



Interleukin-33 and Osteoprotegerin Levels in Gingival Crevicular Fluid and Saliva in Chronic Periodontitis and Their Correlation to Diabetes Mellitus: A Cross-Sectional Study

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Abstract

Background: This study investigates the presence of interleukin (IL)-33 and osteoprotegerin (OPG) in saliva and gingival crevicular fluid (GCF) samples of patients with chronic periodontitis and whether or not they are correlated with diabetes mellitus. **Methods:** Sixty subjects were included in this study: 20 chronic periodontitis patients (CP), 20 diabetic chronic periodontitis patients (CPDM) and 20 systemically and periodontally healthy subjects. GCF and saliva samples were collected from all participants. Enzyme linked immunosorbent assay (ELISA) kits were used for assaying IL-33 and OPG levels. Analysis of variance was used to compare means of the CP and CP-DM groups with the control. Correlation analyses were performed to find the value of Spearman's correlation. A receiver operating characteristic (ROC) curve was constructed to determine the cut-off values of the markers to differentiate between the groups. Areas under the ROC curve (AUCs) were compared using z-statistic. **Results:** IL-33, in both GCF and saliva, was significantly higher in the CP and CP-DM groups compared to the control, and significantly higher in the CP-DM group compared to the CP group in saliva. On the other hand, the level of OPG in GCF and saliva was significantly lower in the CP and CP-DM groups compared to the control, but was of no significance when comparing the CP-DM and CP groups. **Conclusion:** IL-33 seems to play a role in the pathogenesis of periodontal disease, while OPG may have a protective function. Diabetes may affect and influence the expression of IL-33. Thus, they could be utilized as diagnostic biomarkers for chronic periodontitis either in saliva or GCF.

Keywords: Interleukin-33, osteoprotegerin, diabetes mellitus, chronic periodontitis, gingival crevicular fluid, saliva

Introduction

Periodontal diseases, which arise from the complicated interaction between bacterial insult and the host inflammatory-immune response against this insult, are governed by a network of cytokines that lead to the progressive destruction of the tooth supporting tissues and ends with tooth loss.^{1,2} Diabetes mellitus (DM) and chronic periodontitis are chronic disorders previously known to be biologically linked, with inflammation being the main feature of their pathogenesis.^{3,4} Diabetes is considered an important risk factor for periodontitis, thereby influencing the osteoclasts and osteoblasts of the

periodontium through several routes, such as by the over expression of inflammatory mediators and by increasing the receptor activator of nuclear factor kappa-B ligand (RANKL) / osteoprotegerin (OPG) ratio, advanced glycated end-products (AGEs), and reactive oxygen species (ROS) levels.⁴ OPG is structurally similar to receptor activator of nuclear factor kappa-B (RANK) and is therefore a soluble decoy receptor for RANKL. It is also called "bone protector" and is produced by many cells including osteoclasts. As RANKL binds to OPG instead of its original receptor RANK, the differentiation of osteoclasts is prevented leading to a subsequent decrease in bone loss.⁵

The RANKL/OPG ratio plays an important role in bone homeostasis where an increase in the RANKL/OPG ratio, occurring either by increased RANKL or decreased OPG, will increase bone loss and vice versa. In active sites of periodontal disease, the RANKL/OPG ratio was found to be increased correlating with disease activity.^{6,7}

The interleukin (IL)-1 family of cytokines plays a major role in stimulation and regulation of immune and inflammatory processes.² A newly discovered member of the IL-1 family is IL-33, also known as IL-1F11, which is expressed on various cells including activated macrophages, fibroblasts, epithelial cells, keratinocytes, endothelial cells, and dendritic cells. IL-33 serves as the ligand of the ST2 receptor which is a member of the Toll-like receptor/IL-1R superfamily. Also, IL-33 activates many cells including T-helper2 (Th2) cells, mast cells, basophils and eosinophils.⁸

IL-33 has a dual function by acting intracellularly as an anti-inflammatory cytokine and extracellularly as a proinflammatory cytokine. It stimulates the production of IL-4, IL-5, and IL-13 through Th2 cells, and also triggers the secretion of tumor necrosis factor- α (TNF- α), IL-6, and IL-1 β which are proinflammatory mediators.^{1,8}

