopontin concentrations were observed in the periodontitis group (1575.01 and 1273.21 ng/mL, respectively) and the lowest in the healthy group (1194.80 and 476.35 ng/mL, respectively). After treatment of the periodontitis group, the level of osteopontin decreased to 1416.15 in gingival crevicular fluid and to 1051.68 ng/mL in plasma. In all groups the gingival crevicular fluid osteopontin levels showed a statistically significant positive correlation with that of plasma and clinical attachment loss.

Conclusion: Osteopontin levels were highest in the gingival crevicular fluid from sites with periodontal destruction; however, periodontal treatment resulted in the reduction of osteopontin levels. Gingival crevicular fluid and plasma osteopontin levels showed a positive correlation in all of the groups.

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Periodontal diseases are chronic inflammatory diseases of the supporting structures of the teeth. Periodontal disease is triggered by periodontopathogens and the clinical outcome is highly influenced by the host local immune response (1). It is a well-established fact that the host immune products are synthesized locally and appear within the gingival crevicular fluid, and therefore gingival crevicular fluid is ideal for obtaining diagnostic information of periodontal health or disease status (2). The markers identified thus include cytokines, prostaglandins, bacterial- and host-derived enzymes, and connective tissue-degradation products, alongside bone matrix components that are primarily isolated in the gingival crevicular fluid (3). However, recent evidence has indicated that patients with periodontitis present with increased systemic inflammation, as indicated by raised plasma levels of various markers when compared with controls (4,5). This increase in systemic inflammation has been implicated in having a modulating role in cardiovascular disease (6), on an adverse pregnancy outcome (7), on diabetes

mellitus (8) and in respiratory disease (9). In view of these findings, studies correlating the levels of these markers in gingival crevicular fluid and in the peripheral circulation is warranted.

Osteopontin is a noncollagenous, calcium-binding, glycosylated phosphoprotein, abundant in the mineralized phase of bone matrix (10). It is synthesized mainly by pre-osteoblasts, osteoblasts and osteoclastic cells and has an RGD (arginine-glutamine-aspartic acid) sequence, confirming an extracellular matrix-cell adhesion function for attaching bone cells to the bone matrix (10).

Recently, osteopontin has also been shown to be a component of human atherosclerotic plaque, where it is synthesized by resident macrophages, smooth muscle and endothelial cells that contribute to cellular accumulation in atherosclerotic plaques (11). Furthermore, it has been shown that plasma osteopontin levels correlate positively with the extent of coronary atherosclerotic disease, suggesting a role of osteopontin in cardiovascular disease (12).

In our previous study, we have shown that the osteopontin levels in gingival crevicular fluid show an increasing trend from health to gingivitis to periodontitis. In addition, a significant reduction was noted in osteopontin levels after initial, nonsurgical treatment, validating the fact that osteopontin levels in gingival crevicular fluid may be considered a marker of periodontal destruction (13). As an extension to the aforementioned study, the present study was designed to correlate the gingival crevicular fluid osteopontin levels with the osteopontin levels of plasma, in subjects with clinically healthy periodontium, in patients with gingivitis and chronic periodontitis, and after scaling and root planing of periodontitis subjects.

Material and methods

The study population consisted of 30 subjects (15 women, 15 men; 30–59 years of age) attending the outpatient clinic of the Department of Periodontics, Government Dental College and Hospital, Bangalore. Exclusion criteria included: a history of diabetes mellitus; ischemic heart disease or any other conditions contributing to atherosclerosis; smoking and alcoholism; bone disorders and/or on antiresorptive drugs such as bisphosphonates (e.g. Alendronate); treatment with anti-inflammatory drugs, antibiotics, steroids and contraceptives in the last 6 mo; and pregnancy and breastfeeding.

Informed consent was obtained from those subjects who agreed to participate voluntarily in this study after institutional ethical clearance was obtained.

