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ORIGINAL ARTICALE

OSTEOPROTEGERIN IN PERIODONTAL DISEASE A CROSS-SECTIONAL STUDY

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ABSTRACT

Background: Under both normal and pathological conditions, the RANKL-OPG (Receptor Activator of NF-κB Ligand-Osteoprotegerin) system is an essential regulator of osteoclastogenesis and bone resorption. In order to evaluate OPG's potential as a diagnostic biomarker for periodontal disease, this study aimed to assess osteoprotegerin levels in the gingival crevicular fluid of patients with chronic periodontitis, including those with diabetes mellitus, smokers, and systemically healthy individuals.

Materials and Methods: The study included 60 subjects of age group above 18 years and distributed into 3 groups. GCF was collected and samples were analyzed for OPG concentration using ELISA assay.

Results: No significant difference in PI and GI between 3 groups. PPD shows significant difference between smokers and diabetes with periodontitis. Concentration of OPG is lower in diabetes milletus and smokers when compared to healthy periodontitis

Conclusions: OPG is the marker for bone resorption and can show negative correlation with the clinical parameters like PPD, CAL, thus its increase in levels would denote lesser bone resorption.

Keywords: Osteoprotegerin (OPG), Periodontal disease, Diabetes mellitus, Gingival crevicular fluid, ELISA, RANKL, Probing pocket depth, Clinical attachment loss, Alveolar bone resorption, Cytokines, Bone remodeling, Risk factors.

INTRODUCTION

Periodontitis being a longstanding inflammatory condition affecting the supporting tissues of teeth. Although plaque is the primary cause, periodontal disease is worsened by the factors like genetics and environment. Periodontitis is marked by periodontal pocket formation and loss of alveolar bone.

Under normal health conditions, host immune system controls the presence of bacteria through various mechanisms. However, when the equilibrium in host response and subgingival biofilm is disrupted, it triggers

innate, inflammatory, and adaptive responses. These processes lead to the loss of alveolar bone and periodontal tissue support.

The activation of B and T lymphocytes, significantly affects bone loss in periodontitis. These cells are the main sources RANKL activator during periodontal inflammation.

Th1 cells increase interferon-γ levels, which is a crucial factor in the initiation and progression of periodontitis. Th1 cells release cytokines, TNF-α and IL-1β. These cytokines enhance production of chemokines, which facilitates neutrophil activation and increases MMP release.

Both the soluble and membrane-bound forms of RANKL are produced by activated T cells and B cells. RANKL, a cytokine belonging to the TNF family, stimulates osteoclast differentiation and activation, resulting in resorption of bone.

Important molecules in controlling bone metabolism include RANKL, RANK and OPG.

RANKL by interacting with its receptor RANK, promotes the recruitment of osteoclasts and its precursors onto the surface of the bone.

Osteoprotegerin (OPG) is capable of blocking RANKL's biological functions through competitive inhibition.

High alveolar bone resorption in periodontitis is indicated by an elevated RANKL/OPG ratio.

Studies by Belibasakis et.al have shown that increase RANKL/OPG ratio have been seen in the patients who are smokers.1 Thus, smoking being a risk factor for causing periodontal disease. Apart from various other mechanisms, the effect of vasoconstriction, changes in inflammatory reaction leading to periodontal destruction. This increases the levels of RANKL and decreases the levels of OPG.

Diabetes being a chronic metabolic disorder with higher blood glucose levels, or hyperglycemia, as well as abnormalities in protein, lipid, and carbohydrate metabolism brought on by defect in insulin synthesis or its action or both.

A 2-way relationship exists between diabetes mellitus and chronic periodontitis. Diabetic individuals are 2-3 times more likely to develop periodontitis compared to non-diabetic individuals, and glycemic control is a crucial factor in assessing this risk. The important risk factors for periodontitis include and smoking and diabetes.

Periodontal tissues get accumulated with AGEs and the interaction with their receptor (RAGE, primarily found on macrophages) trigger local immune and inflammatory responses. This leads to increased levels of proinflammatory mediators like IL-6, IL-1β and TNF-α. A disturbance in the RANKL, OPG axis favors bone resorption.

AIMS OF STUDY

To evaluate and co-relate the amounts of osteoprotegerin (OPG) in the crevicular fluid of atients with periodontitis, smokers and diabetic milletus patients.

METHOD OF STUDY

The present clinico-biochemical research study was designed and performed at Department of Periodontics. The study included 60 subjects of age group above 18 years and distributed into 3 groups (**Group -A chronic periodontitis who are systemically healthy), Group -B individuals with periodontitis and diabetes, Group-C smoker patients with periodontitis)** based on inclusion criteria. (i)Patients who are systemically healthy with periodontitis. (ii)Patients who have type 2 controlled diabetes mellitus with periodontitis. (iii) Smokers with periodontitis;(iv) Patients with periodontitis who have minimum two teeth in each quadrant with probing depth ≥5mm;(v) Patients with clinical attachment loss ≥ 3mm; (vi)Patients with evidence of bone loss seen on radiological assessment.