IL-33 released by damaged cells acts as an alarm, especially in endothelial or epithelial cells exposed directly to environmental factors. Moreover, the survival, maturation, and adhesion of mast cells are facilitated by IL-33, which in turn recognize IL-33 and become activated to secrete histamine, leukotrienes, prostanoids, proteases, cytokines, and chemokines, thus recruiting neutrophils to the infection site and stimulating an inflammatory response. This suggests that the produced inflammatory mediators and IL-33 cause osteoclast activation by increasing RANKL expression and decreasing OPG production. Thus, IL-33 may have a role in alveolar bone destruction in periodontitis.^{9,10} IL-33 was previously studied with chronic periodontitis in GCF, saliva, and serum.^{8,11}

An ongoing relation exists between diabetes and chronic periodontitis, with the latter being the sixth complication of diabetes and is considered a risk factor.³ Concurrently, there is an increased risk of severity of periodontal disease in diabetic patients due to hyperglycemia, leading to an exaggerated inflammatory immune response.⁴

Consequently, this study investigates the effect of type 2 DM on chronic periodontitis by detecting the levels of IL-33 and OPG in gingival crevicular fluid (GCF) and saliva, which seem to have opposite roles in periodontal inflammation.

Materials and Methods

This study included a total of 60 subjects of the Egyptian population that were categorized into 20 patients presenting with chronic periodontitis (CP) (group I) according to the Armitage et al. 1999 classification,¹¹ 20 patients presenting with chronic periodontitis along with type 2 diabetes mellitus (CP-DM) (group II), and 20 systemically and periodontally healthy control subjects (group III). The study groups can be elaborated as follows:

Group I: CP which was comprised of 13 females and 7 males ranging between the ages of 32 and 48 years (mean of 38.2 ± 4.5)

Group II: CP-DM which was comprised of 13 females and 7 males ranging between the ages of 20 and 41 years (mean of 28.9 ± 6.3)

Group III: Control group which was comprised of 14 females and 6 males (mean age 34.9 ± 6.6) who had PD < 3 mm and no CAL, clinical inflammation, or bone loss

The protocol was approved by the Cairo University Research Ethics Committee, and their outpatient clinic was used for patient selection. Informed consent was obtained from the subjects prior to inclusion in the study. Complete records of the periodontal examination, diagnosis, treatment, and recommended follow-up were maintained.

Diabetic patients were recruited according to the American Diabetes Association¹² defining type 2 diabetes as untreated adult-onset hyperglycemia with a glycated hemoglobin (HbA1c) level of $\geq 6.5\%$ and normoglycemia defined as HbA1c of $\leq 6\%$. Exclusion criteria included any other systemic disease, pregnancy, antibiotic therapy, and long-term use of drugs that can cause gingival changes.

Clinical Parameters

Clinical parameters were recorded for six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) for the whole dentition except for the third molars if present. Parameters were recorded using William's periodontal probe and they included:

(a) gingival index,¹³ (b) plaque index,¹⁴ (c) probing depth (PD),¹⁵ and (d) clinical attachment loss (CAL).¹⁶ The inclusion criteria for chronic periodontitis patients was based on a clinical examination demonstrating the involvement of >30% of sites with a CAL \geq 5mm, and a PD >6mm. Treatment of patients was commenced on the following visit, after recording the clinical parameters, collecting the samples, and delivering oral hygiene instructions in the first appointment.

GCF Sample Collection

Upon removal of supragingival plaque, the required areas were isolated using cotton rolls after being washed with a water spray and dried with an air syringe. GCF was obtained using filter paper strips, which were inserted into the pocket or sulcus with the greatest depth until a slight resistance was felt and were left in place for 30 seconds. The strips were placed into sterile Eppendorf tubes. Paper strips contaminated with blood were excluded. All GCF samples were immediately stored at -80°C until subsequent analysis.