Criteria for subject grouping were followed as per our previous study (13). Briefly, subjects were categorized into three groups based on the clinical examination and modified gingival index scores (14) and radiographic evidence of bone loss. After a full-mouth periodontal probing, bone loss was recorded dichotomously (presence or absence) to differentiate chronic periodontitis patients from other groups without any delineation in the extent of alveolar bone loss. Ten subjects with clinically healthy periodontium (modified gingival index < 1) were designated as the healthy group; the gingivitis group consisted of 10 subjects with gingival inflammation (modified gingival index > 1) and no attachment loss; and the third group consisted of 10 patients with chronic periodontitis showing a probing clinical attachment loss of > 2 mm and a modified gingival index of > 1. Chronic periodontitis patients were treated with scaling and root planing, and gingival crevicular fluid and plasma samples were taken from the same sites 6–8 wk after treatment to constitute the after-treatment group.

Site selection and gingival crevicular fluid sampling

To ensure blinding of the sampling examiner (CGDS), clinical examination and site selection was performed by the second examiner (ARP) on the previous day. The samples were collected on the subsequent day to prevent contamination of gingival crevicular fluid with blood associated with mechanical irritation as a result of probing of inflamed sites. One site per subject was selected as a sampling site. In the healthy group, sampling was predetermined to be from the mesiobuccal region of the maxillary right first molar, in the absence of which the left first molar was sampled. Sites with the highest clinical signs of inflammation (i.e. redness, bleeding on probing, and edema) were selected in the gingivitis group. In patients of the periodontitis group, sites with > 2 mm of clinical attachment loss (and absence of marginal tissue recession), as measured from the clinical cemento–enamel junction to the base of the periodontal pocket using a Williams graduated periodontal probe, were identified, and the site showing the highest clinical attachment loss, along with radiographical confirmation of bone loss, was assigned for sampling. On the subsequent day, after drying the area with a blast of air, supragingival plaque was removed without touching the marginal gingiva, and gingival crevicular fluid was collected using color-coded 1–5 μL calibrated volumetric microcapillary pipettes (Sigma-Aldrich Chemical Co. Ltd, St Louis, MO, USA). From each test site, a standardized volume of 1 μL was collected using the calibration on the micropipette and by placing the tip of the pipette extracrevicularly (unstimulated). The gingival crevicular fluid collected was immediately transferred to a plastic vial and stored at −70°C until the assay.

Plasma collection

A 5-mL blood sample was obtained from the antecubital fossa by venepuncture into a vacutainer coated with 3.2% sodium citrate. The samples were centrifuged (1000 ***g***, 4°C, 10 min) within 30 min of collection. The plasma was aliquoted and stored at −70°C until analysis.

Osteopontin assay

The concentration of osteopontin was determined using a sandwich-type human Osteopontin Enzyme Immunometric Assay kit (TiterZyme®;

Assay Designs Inc., Ann Arbor, MI, USA). All samples and standards were assayed in duplicate, as suggested by the manufacturer. After appropriate dilution of the samples, 100 μL of sample was added to appropriate wells, and the plate was sealed and incubated at 37°C for 1 h. Following incubation and washing seven times, 100 μL of the labeled antibody was pipetted into the wells, followed by incubation at 4°C for 30 min. Later, the plate was washed nine times, and 100 μL of the substrate solution was added to each well followed by incubation at room temperature for 30 min in the dark. Lastly, 100 μL of stop solution was added to each well and the plate was read immediately for absorbance of each well using a microplate (enzyme-linked immunosorbent assay) reader set at a wavelength of 450 nm. The concentration of osteopontin in the tested samples was computed using the standard curve plotted with the optical density values obtained from the assay.

Statistical analysis

All data were analyzed using statistical software SPSS, version 10 (SPSS Inc., Chicago, IL, USA). The Kruskal–Wallis test, Mann–Whitney *U*-test and Wilcoxon signed rank test were carried out to compare osteopontin levels between groups. The Spearman's rank correlation test was used to compare osteopontin levels between the groups and the clinical parameters.

Results

All the assayed samples of gingival crevicular fluid and plasma showed the presence of osteopontin. The mean concentration of osteopontin, both in gingival crevicular fluid and plasma, was observed to be highest in the periodontitis group (1575.01 ng/mL in gingival crevicular fluid and 1273.21 ng/mL in plasma) and lowest in the healthy group (1194.80 ng/mL in gingival crevicular fluid and 476.35 ng/mL in plasma). The mean osteopontin concentration in the gingivitis group (1392.29 in gingival crevicular fluid and 830.18 ng/mL in plasma) and after-treatment group (1416.15 in gingival crevicular fluid and 1051.68 ng/mL in plasma) fell between the highest and lowest values, as shown in Table 1 and Fig. 1.

