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Research Article

EVALUATION OF BONE METABOLISM MARKER SCLEROSTIN AND ITS CORRELATION WITH CLINICAL PARAMETERS IN CHRONIC PERIODONTITIS PATIENTS: AN OBSERVATIONAL STUDY

[1]Dr. Dheeraj B R, [2]Dr.Kranti. Konuganti and [3]Dr. Ashwini s

Department of Periodontics, Faculty of Dental Science, Ramaiah University of Applied Sciences, Bengaluru-560054

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A B S T R A C T

Introduction: Osteocytes are naturally occurring modulators of bone metabolism that regulate the balance between osteoclastic and osteoblastic activity. Sclerostin is a marker of mature osteocytes and affects bone metabolism by inhibiting osteoblast differentiation .There are limited studies in the literature which have evaluated the levels of bone metabolism marker Sclerostin in the GCF .So the aim of the study was to estimate the level of Sclerostin in GCF of patients and find its association with periodontal status

Material and Methods: 82 patients, 41 of whom were healthy and 41 of whom had chronic periodontitis were recruited. GCF samples were collected from each subject and analysed for Sclerostin levels by ELISA

Results: Gingival Index mean scores were 0.87 in group 1 and 1.35 in group 2 and this difference was statistically significant. The Mean Sulcus depth was found to be 2.95 mm in Group 1 while mean PPD was found to be 6.85 mm in Group 2, this carried statistical significance. Sclerostin levels in GCF of the two groups were 0.46 pg/ml and 0.92 pg/ml respectively, the difference was highly statistically significant (p=0.004)

Conclusion: The results of the present study suggest that there is a statistically significant increase in GCF Sclerostin levels in chronic periodontitis patients as compared to healthy subjects. It also correlated with clinical parameters Gingival Index, Probing pocket depth and Clinical attachment loss. Sclerostin could be a feasible biomarker for assessment of periodontal health status of patients

INTRODUCTION

Periodontitis is described as destruction of periodontal tissue as a result of complex host response to polymicrobial infections and subsequent introduction of inflammatory mediators, it is characterized by the loss of periodontal ligament (PDL) and the supporting alveolar bone. Bone remodeling, coordinated with the processes of bone formation by osteoblasts and bone resorption by osteoclasts, maintains bone growth and repair during lifetime. Host inflammatory mediators contain an array of pro-inflammatory mediators which can induce and activate lytic enzymes and induce osteoclastogenesis; while anti-inflammatory mediators which counteract and attenuate disease progression [1].

Bacterial products in biofilm stimulate the inflammatory process and lead to the release of various cytokines and enzymes with tissue breakdown capacity within the body fluids. Cytokines are simple proteins which have an important role in the initiation and progression of inflammatory and immune responses. They act in acute-phase response, which is a primary defence reaction and therefore protects the body against bacterial toxins. Cytokines regulate the synthesis of acute-phase proteins. Acute-phase response occurs in the innate host response to injuries, infections, or ischemic necrosis by releasing various acute-phase proteins. The levels of various acute-phase proteins seen to be increased in periodontal disease in both gingival crevicular fluid (GCF), saliva and serum. Therefore, acute-phase proteins can be susceptibility markers in relation to inflammatory status [2, 3].

Evidence suggests that the estimation of inflammatory biomarker levels in various body fluids is valuable to predict and define the inflammatory activity; it is also proven that the study of saliva and Gingival crevicular fluid (GCF) is a valuable guide for the conclusion of current periodontal condition [4,5].

In studies evaluating the pathogenesis of periodontal disease, it is paramount if the immunological and biochemical marker within saliva or Gingival crevicular fluid mirror the amount of ongoing destruction of periodontal tissue and help forecast the progression of future periodontal disease. Being an exudate, the gingival crevicular fluid could be collected from the

Corresponding author:* **Dr. Dheeraj B R
Department of Periodontics, Faculty of Dental Science, Ramaiah University of Applied Sciences, Bengaluru-560054

periodontal pocket, and can be regarded as an encouraging medium to ascertain the periodontal disease activity. Among more than 50 various vital constituents in Gingival Crevicular Fluid analyzed presently, majority of the vital constituents essentially are part of soft-tissue inflammation events than being specific to alveolar bone destruction [6].