Salivary Sample Collection

Whole unstimulated saliva was collected using standard techniques as described by Navazesh.¹⁷ Subjects refrained from eating, drinking, chewing gum etc., for at least half an hour prior to the evaluation. Samples were collected by asking subjects to swallow first, tilt their head forward and expectorate saliva in a tube for five minutes without swallowing. Following collection, all samples were immediately stored at -80°C until assayed.

Quantitation of IL-33

Quantitative measurements of human IL-33 concentrations were determined using a Human IL-33 ELISA Kit.^a This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for IL-33 was pre-coated onto a microplate. Standards and samples were pipetted into the wells causing any IL-33 present to be bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-33 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and observed for color changes which would be

proportional to the amount of IL-33 bound in the initial step. Once the color development stopped, the intensity of the color was measured.

Quantitation of Osteoprotegerin

OPG was quantified using a Human Osteoprotegerin Instant ELISA Kit.^b An anti-human OPG polyclonal coating antibody was adsorbed onto microwells. Human OPG present in the sample or standard would then bind to antibodies adsorbed onto the microwells, and a biotin-conjugated polyclonal anti-human OPG antibody would bind to human osteoprotegerin captured by the first antibody. Streptavidin-HRP would then bind to the biotin conjugated anti-human OPG. Following incubation, unbound biotin conjugated anti-human OPG and Streptavidin-HRP were removed during a wash step, and a substrate solution reactive with HRP was added to the wells. The formed colored product would be proportional to the amount of soluble human OPG present in the sample. The reaction was then terminated through the addition of an acid, and absorbance was measured at 450 nm. A standard curve was prepared using seven human OPG standard dilutions and the human OPG sample concentration was determined.

Statistical Analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Age data showed normal (parametric) distribution while all other variables showed non-normal (non-parametric) distribution. Parametric data were presented as mean, standard deviation (SD), and 95% Confidence Interval (95% CI) values. Non-parametric data were presented as median, Inter-Quartile Range (IQR), mean, and standard deviation (SD) values.

For parametric data, one-way ANOVA was used to compare between the three groups. Bonferroni's post-hoc test was used for pair-wise comparisons when ANOVA test was significant. As for non-parametric data, Mann-Whitney U test was used for comparisons between the two experimental groups. Kruskal-Wallis test was used to compare between the three groups. Dunn's test was used for pair-wise comparisons. Spearman's correlation coefficient was used to determine significant correlations between markers and different variables.

^a Quantikine, R&D Systems, Inc. Minneapolis, USA, and Catalog Number D3300

^b BMS2021INST provided by eBioscience (Bender MedSystem GmbH, Vienna, Austria)

Qualitative data were presented as frequencies and percentages. Chi-square test was used for the comparisons.

Receiver operating characteristic (ROC) curve was constructed to determine the cut-off values of different markers for differentiation between the three groups. Areas under the ROC curve (AUCs) were compared using z-statistic.

The significance level was set at $P \leq 0.05$. Statistical analysis was performed using IBM SPSS Statistics Version 20 for Windows. ROC curve analysis was performed using MedCalc Version 11.3 for Windows (MedCalc Software bvba).

Sample Size Calculation

The power analysis was for one-way fixed effect Analysis of Variance (ANOVA) for comparison between the three groups. Calculation utilized IL-33 level as the primary outcome based upon the results of Sağlam et al. (2017). The effect size (f) was 24.3, using alpha (α) level of 0.05 (5%) and Beta (β) level of 0.10 (10%) i.e. power = 90%; the minimum estimated sample size was a total of 18 subjects (6 subjects per group). Oversampling was employed for the sake of reliable results. Sample size was calculated using IBM SPSS SamplePower (IBM® Corporation, NY, USA. SPSS®, Inc., an IBM Company), Release 3.0.1.

Results

There was no statistically significant difference between gender distributions in the three groups.

As regards to age, there was no statistically significant difference between the CP and control group; both showed significantly higher mean age values than the CP-DM group.