The Kruskal–Wallis and Mann–Whitney *U*-tests were carried out to determine whether there were any significant differences in the gingival crevicular fluid and plasma osteopontin levels between the study groups (Tables 2 and 3). The results implied that osteopontin levels, both in gingival crevicular fluid and plasma, increase progressively from healthy to periodontitis patients.

When the after-treatment group and the periodontitis group were compared using the Wilcoxon signed rank test, the difference in concentration of osteopontin was statistically significant ($p = 0.005 < 0.05$), indicating that, after scaling and root planing, osteopontin levels decreased considerably both in gingival crevicular fluid (1575.01–1416.15 ng/mL) and proportionally in the plasma (1273.21–1051.68 ng/mL), as shown in Table 4, in accordance with a decrease in clinical attachment loss.

Table 1. Descriptive statistics for osteopontin levels in gingival crevicular fluid (GCF) and plasma

	Osteopontin levels (mean ± SD) (ng/mL)	
Groups	GCF	Plasma
HG	1194.80 ± 64.76	476.35 ± 58.45
GG	1392.29 ± 73.07	830.18 ± 77.70
PG	1575.01 ± 103.91	1273.21 ± 163.04
AG	1416.15 ± 82.25	1051.68 ± 88.26

AG, after-treatment group; GG, gingivitis group; HG, healthy group; PG, periodontitis group.

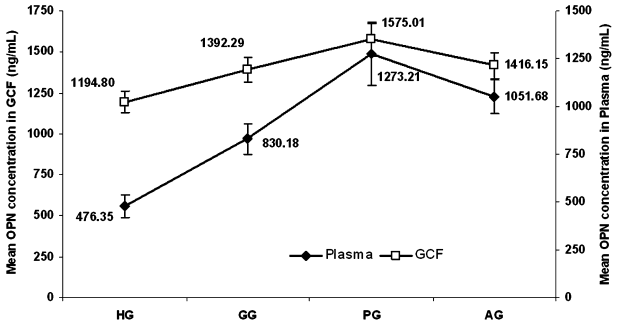

Fig. 1. Mean concentration of osteopontin in gingival crevicular fluid and plasma in all the groups. AG, after-treatment group; GCF, gingival crevicular fluid; GG, gingivitis group; HG, healthy group; OPN, osteopontin; PG, periodontitis group.

Table 2. Kruskal–Wallis test comparing the mean osteopontin concentrations in gingival crevicular fluid (GCF) and plasma

		GCF		Plasma	
Groups	*n*	Mean rank	*p*-value	Mean rank	*p*-value
HG	10	5.5	0.00*	5.5	0.00*
GG	10	19.6		15.6	
PG	10	34.3		32.8	
AG	10	22.6		28.1	

AG, after-treatment group; GG, gingivitis group; HG, healthy group; PG, periodontitis group.
*$p < 0.05$.

Table 3. Mann–Whitney U-test to compare gingival crevicular fluid (GCF) and plasma osteopontin concentrations

		GCF		Plasma	
Groups	*n*	Mean rank	*p*-value	Mean rank	*p*-value
HG	10	5.5	0.00*	5.5	0.00*
GG	10	15.5		15.5	
HG	10	5.5	0.00*	5.5	0.00*
PG	10	15.5		15.5	
GG	10	5.5	0.00*	5.5	0.00*
PG	10	15.5		15.5	

GG, gingivitis group; HG, healthy group; PG, periodontitis group.
*$p < 0.05$.

Table 4. Wilcoxon Signed Rank test to compare the osteopontin concentration in gingival crevicular fluid (GCF) and plasma in patients before and after treatment

		Mean osteopontin concentration (ng/mL)					
Groups	*n*	GCF	*Z*	*p*-value	Plasma	*Z*	*p*-value
PG	10	1575.01	−2.803	0.005*	1273.21	−2.803	0.005*
AG	10	1416.15			1051.68		

AG, after-treatment group; PG, periodontitis group.
*$p < 0.05$.