The following were the exclusion criteria:-(i) Uncooperative patients;(ii) Patients who have received periodontal therapy within the last three months. ;(iii) Systemic diseases other than diabetes mellitus;(iv) Use of antibiotics in past 3 months;(v) patients with alcohol and drug consumption.

All subjects in the study met the study criteria and the study design received approval from the Institutional Ethical Committee, and written consent was signed by all the participants.

Clinical examination

A detailed case history was recorded including the Gingival Index (Loe.H and Silness.J, 1963); the Plaque Index (Silness.J and Loe.H, 1964); the UNC-15 periodontal probe for measuring the Probing Pocket Depth (PPD); and Clinical Attachment Loss (CAL).

GCF Sampling

Collection of GCF samples was done using micro pipettes ranging 1 to 5 µl. Micropipettes contaminated with saliva and blood were thrown away. As soon as possible, the collected GCF was placed in aliquots and kept cold until the assay was scheduled.

Measurement of OPG in GCF samples:

GCF samples were analyzed for OPG concentration using ELISA assay. The GCF samples, proteins were quantified using an OPG ELISA development kit. For every assay, the manufacturer's instructions were followed and the OPG concentration is expressed in picograms per milliliter (pg /ml).

Statistical analysis:

All the data obtained from the clinical examination and OPG measurements using ELISA were tabulated against respective subjects using excel sheets, descriptive

analysis was done using mean and standard deviation for which inferential statistics was applied that includes one-way ANOVA with Tukey HSD analysis. The results thus obtained is shown below in the following tables.

RESULTS

Table 1 shows the mean, standard deviation (SD) and 95% confidence intervals (CI) for clinical parameters across the chronic periodontitis, diabetes mellitus with periodontitis and smokers with periodontitis groups. Plaque index (PI) and Gingival index (GI) scores the highest in the smoker with periodontitis group (mean I: 1.17; SD: 0.22; mean GI: 1.05; SD: 0.47) and were slightly lower in diabetes mellitus with periodontitis (mean PI: 0.98; SD: 0.23; mean GI: 1.15; SD: 0.39) and chronic periodontitis (mean PI: 0.96; SD: 0.22; mean GI: 1.20; SD: 0.38). Probing pocket depth was significantly greater in smokers with periodontitis mean (5.78; SD 0.47) and clinical attachment level (CAL) with higher values in smokers (mean 4.19; SD 0.48) than the diabetes with periodontitis and chronic periodontitis. Table 2 shows the mean, standard deviation (SD) and 95% confidence intervals (CI) for Osteoprotegerin (OPG) concentration in gingival crevicular fluid in the respective groups. OPG concentration is highest in chronic periodontitis group (mean 91.38 pg/mL; SD 4.15), significantly lower in diabetes mellitus with periodontitis (mean 26.37 pg/mL; SD 1.78), and in smokers with periodontitis (mean 28.44 pg/mL; SD 6.72). Table 3 shows the comparison of OPG between the groups were the mean OPG for diabetes mellitus with periodontitis was significantly lower than chronic periodontitis ($p < 0.001$). Furthermore, smokers with periodontitis showed significantly lower OPG than the chronic periodontitis ($p < 0.001$). However, there was no significant difference in OPG between diabetes milletus with periodontitis and smokers with periodontitis ($p = 0.349$).

Table 1. Mean, standard deviation (SD) values for plaque index (PI), gingival index (GI), pocket probing depth (PPD), clinical attachment level (CAL) in 3 groups

Clinical parameters	Chronic periodontitis (n=20)				Diabetes milletus (n=20)				Smokers (n=20)			
	Mean	95% CI mean		SD	Mean	95% CI mean		SD	Mean	95% CI mean		SD
		upper	Lower			upper	lower			upper	lower	
PI	0.960	1.062	0.858	0.219	0.980	1.085	0.875	0.225	1.170	1.354	0.987	1.700
GI	1.204	1.383	1.026	0.381	1.146	1.374	0.918	0.486	1.047	1.217	0.877	1.833
PPD	5.191	5.358	5.025	0.355	5.182	5.398	4.967	0.460	5.779	6.077	5.480	6.860
CAL	3.647	3.871	3.422	0.480	3.544	3.738	3.350	0.414	4.194	4.553	3.835	5.610

Table 2. Mean, standard deviation (SD) for the Osteoprotegerin (OPG) measurements from the GCF samples of respective group.