Figure 1. ROC curves of markers for differentiation between the CP and control groups

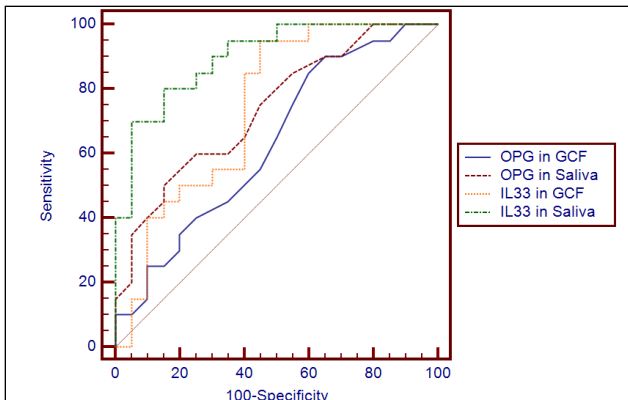
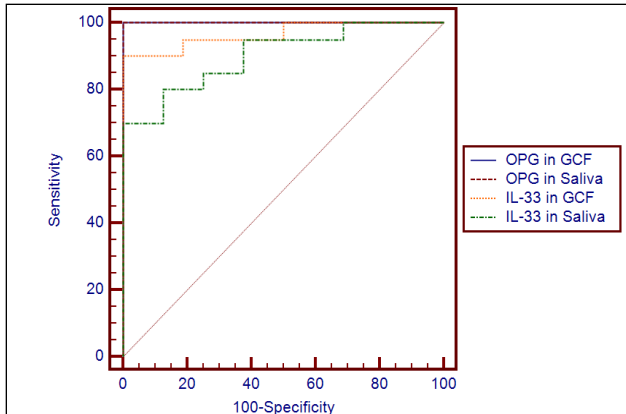


Figure 2. ROC curves of markers for differentiation between the CP and the CP-DM groups



The CP group showed a significantly higher median PI and GI than the CP-DM group. There was no statistically significant difference between the median PD for the two groups. The CP group showed a significantly lower median CAL than the CP-DM group.

Regarding OPG concentrations in GCF and saliva, the control group showed a significantly higher mean level than the other groups; there was no statistically significant difference between the CP and CP-DM groups.

As for IL-33 concentrations in GCF, there was no statistically significant difference between the CP and CP-DM groups; both showed a significantly higher mean IL-33 level than the control group.

Regarding IL-33 levels in saliva, the CP-DM group showed a significantly higher mean level than the other groups. The CP group showed a significantly lower mean value, and the control group showed the lowest mean level, which was of statistical significance (Table 1).

The ROC curve analysis of the markers for differentiation between the CP and control subjects showed cut-off values of 101, 70, 13.9 and 16.8 pg/ml for OPG in GCF, OPG in saliva, IL-33 in GCF, and IL-33 in saliva, respectively. OPG in GCF and saliva showed the highest diagnostic accuracy (100% for each marker). IL-33 in GCF showed a lower diagnostic accuracy of 94.4%, and IL-33 in saliva showed the lowest diagnostic accuracy of 83.3%. Pair-wise comparison of the AUCs for the markers showed a statistically insignificant difference (Table 2; Figure 1).

The ROC curve analysis of the markers for differentiation between CP-DM and control subjects showed cut-off values of 123, 77, 13.9 and 16.8 pg/ml for OPG in GCF, OPG in saliva, IL-33 in GCF, and IL-33 in saliva,

Table 1. Descriptive statistics and results of Kruskal-Wallis test for comparisons between markers’ levels in the three groups

	CP (n = 20)	CP-DM (n = 20)	Control (n = 20)	P-value
OPG in GCF (pg/ml)				
Median (IQR)	66.5 (60 – 71.5) B	69 (65.3 – 78.3) B	219.5 (185.3 – 233) A	<0.001*
Mean (SD)	67.7 (11.4)	74.4 (16.2)	212.4 (41.1)	
OPG in Saliva(pg/ml)				
Median (IQR)	59 (53 – 64.5) B	65.5 (59.3 – 69) B	134 (120.3 – 150.8) A	<0.001*
Mean (SD)	58.4 (7.0)	64.5 (6.5)	134.7 (18.1)	
IL-33 in GCF (pg/ml)				
Median (IQR)	22.8 (17.4 – 30.2) A	30.5 (27 – 36.8) A	10.8 (10 – 11.6) B	<0.001*
Mean (SD)	25.1 (10.1)	31.9 (6.5)	10.9 (1.4)	
IL-33 in Saliva (pg/ml)				
Median (IQR)	20.4 (15.8 – 25.1) B	32.5 (27 – 40) A	12.4 (11.2 – 15) C	<0.001*
Mean (SD)	20.7 (6.1)	33.1 (7.5)	12.9 (2.3)	