Spearman's rank correlation test, performed to establish any correlation between the gingival crevicular fluid and plasma osteopontin concentration, showed a positive correlation in all the four groups suggesting that plasma osteopontin levels were commensurate with that of gingival crevicular fluid, and vice versa (Table 5). When the osteopontin levels in gingival crevicular fluid and plasma were analysed for correlation with the periodontal disease severity measures, a positive correlation in all the four groups for modified gingival index and for clinical attachment loss in periodontitis group and the after-treatment group was observed. This suggests that osteopontin levels, both in gingival crevicular fluid and plasma, show a positive correlation with the severity of the periodontal disease.

The Kruskal–Wallis test was carried out to compare the mean osteopontin concentration in gingival crevicular fluid and plasma at different clinical attachment loss levels (before and after treatment). A significant reduction of osteopontin levels in gingival crevicular fluid and plasma was found after treatment, as shown in Table 6 and Fig. 2.

In summary, the results of the study suggest that the mean concentration of osteopontin in gingival crevicular fluid and plasma is highest in the periodontitis group and differs significantly from that of the healthy group, the gingivitis group and the after-treatment group. Furthermore, both the gingival crevicular fluid and plasma osteopontin concentration increases proportionally with progressive periodontal disease and decreases after treatment aimed at reducing periodontal inflammation and arresting alveolar bone loss.

Discussion

Osteopontin, known to act as an anchor for osteoclasts by virtue of the RGD motif, can be one of the principal mediators of alveolar bone destruction in progressive periodontal disease, as described in our previous study (13). To date, only two studies have detected osteopontin in gingival crevicular fluid and explained the possible role of osteopontin in periodontal disease (13,15), and none have correlated the osteopontin levels in gingival crevicular fluid and plasma of healthy, diseased periodontium and after treatment.

Hence, the present study was undertaken to determine the potential role of osteopontin, as a mediator of periodontal inflammation and alveolar bone destruction, and correlate periodontal disease status with that of plasma osteopontin levels.

The results of the present study are in accordance with our previous study (13). The mean concentrations of osteopontin in gingival crevicular fluid were found to increase progressively from health to periodontitis. More-

Table 5. Spearman's rank correlation (r) test comparing gingival crevicular fluid (GCF) and plasma osteopontin (OPN) levels, modified gingival index (MGI) and clinical attachment loss within the groups

Groups	GCF and plasma OPN levels	GCF OPN levels and MGI	Plasma OPN levels and MGI	GCF OPN levels and CAL	Plasma OPN levels and CAL
HG	0.879*	0.867*	0.988*	–	–
GG	1.000*	1.000*	1.000*	–	–
PG	0.927*	1.000*	0.927*	0.798*	0.798*
AG	1.000*	0.321*	0.321*	0.853*	0.853*

AG, after-treatment group; GG, gingivitis group; HG, healthy group; PG, periodontitis group.
*If the 'r' value is between 0 and 0.5, there is a weakly positive correlation; if the 'r' value is between 0.5 and 1, there is a strongly positive correlation; and if r is 1, there is 100% positive correlation between the two sets of data compared.

Table 6. Kruskal–Wallis test comparing the mean concentration of gingival crevicular fluid (GCF) and plasma osteopontin with respect to clinical attachment loss (CAL)

Study group	CAL (mm)	*n*	Mean osteopontin concentration (ng/mL)		*p*-value
			Mean	SD	
PG					
GCF	3	7	1519.36	39.87	0.02*
	4	3	1704.86	87.68	
Plasma	3	7	1188.20	83.54	0.01*
	4	3	1471.58	119.82	
AG					
GCF	1	4	1346.66	43.95	0.011*
	2	6	1462.47	67.68	
Plasma	1	4	974.16	21.09	0.008*
	2	6	1103.37	75.78	

AG, after-treatment group; PG, periodontitis group.
*$p < 0.05$.