Osteocytes are naturally occurring modulators of bone metabolism that regulate the balance between osteoclastic and osteoblastic activity. Sclerostin is a marker of mature osteocytes and affects bone metabolism by inhibiting osteoblast differentiation [7]. It is believed to act by promoting osteoclast formation via a RANKL-dependent pathway as well as by interacting with osteoblasts [8]. At the molecular level, osteocytes regulate bone homeostasis through at least three key molecules: sclerostin, OPG, and RANKL .Sclerostin is a mature osteocyte marker which is produced by osteocytes, similar to RANKL and OPG.[9] Its expression, which suppresses osteoblastogenesis and reduces the viability of osteoblasts and osteocytes, leads to unbalanced bone turnover in favor of bone resorption.

As a bone metabolism marker Sclerostin inhibits Wnt- B (Wingless integrated) catenin signalization pathway, which is known to stimulate bone morphogenic proteins and cause formation of bone [10]. A deficiency of sclerostin leads to sclerosteosis and van Buchem disease, characterized by high bone mass [11]. Sclerostin (SOST) is known to have a negative impact on bone mass and differentiation of osteoblast and could cause an inhibition of bone formation [12].

Moreover, osteocyte cell cultures showed a catabolic action on bone formation upon being treated with recombinant Sclerostin, this effect was dose-dependent [13]. Also, in animal model, upon deleting the gene coding for sclerostin with the use of monoclonal antibodies, periodontal ligament and bone defects were significantly restored [14].Circulating levels of sclerostin are associated with porosity of cortical bone, which means that changes sclerostin plays a major role in defining cortical structure of bone. [15]

There are limited studies in the literature which have evaluated the levels of bone metabolism marker Sclerostin in the GCF. So the aim of the study is to compare the GCF levels of Sclerostin in healthy individuals and individuals with chronic periodontitis.

MATERIALS AND METHODS

Eighty two patients were recruited from the outpatients, Department of Periodontology, Faculty of Dental Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India. The ethical clearance for the present study was obtained from the Ethical committee of the institution. Healthy subjects with ≥ 20 teeth, subjects with chronic periodontitis having PPD ≥ 5 mm, CAL ≥ 3mm, bone loss > 30% of sites were included and subjects having less than 20 teeth, Subjects suffering from systemic and immunologic diseases and infections, Pregnant and lactating women, former/ current smokers; those who received antibiotics within the previous 3 months; those who underwent treatment for periodontal disease within the last 6 months were excluded from the study. For the 82 patients who were selected based on inclusion and exclusion criteria, the Gingival Crevicular fluid (GCF) from the sulcus of tooth most representative of the periodontal disease were collected and stored at – 80˚ C. At the same time Clinical Parameters such as Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque Index (PI, Silness and Loe, 1964), Gingival index

(GI, Loe and Silness- 1963) were also recorded. Levels of Sclerostin was assessed in the stored GCF samples at the end of the study using ELISA which was performed according to manufacturer's Instructions and the individual values were recorded for every patient. Probing pocket depth was noted from the gingival margin to the base of the pocket at 6 sites and clinical attachment level was noted from CEJ to the base of the pocket using UNC-15 Periodontal Probe.

GCF was collected from the sulcus around the tooth which exhibit the deepest pocket (most representative) by placing a capillary tube parallel to the tooth surface so as to collect 5 microliters of GCF from the sulcus after proper isolation using cotton rolls. The Samples were then transferred to the vials and mixed with 0.5cc of Phosphate buffer saline to attain a neutral pH. The same was then stored at -75^{o} C until all the samples were collected.

Sclerostin analysis

The 96-wells commercially purchased ELISA kit for Sclerostin (Elabscience co, USA) was used to analyse its concentration in GCF. The kit used a double-antibody Sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the level of Sclerostin in samples. The ELISA was run according to manufacturer's instructions. The O.D absorbance was read at 450 nm in microplate reader immediately after adding the stop solution.

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0.2013. Armonk, NY: IBM Corp. was used to perform statistical analyses. Pearson correlation test used to estimate the relation between Sclerostin in GCF & Periodontal parameters. Multiple linear regression analysis was performed to estimate the impact of periodontal parameters on GCF Sclerostin. The level of significance [P-Value] was set at $P<0.01$

RESULTS

41 Study subjects in the Gingivitis group were within the age range of 37- 53 years with a Mean age of 45.7 years and a Standard Deviation of 4.5. The 41 subjects designated to the chronic periodontitis group were between 39- 68 years of age with a mean age of 47.5 years, the standard deviation here was 11.6. There were no statistically significant correlation between Age and Periodontal disease. 47 among the 82 patients were males that attributed to 57% of the total subjects, and 35 were females which attributed to 43% of the study population. Here the Healthy group consisted of 24 males who formed 58.5% of the group and 17 were females who attributed to 41.5% of the Healthy group. In the Chronic periodontitis group 23 were males and constituted 56.1% of the group and 18 were females who formed 43.9% of the chronic periodontitis group.