Variable	Chronic periodontitis (n=20)				Diabetes milletus (n=20)				Smokers (n=20)			
	Mean	95% CI mean		SD	Mean	95% CI mean		SD	Mean	95% CI mean		SD
		Upper	Lower			upper	lower			upper	lower	
OPG (pg/ml).	91.380	93.322	89.438	4.150	26.370	27.202	25.538	1.777	28.435	31.580	25.290	6.72

Table 3. Osteoprotegerin (OPG) values compared in all 3 groups. Here the comparison is done by one-way ANOVA and Tukey HSD analysis was applied.

comparison	Mean Difference	SE	T	p value
Group C - Group A	-62.945	1.478	-42.584	<0.001
Group C - Group B	2.065	1.478	1.397	0.349
Group A - Group B	65.010	1.478	43.982	<.001

DISCUSSION

In this study, we analyzed GCF concentrations in healthy individuals, those with diabetes mellitus, and smokers with periodontitis, following assessment of their clinical parameters. Plaque and Gingival indices showed no significance with mean value being 0.960 in Group A,0.980 in Group B,1.170 in Group C as shown in Table 1. Patino Marin [2] and Mazhari et al. [3] found no significant difference in plaque index (PI) between diabetics and non-diabetics. In contrast, Siudikiene et al.[4] demonstrated that the PI in diabetics was significantly higher than in non-diabetics. Buduneli et al.[5] found no significant difference in the mean plaque index (PI) between smoking & non-smoking individuals when oral hygiene levels were comparable.[2] In contrast, Bergstrom et al. (1991)[6] proposed that tobacco has a direct impact on periodontal health, regardless of plaque infection. Thus, systemic influences on the plaque deposition would have much lesser effect than the effect of plaque control measures that have direct effect on the quality and quantity of the plaque deposition.

Probing pocket depth shows significant difference between smokers with periodontitis and diabetes with periodontitis (p value < 0.05.) mean PPD values 5.779 in smokers and 5.182 in diabetes group. On the other hand, there is no significant difference in PPD between healthy (PPD 5.191) and diabetes mellitus subjects (PPD 5.182) P value > 0.05 (table 1). The inference of these finding would be that smoking might cause more destruction of the periodontium when compared to the systemic effects of increased glycemic control.

Likewise, because the probing pocket depth increases would also influence the clinical attachment levels directly, thus CAL when compared between three groups shows significant difference between smokers with periodontitis and diabetes milletus with periodontitis group with CAL values 4.194 and 3.544 respectively P value < 0.05. Significant difference was not found in CAL among group A and group B, with values 3.647 and 3.44, respectively (p > 0.05) (Table 1).

In our present study mean concentration of OPG is lower in group B (diabetes milletus and periodontitis) at 26.370 pg/µl and group C (smokers and periodontitis) at 28.435pg/µl than group A (healthy periodontitis) which was at 91.380pg/µl as seen in table 2. Xin-Ran Xu et al.,[7] compared gingival crevicular fluid (GCF) and serum inflammatory mediators in patients with periodontitis, type 2 diabetes mellitus (T2DM) and healthy individuals. The research found notable high levels of Interleukin-17 and the RANKL/OPG ratio in the diabetes-associated periodontitis (DC) group compared to both the periodontitis (CP) group and the healthy individuals, elevated levels also found in the CP group compared to the healthy group. Although precise concentrations of RANKL and OPG in GCF varied across different studies, a significant hike in RANKL/OPG ratio in periodontitis cases compared to healthy controls was consistently reported, which aligns with the findings of our study.

Bostanci et al.[8] observed a correlation among RANKL/OPG ratio in GCF and probing depth (PD) in periodontitis patients. Similarly, Tabari et al. found positive correlations between attachment loss (CAL) and plaque (PI) with salivary levels of soluble RANKL (sRANKL), the sRANKL/OPG ratio. Our study results are similar to that reported by Bostanci and Tabari et al.

Our study was conducted to know variation in OPG level which is the chemical mediator, that is released from the resident cells in the connective tissue of the periodontium. Cells like fibroblasts, chondroblasts and osteoblasts have been shown to release this cytokine that acts by acting as a decoy for the RANK receptor present on the cells of the haemopoietic linage that acts as a precursors to the osteoclasts, the cells that has these RANK receptors on their cell membrane when activated by the ligand RANKL can lead to their differentiation to form themselves into bone resorbing cells osteoclasts, the action of RANK can not only lead to differentiation but also their further proliferation and their function by secreting various enzymes which are important in the resorption of the bone. Thus, a protective mechanism exists wherein the OPG will selectively bind on these RANK receptors giving no further space for the attachment of RANKL and its functions.