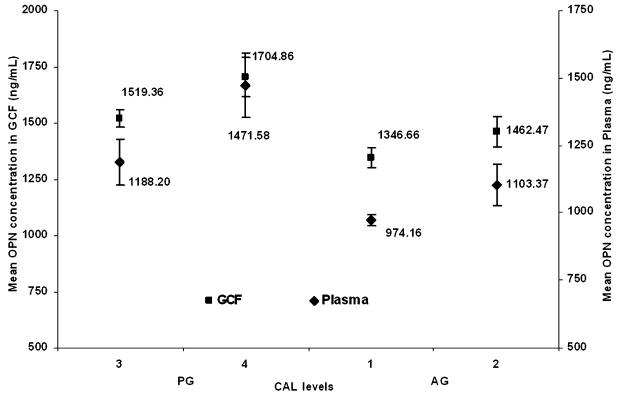

Fig. 2. Gingival crevicular fluid and plasma osteopontin levels in the periodontitis group and the after-treatment group at different clinical attachment loss levels. AG, after-treatment group; GCF, gingival crevicular fluid; HG, healthy group; OPN, osteopontin; PG, periodontitis group.

over, the mean osteopontin levels in plasma were observed to be highest in the periodontitis group and lowest in the healthy group, with intermediate values for the gingivitis group. Thus, the mean concentration of osteopontin increased progressively from health to gingivitis to periodontitis, both in gingival crevicular fluid and, proportionately, in plasma.

When pairwise comparison, using the Mann–Whitney *U*-test, was performed between the groups, the results confirmed that the osteopontin levels, both in gingival crevicular fluid and proportionally in plasma, increased progressively from health to periodontitis.

Subjects in the periodontitis group were treated by nonsurgical periodontal therapy (scaling and root planing), and strict oral hygiene measures were instituted. The mean concentration of osteopontin in the gingival crevicular fluid and plasma in the periodontitis group decreased from 1575.01 and 1273.21 ng/mL, respectively, to after-treatment levels of 1416.15 and 1051.68 ng/mL, respectively, a statistically significant reduction. Also, the mean modified gingival index score of the periodontitis group decreased from 2.41 to 1.36 (data not shown) after treament, commensurate with that of the clinical attachment loss levels and the osteopontin levels in gingival crevicular fluid and plasma.

As suggested in our previous study, the source of osteopontin in gingival crevicular fluid seems to be neighboring tissues, including alveolar bone and cementum, macrophages in periodontal tissues, blood and salivary glands (13). Furthermore, the concomitant increase of osteopontin in plasma, as noted in our study, may be caused by the spillage or overflow of osteopontin from the diseased periodontal tissues, or produced by the circulating activated macrophages.

The role of osteopontin in atherosclerotic disease has been previously studied, and various findings supporting the pathogenic effects, have been reported. Osteopontin is known to be specifically associated with, and have an important role in, the onset and progression of disease in human coronary atheroma and, ultimately, to alter vessel compliance (16). In an *in vitro* study, it was reported that osteopontin mRNA was expressed by smooth muscle-derived foam cells in human atherosclerotic lesions of the aorta, and the magnitude of its expression was proportional to the stage of atherosclerosis (17). However, most of these studies attribute the increase in osteopontin levels to local production by activated macrophages and smooth muscle cells. By contrast, one study associated an increase in plasma osteopontin levels with calcified coronary atherosclerotic plaques, and this increase correlated with the number of stenotic coronary vessels, with the highest levels reported in three-vessel disease (12). These findings delineate the role of osteopontin in the pathogenesis of atherosclerosis and, in turn, increase the risk of cardiovascular and cerebovascular accidents.

In our study, the highest mean plasma concentration (in the periodontitis group) was 1273 ± 163.04 ng/mL, which is much higher than the concentration described in an earlier study (12). Based on the above findings, it can be hypothesized that the increase in plasma osteopontin levels caused by progressive periodontal disease could act as a risk factor for coronary artery disease. However, this needs to be confirmed by conducting longitudinal, prospective studies involving larger populations.

Hence, within the limits of our study, it can be postulated that with a greater the extent of periodontal destruction there is substantial increase in osteopontin concentration locally, in the gingival crevicular fluid, and this is associated with an increase in plasma osteopontin concentration. However, further longitudinal studies should be undertaken to validate osteopontin as a 'biochemical marker' of periodontal destruction and a 'risk factor' for atherosclerotic diseases.

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