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OSTEOPONTIN LEVELS IN THE GCF OF HEALTHY, CHRONIC GINGIVITIS AND CHRONIC PERIODONTITIS PATIENTS

Dental Science

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ABSTRACT

AIM: The purpose of the present study was to determine the levels of osteopontin in GCF of healthy, chronic gingivitis and chronic periodontitis subjects and to investigate the relationship between GCF osteopontin levels between healthy and diseased subjects and to explore the possibility of using OPN in GCF as a biomarker of periodontal destruction

MATERIALS AND METHODS: Total of 45 subjects were divided into three groups based on MGI,PPD,CAL and radiographic evidence of bone loss.

Group-1 (healthy) n=15
Group -2 (chronic gingivitis) n=15
Group -3 (chronic periodontitis) n=15

GCF samples were collected by micro papillary pipettes from each subject and the samples were quantified for osteopontin using enzymatic immunometric assay

RESULTS: The results in the present study showed an increase in GCF osteopontin levels in chronic periodontitis compared to healthy controls and chronic gingivitis. Significant difference was found to be between GCF osteopontin (OPN) levels in chronic gingivitis and chronic periodontitis patients.

KEYWORDS

GCF, osteopontin

INTRODUCTION:

Human periodontal diseases result from heterogeneous aetiologies which includes complex biofilm in the sub gingival microenvironment, social and behavioural modulations, and genetic traits of host, each of which is influenced by host immune and inflammatory responses.[1] However, it is widely accepted that periodontal pathogens are the perpetrator for initiation and sustaining periodontal disease and inflammatory process is crucial for destruction of mineralized and non-mineralized extracellular matrices in periodontal tissues.[2]

Bone tissue, including tooth supporting alveolar bone, can only be resorbed by osteoclasts and, therefore, if bone loss is to occur in periodontitis a local stimulation of osteoclastic differentiation and its action is essential. Inflamed periodontal tissues contain a wide array of inflammatory mediators, some of which are able to cause bone resorption by stimulating osteoclast differentiation, chemo taxis, adhesion and/or enhancing its activity [3] which includes Interleukin-1 (IL-1), Tumour necrosis factor-α (TNF-α), Interleukin-6 (IL-6), Prostaglandin E_2(PGE_2), [4-5] and Osteoprotegrin.[6] Recent addition to this class of proteins is a bone matrix-derived and multifunctional cytokine, Osteopontin which has been detected in gingival crevicular fluid (GCF).[7]

Osteopontin (OPN) is a non-collagenous, highly phosphorylated, sialic acid-rich, calcium binding glycosylated phosphoprotein, which has an RGD (arginine-guanidine-aspartic acid) sequence.[8] It is an extracellular matrix cell adhesion protein, which is abundant in bone and is synthesized mainly by preosteoblasts, osteoblasts, and osteoclastic cells that are localized in the mineralized phase of bone matrix.[8] However, this protein is also found in kidney, blood, mammary gland, salivary glands and calculus.[7] Thus, OPN exists both as an immobilized extracellular matrix molecule in mineralized tissues and as a multifunctional cytokine in body fluids where it is secreted by activated macrophages, leukocytes, activated T-cells and natural killer cells.[9]

Further, OPN is intimately involved in the regulation of both physiological and pathological mineralization.[10] OPN serves both to attach bone cells to bone matrix and to generate intracellular signals essential for normal osteoclast motility on bone.[9]

In the inflammatory process, OPN is known to be a multifunctional cytokine and is chemotactic for various cell types including monocytes/macrophages, which are attracted to Sites of infection and inflammation. It is essential for the cell-mediated immunity and normal Th-1 cytokine response during granuloma formation .[9]

OPN has also been shown to be a component of human atherosclerotic plaque and may be a mediator of arterial neointima formation. It is synthesized by resident macrophages, smooth muscle and endothelial cells in primary and restenotic human coronary atherosclerotic plaques, which contribute to cellular accumulation, and dystrophic calcification in atherosclerotic plaques.[11]

OPN has also been implicated in carcinogenesis and spread of cancer cells.[8] Further the serum OPN levels have been associated with survival rates in breast cancer patients.[12]

Recently, OPN molecule was shown to be present in GCF and its levels increases with the progression of periodontal diseases and indicated that OPN levels in GCF may become a marker of periodontal disease, especially alveolar bone resorption.[7]

Hence in light of the above mentioned facts, this clinico-biochemical study was designed to estimate the levels of OPN in GCF of subjects with clinically healthy periodontium, gingivitis, and chronic periodontitis.

Criteria for Subject Grouping:

The subjects were categorized into 4 groups, each group comprising of 15 patients based on modified gingival index (Loe & Sillness 1986), probing pocket depth (PPD) ≥5mm and clinical attachment loss (CAL), with radiographic evidence of bone loss.