There were no statistically significant correlation between Gender and Periodontal disease.

Clinical Parameters

The Plaque Index (PI) mean was found to be 1.00 in Group 1 (Healthy group) with a standard deviation of 0.66 while it was 1.11 in Group 2 (Chronic periodontitis group) with a standard deviation of 0.69. The mean difference was -0.11. Plaque Index between the two groups had no statistically significant difference as the p value was 0.52.Gingival Index (GI) mean was found to be 0.87 in Group 1 with a standard deviation of 0.55 and it was 1.35 in Group 2 with a standard deviation of 0.50, the mean difference hence was of -0.48 and the Z value was -3.944.Gingival

Index scores were found to have statistically significant difference with a p value of p< 0.001.(Graph-1)

The Mean Sulcus depth was found to be 2.95 mm in Group 1 with a standard deviation of 0.86 while mean Probing Pocket depth (PPD) was found to be 6.85 mm in Group 2 with a standard deviation of 1.06. The mean difference between the two groups was found to be -3.90 and the Z value was -7.903. This difference was found to be highly statistically significant with a P value of p< 0.001. (Graph-2)

By virtue there was no Clinical attachment loss in Group 1, hence the standard deviation remained 0. A mean loss of 5.71 mm was found in Group 2 with a standard deviation 1.72. The mean difference was -5.71 mm and the Z value was -8.354.Clinical Attachment Loss was highly statistically significant with a P value of p< 0.001.

Biochemical Parameters

Sclerostin levels in GCF of the two groups (Group 1 and 2) were compared using Mann Whitney test. For Group 1 the mean Sclerostin level was 0.46 pg/ml and standard deviation was 0.50. For Group 2 mean Sclerostin levels were 0.92 pg/ml and standard deviation was 0.84.The difference in mean Sclerostin levels between the groups was found to be 0.46 pg/ml with a Z value of -2.894. Sclerostin levels between the two groups were highly statistically significant with a P value of p=0.004.(Table-1)

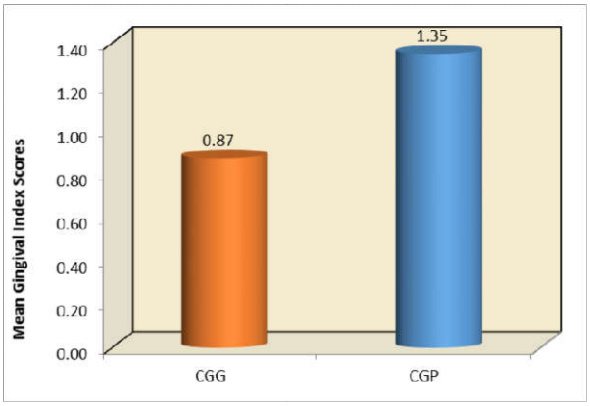
Correlation between clinical and biochemical parameters

Relationship between Sclerostin level of both the groups (1 and 2) and clinical parameters in each group were assessed using Spearman's correlation test. In terms of Plaque Index, the correlation coefficient (rho) between Plaque Index and GCF Sclerostin levels was found to be 0.37 hence a weak correlation could be found and it was not statistically significant as the P value was

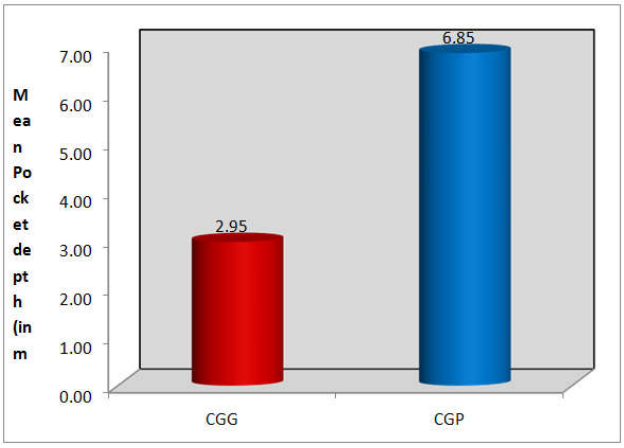
0.78. The correlation coefficient (rho) between GCF Sclerostin levels and Plaque Index in Group 2 was -0.07 hence a very weak negative correlation was found and this was statistically significant with a P value of 0.005.