Hence, we tested the presence of this chemical mediator OPG and their relation to the risk factors that are associated strongly with periodontal destruction i.e.; smoking and diabetes milletus levels hypothesizing that their actions will have an effect on the levels of OPG and GCF was collected using micropipettes. As seen in tables 1,2,3 periodontal destruction is significantly more in Group C when compared to Group B patients but significantly lesser in the Group A subjects. Likewise, when biochemical analysis was done to check the levels of OPG it showed significantly higher in Groups C and B subjects than Group A subjects, thus denoting the effects of risk factors that can cause periodontal destruction corelating the significant destruction of periodontal tissue with the decrease in the levels of OPG.

If we would have chosen a larger sample size or addition of more risk factors as groups or a longitudinal study design, a cohort study design would have got a better robust result and thus we consider the above listed as the limitations of this study.

CONCLUSION

We would like to say that OPG is the marker for bone resorption and can show negative corelation with the clinical parameters like PPD, CAL, thus its increase in

levels would denote lesser bone resorption. Also, the risk factors that cause increased periodontal destruction like smoking and diabetes could be due to the decrease in levels of osteoprotegrin.

DECLARATIONS

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REFERENCES

1. Belibasakis GN, Bostanci N. The RANKL-OPG system in clinical periodontology. J Clin Periodontol. 2012 Mar;39(3):239-48.
2)Patiño Marín N, Loyola Rodríguez JP, Medina Solis CE, Pontigo Loyola AP, Reyes Macías JF, Ortega Rosado JC, Aradillas García C. Caries, periodontal disease and tooth loss in patients with diabetes mellitus types 1 and 2. Acta Odontol Latinoam. 2008;21(2):127-33.
3) Mazhari F, Khordi Mood M, Esmaeili H, Tootooni H. Prevalence of periodontal diseases and oral health status, in children with type I diabetes in Khorasan, Iran in 2002. *Journal of Islam Dental Association.* 2007;19(2):29–34.
4)Siudikiene J, Machiulskiene V, Nyvad B, Tenovuo J, Nedzelskiene I. Dental caries increments and related factors in children with type 1 diabetes mellitus. Caries Research. 2008;42(5):354–62.
5)Buduneli N, Buduneli E, Kütükçüler N. Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and non-smoking patients with chronic periodontitis during initial periodontal treatment. J Periodontol. 2009.
6)Bergström J, Eliasson S, Preber H. Cigarette Smoking and Periodontal Bone Loss. Journal of Periodontology. 1991 Apr;62(4):242–6.
7)Xu XR, Xu JL, He L, Wang XE, Lu HY, Meng HX. Comparison of the inflammatory states of serum and gingival crevicular fluid in periodontitis patients with or without type 2 diabetes mellitus. J Dent Sci. 2023 Jul;18(3):1125-1133.
8)Bostanci N, Ilgenli T, Emingil G, Afacan B, Han B, Töz H, Atilla G, Hughes FJ, Belibasakis GN. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. J Clin Periodontol. 2007 May;34(5):370-6.
9) Bunaes DF, Mustafa M, Mohamed HG, Lie SA, Leknes KN. The effect of smoking on inflammatory and bone remodeling markers in gingival crevicular fluid and subgingival microbiota following periodontal therapy. J Periodontal Res. 2017 Aug;52(4):713-724.
10) Tabari ZA, Azadmehr A, Tabrizi MAA, Hamissi J, Ghaedi FB. Salivary soluble receptor activator of nuclear factor kappa B ligand/osteoprotegerin ratio in periodontal disease and health. Journal of Periodontal & Implant Science. 2013;43(5):227.
11)Priscila Paganini Costa, Trevisan GL, Macedo GO, Daniela Bazan Palioto, Scombatti L, Grisi M, et al. Salivary Interleukin-6, Matrix Metalloproteinase-8, and Osteoprotegerin in Patients with Periodontitis and Diabetes. Journal of Periodontology. 2010 Mar 1;81(3):384–91.
12)Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation, Nature Reviews Immunology [Internet]. 2014 Dec 23;15(1):30-44.
13)Rochette L, Meloux A, Rigal E, Zeller M, Cottin Y, Vergely C. The Role of Osteoprotegerin and Its Ligands in Vascular Function. International Journal of Molecular Sciences [Internet]. 2019 Feb 6;20(3):705.
14)Hernández M, Baeza M, Contreras J, Sorsa T, Tervahartiala T, Valdés M, et al. MMP-8, trap-5, and OPG levels in GCF diagnostic potential to discriminate between healthy patients', mild and severe periodontitis sites. Biomolecules. 2020 Oct 30;10(11):1500.
15)Asif S, Ahmad B, Hamza SA, Taib H, Kassim NK, Zainuddin SL. Investigation of salivary RANKL and OPG levels in periodontitis patients at Hospital University Sains Malaysia. European Journal of Dentistry. 2021 Sept 27;16(01):173–8.