- **Group 1(Healthy):** Consisted of 15 subjects with clinically healthy periodontium, and with no evidence of disease. The score obtained after assessing the gingival status using MGI was <1, PPD ≤3mm and CAL=0 with no crestal bone loss as determined from radiograph.
- **Group 2 (Gingivitis):** Consisted of 15 subjects whose gingivae showed clinical signs of inflammation but with no evidence of attachment loss i.e., CAL=0. The radiographs did not show any bone loss. The score between 1 and 2 was obtained after recording MGI.
- **Group 3 (Chronic Periodontitis):** Consisted of 15 subjects, both male and female in the age range of 38-50 yrs who showed clinical signs of gingival inflammation and attachment loss with

radiographic evidence of bone loss and with PPD ≥5mm in multiple sites in all sites of quadrants. The MGI score between 2 and 4 was obtained for these patients.

Procedure For Gingival Crevicular Fluid Collection:

After making the subjects sit comfortably in an upright position on the dental chair, the selected test site was air dried and isolated with cotton rolls. Without touching the marginal gingiva, supragingival plaque was removed to avoid contamination and blocking of the micro capillary pipette. GCF was collected by placing white color-coded 1-5µL calibrated volumetric micro capillary pipettes obtained from Sigma-Aldrich Chemical Company, USA (Catalogue no. p 0549)[§§]. By placing the tip of the pipette extracrevicularly (unstimulated) for 5-20 minutes, a standardized volume of 1µl GCF was collected using the calibration on the micropipette from each test site.

The test sites, which did not express standard volume (1µl) of GCF, and micropipette contaminated with blood and saliva, were excluded or discarded. The GCF collected was immediately transferred to plastic vial and stored at –70^{0}C till the time of the assay.

The samples were then assayed for OPN levels by using Quantakine Human Osteopontin kit (DOST00)[‡‡]. Samples were assayed at the department of molecular and cellular biology, NIN (National institute of Nutrition) by ELISA method

‡‡ R&D Systems, Minneapolis,
United States of America
§§ Sigma Aldrich

RESULTS:

In this present study an attempt was made to evaluate the levels of osteopontin in healthy, chronic gingivitis and chronic periodontitis patients. A total of 45 subjects were included in the study between 24-50 yrs. All the samples assayed showed the presence of osteopontin. The mean concentration of osteopontin in GCF in healthy, chronic gingivitis and chronic periodontitis were 60.08±31.54 ng/ml, 137.36± 54.44ng/ml, 238.06± 99.76ng/ml respectively (TABLE 4). The differences in the mean osteopontin levels between groups 1& 2, 1 &3 and 2& 3 were found to be statistically significant.

The mean probing depth in healthy was 2.35± 0.36, chronic gingivitis 2.79± 0.30 and chronic periodontitis was 6.66±0.90. The difference in mean probing depth was found to be statistically significant between healthy and chronic periodontitis, chronic gingivitis and chronic periodontitis groups. Mean probing depth between healthy and chronic gingivitis was statistically insignificant.

Table 1: Mean MGI (Modified Gingival Index Score)

	GROUP 1	GROUP 2	GROUP 3
MEAN	0.30	1.65	1.58
SD	0.11	0.20	0.54

Table 1a: Comparison Of MGI Scores Between Groups Using ANOVA

GROUP	GROUP COMPARED	MEAN DIFFERENCE	P value	significance
1	2	-1.3500	<0.001**	S
	3	-2.0787	<0.001**	S
2	3	-0.7281	<0.001**	S

Table 2: Mean Probing Depth Among The Group

	GROUP 1 Healthy	GROUP 2 Chronic gingivitis	GROUP 3 Chronic periodontitis
MEAN	2.35	2.79	6.66
SD	0.36	0.30	0.90

Table 2a: Comparison Of Probing Depth Scores Between Groups Using ANOVA

Group	Group Compared	Mean Difference	P value	significance
1 healthy	2 chronic gingivitis	0.4357	0.245	NS
	3 chronic periodontitis	-4.3085	<0.001**	S
2 chronic gingivitis	3 chronic periodontitis	-3.8729	<0.001**	S

Table 3: Mean CAL (Clinical Attachment Level)

	Group 3 Chronic periodontitis
Mean	5.37
SD	0.80
P value	0.873

Table 4: Mean OPN Levels Among The Group

	GROUP 1 healthy	GROUP 2 Chronic gingivitis	GROUP 3 Chronic periodontitis
MEAN	60.08ng/ml	137.36ng/ml	238.ng/ml
SD	31.54	54.44	99.76

Table 4a: Comparison Of Osteopontin Levels Between Groups Using ANOVA

Group	Group Compared	Mean Difference	P value	Significance
1 healthy	2 chronic gingivitis	-77.2833	0.021	S
	3 chronic periodontitis	-177.9773	<0.001**	S
2 chronic gingivitis	3 chronic periodontitis	-100.6940	0.001	S

Table 5: Pearsons Correlation Between Different Variables And OPN (Osteopontin)