With Gingival Index, the correlation with GCF Sclerostin had a correlation coefficient (rho) of -0.08 hence a very weak negative correlation was found but it was also not statistically significant as the P value was 0.42. Within Group 2 the correlation coefficient was found to be 0.13, hence a very weak negative correlation was found and this was not statistically significant as the P value was 0.60.

In terms of Probing pocket depth the correlation coefficient (rho) of -0.10 was found, hence a strong correlation could be interpreted but it was also not statistically significant as the P value was 0.81. In Group 2 the correlation coefficient was found to be -0.67, hence a strong negative correlation was found and this was highly statistically significant as the P value was 0.01. Pertaining to Clinical attachment loss applicable to Group 2 the correlation coefficient with Sclerostin was found to be -0.46, hence a moderate negative correlation was found and this was also highly statistically significant as the P value was 0.03.(Graph-3 & Graph-4)



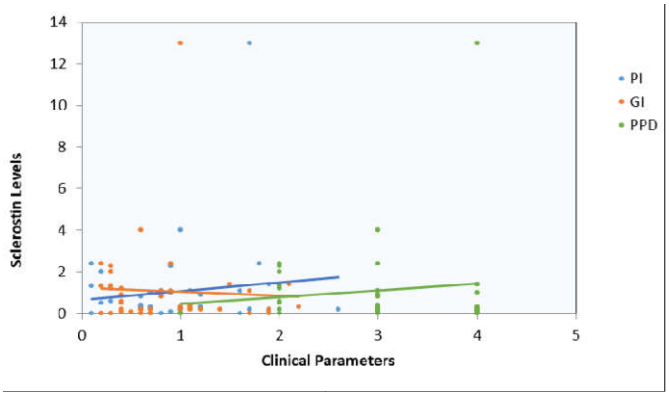
Graph 1 Comparison of mean Gingival Index Scores between 2 groups



Graph 2 Comparison of mean Pocket depth between 2 groups

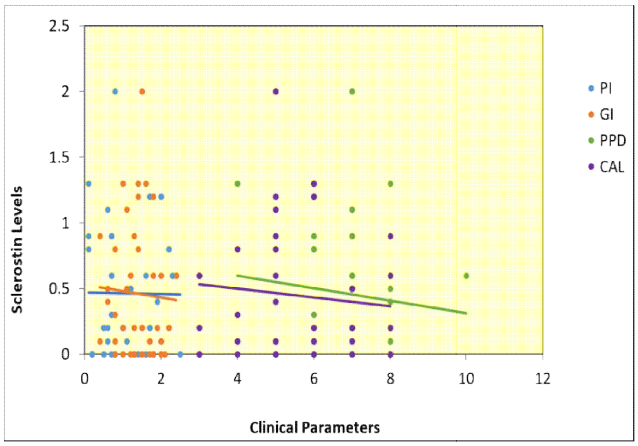
Table 1 Comparison of mean Sclerostin levels between 2 groups

Variable	Group	N	Mean	Standard Deviation	Mean Difference	Z	P-Value
Sclerostin	CGG	41	0.46	0.84	0.46	-2.894	0.004*
	CGP	41	0.92	0.50			



Graph 3 Scatterplot depicting the relationship b/w clinical parameters and Sclerostin levels in

Healthy Group



Graph 4 Scatterplot depicting the relationship b/w clinical parameters and Sclerostin levels in

CGP Group

DISCUSSION

The turnover of bone, which encompasses the coordination of bone deposition by the osteoblasts and resorption by osteoclasts, leads to skeletal repair and growth maintenance throughout life. Control of bone metabolism contains complex processes that involve multiple signal transduction systems [16]. Having a clear understanding of the coupling mechanism between the formation of bone and its resorption is tantamount for understanding and management of periodontal disease.