*: Significant at P ≤ 0.05; Different superscripts in the same row are significantly different

Table 2. Cut-off values for different markers and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, area under the ROC curve (AUC), and 95% confidence interval (95% CI) of the (AUC) for differentiation between the CP and control groups

Marker	Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic Accuracy %	AUC	95% CI
OPG in GCF	101	100	100	100	100	100	1.000	0.903 – 1.000
OPG in Saliva	70	100	100	100	100	100	1.000	0.903 – 1.000
IL-33 in GCF	13.9	90	100	100	88.9	94.4	0.966	0.844 – 0.998
IL-33 in Saliva	16.8	70	100	100	72.7	83.3	0.903	0.758 – 0.976

+PV: Positive predictive value; -PV: Negative predictive value; AUC: Area under the curve; CI: Confidence interval

respectively. All markers showed 100% diagnostic accuracy.

The ROC curve analysis of the markers for differentiation between the CP and CP-DM groups showed cut-off values of 61, 63, 23, and 25.6 pg/ml for OPG in GCF, OPG in saliva, IL-33 in GCF, and IL-33 in saliva, respectively. IL-33 in saliva showed the highest diagnostic accuracy (82.5%). IL-33 in GCF showed a lower diagnostic accuracy of 75%, followed by OPG in saliva at 67.5%. OPG in GCF showed the lowest diagnostic accuracy at 62.5%. Pair-wise comparison of the AUCs for the markers demonstrated that IL-33 in saliva had a significantly higher mean AUC than OPG in GCF and OPG in saliva (Table 3; Figure 2).

There was a statistically significant, direct, positive correlation between OPG in GCF and OPG in saliva, i.e. an increase in OPG in GCF is associated with an increase in OPG in saliva and vice versa. There was also a significant, inverse, negative correlation between OPG in GCF and IL-33 in GCF and saliva, i.e. an increase in OPG in GCF is associated with a decrease in IL-33 in GCF and saliva and vice versa. There was no significant correlation between OPG in GCF and other variables (Table 4).

The results demonstrated a statistically significant, inverse, negative correlation between OPG in saliva and PI, i.e. an increase in PI is associated with a decrease in OPG in saliva and

vice versa. There was a significant, inverse, negative correlation between OPG in saliva and CAL, i.e. an increase in CAL is associated with a decrease in OPG in saliva and vice versa. There was also a significant, inverse, negative correlation between OPG in saliva and IL-33 in GCF and saliva i.e. an increase in OPG in saliva is associated with a decrease in IL-33 in GCF and saliva. There was no significant correlation between OPG in saliva and other variables.

Table 3. Cut-off values for different markers and the corresponding sensitivity, specificity, predictive values, diagnostic accuracy, area under the ROC curve (AUC), and 95% confidence interval (95% CI) of the (AUC) for differentiation between the CP and CP-DM groups

Marker	Cut-off	Sensitivity %	Specificity %	+PV %	-PV %	Diagnostic Accuracy %	AUC	95% CI
OPG in GCF	61	90	35	58.1	77.8	62.5	0.631	0.464 – 0.778
OPG in saliva	63	60	75	70.6	65.2	67.5	0.738	0.575 – 0.864
IL-33 in GCF	23	95	55	67.9	91.7	75	0.740	0.577 – 0.866
IL-33 in saliva	25.6	80	85	84.2	81	82.5	0.900	0.763 – 0.972

+PV: Positive predictive value; -PV: Negative predictive value; AUC: Area under the curve; CI: Confidence interval

Furthermore, a statistically significant, direct, positive correlation was observed between IL-33 in GCF and both PI and GI i.e. an increase in PI and GI is associated with an increase in IL-33 in GCF and vice versa. Comparing IL-33 in GCF and both PD and CAL, a significant, direct, positive correlation was observed, i.e. an increase in PD and CAL is associated with an increase in IL-33 in GCF and vice versa. A significant, direct, positive correlation was also found between IL-33 in GCF and IL-33 in saliva, i.e. an increase in IL-33 in GCF is associated with an increase in IL-33 in saliva and vice versa.