Pearson's correlation co-efficient -r P value	Group 1 Healthy	Group 2 Chronic Gingivitis	Group 3 Chronic Periodontitis
OPN vs. MGI	0.492 (0.062)	-0.010 (0.973)	-0.198 (0.479)
OPN vs. PD	0.631 (0.012)*	0.519 (0.048)*	0.874 (<0.001)**
OPN vs. CAL	-	-	0.567 (0.028)*

*indicates P = <0.05 significant
**indicates P = <0.001 highly significant

DISCUSSION:

Periodontal diseases are a group of inflammatory conditions affecting the supporting structures of the dentition. Though the unequivocal role of microbial challenge in the aetiology of periodontal disease has been well studied, it is the paradoxical impact of the susceptible host's inflammatory response to microbial challenge that ultimately leads to the destruction of the periodontal structures and subsequent tooth loss via osteoclastic action.[13] The process involves sequestration of bone related proteins like ICTP, ON (osteonectin), OCN (osteoclacin) and bone phosphoprotein in GCF, and thus act as important markers of bone resorption in periodontal disease.[14]

Traditional periodontal diagnostic methods like assessment of clinical parameters and radiographs though efficient, are inherently limited in the, determination of only historical perspective, not current appraisal; of disease status can be determined. Advances in the use of oral fluids as possible biological samples for objective measures of disease state, treatment monitoring and prognostic indicators have boosted GCF and other fluids to the forefront of technology. GCF contains locally and systemically derived mediators of periodontal disease including microbial, host response and bone specific resorptive markers . Although most biomarkers in GCF most lack specificity to alveolar bone destruction and essentially constitute soft tissue inflammatory events.[15]

Several specific collagen degradation and bone turnover related molecules have emerged as possible measures of periodontal disease activity, one of such bone related protein is osteopontin (OPN).

Osteopontin is known to act as an anchor for osteoclasts by virtue of its RGD motif, can be one of the principal mediators of alveolar bone destruction in progressive periodontal disease.[14]

Hence this present study was done to determine the osteopontin levels in chronic periodontitis patients and compare them with chronic gingivitis and healthy subjects.

The results of the present study showed an increase in OPN levels in chronic periodontitis subjects compared to healthy controls and chronic gingivitis subjects. This increase in OPN levels in periodontitis subjects is in accordance with the results of earlier study

by ***Kido J et al,*** they reported the presence of OPN in GCF and found that increase in osteopontin level was associated with progression of periodontal disease.

The OPN levels were found to be significantly higher in chronic periodontitis subjects compared with healthy controls and chronic gingivitis subjects and statistically significant difference was found in the OPN levels between chronic gingivitis and chronic periodontitis subjects. The reason for this difference in OPN levels between chronic gingivitis and periodontitis subjects is due to the intimate involvement of osteopontin in the regulation of both physiological and pathological mineralization.

Another important finding in this study is the correlation between periodontal parameters and OPN levels. Periodontal probing depth showed a positive correlation with OPN levels in all the groups. This correlation between PD and OPN levels was found to be statistically significant in healthy controls and chronic gingivitis groups and statistically highly significant in chronic periodontitis group. This finding is in accordance with study by ***Kido et al*** who demonstrated that as the pocket depth increases, OPN levels also increases.

Also in the present study a positive correlation between CAL & OPN was found to be statistically significant in chronic periodontitis groups. This finding is similar to the results of the earlier study by ***Sharma CG, Pradeep AR***.

Therefore the findings from this study and from those of the earlier studies indicates that the increase in OPN levels in periodontitis patients can be used as reliable indicator of disease activity as evidenced by increase in pocket depth and attachment loss. Given their specificity for bone resorption, the OPN group of bone related proteins, represent a potentially diagnostic aid in periodontics because of biochemical markers specific for bone degradation may be useful in differentiating between the presence of gingival inflammation and active periodontal destruction.

Though the present study has demonstrated increase in GCF OPN levels in periodontitis subjects,

CONCLUSION:

From this study it can be said that osteopontin in seen in GCF of healthy, chronic gingivitis and chronic periodontitis subjects. An elevated level of osteopontin in GCF of chronic periodontitis patients was noticed when compared with chronic gingivitis and healthy subjects. A positive correlation was observed between probing depth & CAL in chronic perioodntitis group.it can be concluded that OPN can be used as reliable indicator of disease activity and may be predictive for future attachment loss.

However the results of this present study cannot be used to validate osteopontin as a biomarker due to limitations, one being small sample size and the study being cross sectional.

The results of the present study showed an increase in GCF osteopontin in chronic periodontitis compared with chronic gingivitis and healthy controls.

A positive correlation was seen between probing depth and GCF OPN levels. This correlation was found to be statistically significant in healthy and chronic gingivitis groups and statistically highly significant in chronic gingivitis and periodontitis groups.

A positive correlation was found between CAL and OPN levels in chronic periodontitis subjects which were statistically significant. Therefore the findings from this study indicate that the increase in GCF OPN levels in periodontitis patients can be used as a reliable indicator of disease activity and may be predictive for future attachment loss.

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