Lip polysaccharides and endotoxins secreted by bacteria with the progress of periodontal disease lead to an increase in the inflammatory state, this in turn causes reduction in growth factor levels and increase in secretion of interleukins and inflammatory biomarkers which are secreted primarily into the plasma, traces of which are found in gingival crevicular fluid and other body fluids. These biological fluids can therefore be utilised to detect bone biomarker levels. Evaluation of biomarkers in tissue fluids have considerable shortcomings. Serum analysis is complex and invasive as drawing of blood is involved, saliva has higher chances of contamination and has poor levels of these biomarkers [17]. Sclerostin is one such biomarker which impacts bone deposition negatively by antagonizing Wingless Integrated (Wnt) signalling pathway that modulates osteoblastogenesis. Sclerostin suppression has been known to cause a hike in bone deposition, volume, mineral density and strength in animal models [18].The present study was conducted with an objective to evaluate level of newer bone metabolism marker Sclerostin in Gingival crevicular fluid and to correlate the same with the periodontal status.

The results of the present study revealed that SOST levels in Gingival crevicular fluid is increased in Chronic periodontitis, this finding is in accordance with the findings of a study where GCF Sclerostin and RANKL/OPG ratio was found to be up-regulated in the gingival crevicular fluid samples of chronic periodontitis patients, it was reported that a spike in gingival crevicular fluid Sclerostin levels had a strong positive correlation with probing pocket depth and Clinical attachment loss[19]. While the study showed no change in the RANKL/OPG ratios after Nonsurgical periodontal therapy, it showed a reduction in SOST levels post treatment and the authors speculated that SOST may be involved in alveolar bone loss and its assessment could be useful to monitor response to periodontal treatment.

A study was where they assessed several bone biomarkers in the Peri-implant sulcular fluid so as to identify potential prognostic markers of peri-implantitis. They found that increased RANK, sRANKL, OPG and sclerostin in PISF were associated with peri-implantitis. OPG and sclerostin levels chiefly showed positive association with all clinical parameters except with Plaque Index [20]. These findings are in agreement with the present study where moderately strong correlation was found between GCF SOST levels and clinical attachment loss and strong correlation was found between SOST and probing pocket depth, however a very weak correlation was found between SOST and Plaque index.

In a study authors studied the gingival biopsies and serum samples of subjects with and without Chronic periodontitis for mRNA and protein samples of Sclerostin and the authors concluded that greater circulating levels of SOST were encountered and also its increased presence in the periodontal tissues of chronic periodontitis subjects were found, suggesting a possible role of these molecules on periodontal tissues [21]. These findings are in conformance with the present study where an increased association could be seen between GCF Sclerostin levels and extent of periodontal destruction in the form of loss of clinical attachment and probing pocket depth formation.

In the present study, the findings suggest that increased levels of GCF Sclerostin are associated with bone loss and increased probing pocket depth suggestive of an increase in periodontal destruction that could be associated with an increase in SOST levels, this finding however is contradicted according to an in-vitro study conducted where human and animal model teeth specimen when subjected to mineralization treatment, increasing levels of sclerostin protein could be verified. [22]

In a more recent study who studied both GCF and PISF among healthy subjects and Chronic periodontitis/ Peri- implantitis subjects the authors found positive correlations between Sclerostin levels in the sulcular fluid with increase in probing pocket depth around both periodontally compromised teeth and implants as compared to healthy ones.[23] These findings are in accordance with the present study where strong positive correlations were found between GCF Sclerostin levels and clinical attachment loss.

In a study where gingival samples were analysed for differential expression profile of Sclerostin among other bone biomarkers between healthy and Severe Chronic periodontitis subjects, the authors concluded that a 3-4 fold increase in Sclerostin expression in the biopsy samples was noted among severe chronic periodontitis as compared to healthy subjects[24]. This is in agreement with the present study where there is a 2 fold increase in sclerostin expression in the gingival crevicular fluid of chronic periodontitis subjects as compared to healthy subjects.

In light of the above findings, within the limitations of this study it is reasonable to implicate that Sclerostin could be a potential biomarker for identification of periodontal disease status.

CONCLUSION

Sclerostin is a pro- inflammatory bone biomarker discovered recently and is currently subjected to extensive research. The assessment of Sclerostin in GCF among healthy and chronic periodontitis groups was done in the present study. The results revealed a statistically significant increase in GCF levels of Sclerostin in Chronic periodontitis cases compared to healthy individuals. A statistically significant correlation was also noticed between GCF levels of Sclerostin with that of clinical parameters that included gingival index, probing pocket depth and Clinical attachment loss. Hence, Sclerostin could be a feasible biomarker to assess periodontal health status of a patient.

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