A statistically significant, direct, positive correlation between IL-33 in saliva and PI was demonstrated i.e. an increase in PI is associated with an increase in IL-33 in saliva and vice versa. As for IL-33 in saliva and CAL, a significant, direct, positive correlation was observed i.e. an increase in CAL is associated with an increase in IL-33 in saliva and vice versa. No significant correlation was found between IL-33 in saliva and other variables.

Discussion

The relationship between periodontitis and systemic diseases such as cardiovascular disorders and DM has been previously described.¹⁸ Previous research stated that uncontrolled diabetic conditions exhibit a more severe breakdown of the periodontium via

vascular changes such as gingival microangiopathy and increased serum proinflammatory cytokines that alter periodontal tissue response and delay wound healing.¹⁹ By preventing osteoclast differentiation, OPG functions as a natural inhibitor of alveolar bone destruction by acting as a decoy receptor for RANKL, thus inhibiting its binding to RANK.²⁰

Interleukin-33 plays an important role in the immune response for diseases such as rheumatoid arthritis, asthma, cardiovascular disease, and diabetes. It activates nuclear factor kappa-B (NF-κB) via its receptor ST2 present on mast cells and Th2-cells, and it has been recently linked to periodontitis due to its stimulation of RANKL.²¹

In the present study, IL-33 levels in both GCF and saliva were found to be higher in the CP and CP-DM groups compared to the healthy control, indicating and highlighting the role of IL-33 in the inflammatory process that takes place in periodontitis. Moreover, the results demonstrated that IL-33 levels in the GCF and saliva of the CP-DM group was higher than that of the CP group, marking the adverse effect of diabetes on periodontal disease. In 2014, Casanova et al²² stated that periodontal disease was found to affect levels of HbA1c in non-diabetic patients. They also stated that a number of studies proved that periodontal treatment acts as a positive factor in controlling hyperglycemia in diabetic patients.

Table 4. Results of Spearman's correlation coefficient for different variables

Variables	OPG in GCF		OPG in Saliva		IL-33 in GCF		IL-33 in Saliva	
	Correlation Coefficient	P-value	Correlation Coefficient	P-value	Correlation Coefficient	P-value	Correlation Coefficient	P-value
Age	0.007	0.958	-0.070	0.607	-0.149	0.273	-0.184	0.175
PI	-0.099	0.543	-0.439	0.005*	0.324	0.041*	0.366	0.025*
GI	-0.094	0.562	-0.134	0.411	0.385	0.014*	-0.218	0.177
PD	0.135	0.407	0.296	0.064	0.404	0.010*	0.307	0.054
CAL	0.274	0.087	-0.345	0.029*	0.347	0.028*	0.416	0.008*
OPG in GCF	-	-	0.621	<0.001*	-0.583	<0.001*	-0.468	<0.001*
OPG in Saliva	0.621	<0.001*	-	-	-0.500	<0.001*	-0.357	0.007*
IL-33 in GCF	-0.583	<0.00*	-0.500	<0.00*	-	-	0.651	<0.001*
IL-33 in Saliva	-0.468	<0.001*	-0.357	0.007*	0.651	<0.001*	-	-

*: Significant at P ≤ 0.05

The results of the present study were partially in accordance with that of the study carried out by Saglam et al.,⁸ who found that the total amount of IL-33 in GCF was greater in gingivitis and chronic periodontitis compared to healthy control subjects. Unlike the present study however, salivary IL-33 levels were similar for all groups.

The results concerning IL-33 levels in GCF were in contrast to what was documented in the study carried out by Kursunlu et al.,² who stated that there was no statistical significance between chronic periodontitis patients and healthy subjects regarding IL-33 in GCF. The results were also in contrast to Papathanasiou et al.,¹ who failed to detect IL-33 in the GCF of patients with chronic periodontitis. Moreover, although Buduneli et al.³ detected IL-33 in chronic periodontitis patients and the healthy controls, they concluded that chronic periodontitis and a clinically healthy periodontium could not be differentiated based on IL-33 level. This contrast may be due to different populations, levels of tissue destruction and gingival inflammation, different kits used to register IL-33 concentrations, utilization of different techniques, or different sample sizes.

As for OPG, the results of the present study demonstrated significantly higher levels in the GCF and saliva of the control group compared to the CP and CP-DM groups. This may reflect the destruction of alveolar bone taking place in periodontal disease in the absence of the protective role of OPG, thus stressing its effect on the inhibition of osteoclast

differentiation. The OPG results were similar to those found in the study performed by Ochanji et al.⁵ who measured OPG levels in saliva only and found that the RANKL/OPG ratio was positively correlated with the severity of periodontitis. Regarding OPG in GCF, similar findings were reported by Mogi et al.²³ who stated that the mean OPG value was significantly lower in patients with periodontitis compared to controls.

On comparing the diagnostic accuracy of OPG and IL-33 in GCF and saliva of the CP and control groups, all were above the cut off value, but OPG had the highest diagnostic accuracy at 100%, while IL-33 demonstrated lower diagnostic accuracy in GCF followed by saliva. This indicates that OPG as a marker is more accurate, whereby it increases in a state of health in both GCF and saliva. Additionally, on comparing the CP-DM and control groups, all markers demonstrated 100% accuracy, thus supporting the use of OPG as a biological marker, which exhibited higher levels in the healthy state compared to the diseased state, and IL-33, for which higher levels were recorded in the diseased state compared to the healthy state. As for the diagnostic accuracy of OPG and IL-33 in the CP and CP-DM groups, IL-33 in saliva demonstrated the highest diagnostic accuracy. These data help in distinguishing the validity of the use of these biological markers for the conditions presented in this study. We are unaware of any other studies that utilize the ROC curve in investigating the diagnostic

accuracy of IL-33 and OPG when used biological markers.

As for the correlation of the clinical parameters with our markers, the results demonstrated a positive correlation between IL-33, in both GCF and saliva, and PI and CAL, while a negative correlation was found between IL-33, in both GCF and saliva, and OPG, in both GCF and saliva. Furthermore, a negative correlation was determined between OPG in saliva and PI and CAL, while a positive one was found between OPG in saliva and OPG in GCF. These findings indicate that both OPG and IL-33 correlate well with clinical parameters. Moreover, they demonstrate that saliva could be of equal importance compared to GCF in the detection of biomarkers of periodontal disease, and possibly a superior tool that is characterized by easier and less time-consuming collection that does not require special equipment or trained personnel.

The previously mentioned data concerning IL-33 and OPG serve as evidence regarding their significant role in periodontal disease pathogenesis, and the influence of diabetes on periodontitis reflected through their expression. This signifies their possible utilization in either GCF or saliva for the diagnosis and differentiation of periodontal disease.

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References

1. Papathanasiou E, Teles F, Griffin T, et al. Gingival crevicular fluid levels of interferon-gamma, but not interleukin-4 or -33 or thymic stromal lymphopoietin, are increased in inflamed sites in patients with periodontal disease. *J Periodontol Res.* 2014; 49(1):55–61.
2. Kursunlu SF, Ozturk VO, Han B, Atmaca H, Emingil G. Gingival crevicular fluid interleukin-36 beta (-1F8), interleukin-36 gamma (-1F9) and interleukin-33 (-1F11) levels in different periodontal disease. *Arch Oral Biol.* 2015; 60(1):77–83.

3. Bascones-Martínez A, González-Febles J, Sanz-Esporrín J. Diabetes and periodontal disease. Review of the literature. *Am J Dent.* 2014; 27(2):63-7.
4. Wu YY, Xiao E, Graves DT. Diabetes mellitus related bone metabolism and periodontal disease. *Int J Oral Sci* 2015;7(2):63-72.
5. Ochanji AA, Matu NK, Mulli TK. Association of salivary RANKL and osteoprotegerin levels with periodontal health. *Clin Exp Dent Res* 2017;3:45–50.
6. Silva N, Dutzan N, Hernandez M, et al. Characterization of progressive periodontal lesions in chronic periodontitis patients: Levels of chemokines, cytokines, matrix metalloproteinase-13, periodontal pathogens and inflammatory cells. *J Clin Periodontol* 2008;35:206-14.
7. Behfarnia P, Saied-Moallemi Z, Javanmard SH, Naseri R. Serum, saliva, and GCF concentration of RANKL and osteoprotegerin in smokers versus nonsmokers with chronic periodontitis. *Adv Biomed Res.* 2016;5:80.
8. Saglam M, Koseoglu S, Aral CA, Savran L, Pekbagriyanik T, Cetinkaya A. Increased levels of interleukin-33 in gingival crevicular fluids of patients with chronic periodontitis. *Odontology.* 2017; 105(2):184-190.
9. da Luz FA, Oliveira AP, Borges D, Brigido PC, Silva MJ. The physiopathological role of IL-33: new highlights in bone biology and a proposed role in periodontal disease. *Mediat Inflamm.* 2014; 2014:342410.
10. Koseoglu S, Hatipoglu M, Saglam M, Enhos S, Esen HH. Interleukin-33 could play an important role in the pathogenesis of periodontitis. *J Periodontol Res.* 2015;50 (4):525–34.
11. Buduneli N, Özçakmak O and Nalbantsoy A. Interleukin-33 Levels in Gingival Crevicular Fluid, Saliva, or Plasma Do Not Differentiate Chronic Periodontitis. *J Periodontol.* 2012 ;83 (3) 362:368.
12. Armitage G. Development of a classification system for periodontal diseases and conditions. *Am Periodontol;* 4:1-6, 1999.
13. Ainamo J. & Bay I. Periodontal indexes for and in practice, *Tandlaegebladet,* 80:149-152, 1976.

14. Davies, G.N., Horowitz, A.S. & Wanda, W. The assessment of periodontal disease for public health purposes. *Journal of Periodontal Research*. 1974; 9, 62-70.
15. Glavind, L. and Löe, H. Errors in the clinical assessment of periodontal destruction. *Journal of Periodontal Research*, [1967], 2: 180-184. doi:10.1111/j.1600-0765.1967.tb01887.
16. Caton J. Periodontal diagnosis and diagnostic aids in proceedings of the world workshop in clinical periodontics. [1989] Chicago: the American academy of periodontology.
17. Navazesh M. Methods for collecting saliva. *Jam N.Y. Acad Sci*. 20: 72-77; 1993.
18. Özçaka Ö, Ceyhan-Öztürk B, Gümüş P, Akcalı A, Nalbantsoy A, Buduneli N. Clinical Periodontal Status and Inflammatory Cytokines in Gestational Diabetes Mellitus. *Arch Oral Biol*. 2016 Dec;72:87-91.
19. Goel K, Pradhan S, Bhattarai MD. Effects of nonsurgical periodontal therapy in patients with moderately controlled type 2 diabetes mellitus and chronic periodontitis in Nepalese population. *Clin Cosmet Investig Dent*. 2017.17;9:73-80.
20. Lacey, D. L., Timms, E., Tan, H. L., Boyle, W. J. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell*, (1998) 93, 165–176.
21. Severino VO, Beghini M, de Araújo MF, de Melo MLR, Miguel CB, Rodrigues WF, de Lima Pereira SA. Expression of IL-6, IL-10, IL-17 and IL-33 in the peri-implant crevicular fluid of patients with peri-implant mucositis and peri-implantitis. *Arch Oral Biol*. 2016 ;72:194-199.
22. Casanova L, Hughes F and Preshaw P. Diabetes and periodontal disease: a two-way relationship. *British dental journal* 2014. 217(8) :433;437.
23. Mogi M, Otogoto J, Ota N, Togari A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. *J of Dent Res* 2004; 83, 2, 166–169.

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