Correlations between Interleukin-33 and -1α Levels in Gingival Crevicular Fluid and Plasma in Patients with Chronic or Aggressive Periodontitis and Disease-free Subjects

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Abstract

Interleukin (IL)-33 is a dual-function protein that may play important roles as both a cytokine and intracellular nuclear factor. It may also function similarly to prototypical alarmin IL-1 α , as an endogenous danger signal to alert innate immune system cells to tissue damage during trauma or infection, as it can be released in the extracellular space after endothelial cell damage or mechanical injury. The aim of this study was to determine possible correlations between concentrations of IL-33 and IL-1 α in gingival crevicular fluid (GCF) and plasma obtained from healthy patients, those with chronic periodontitis (CP), and those with generalized aggressive periodontitis (GAP) patients. Forty-five patients with an age range of 20–60 years were enrolled and divided into 3 groups: Group H, 30 samples (15 GCF and 15 plasma) from 15 patients with healthy periodontium; Group CP, 30 samples from 15 patients with CP; and Group GAP, 30 samples from 15 patients with GAP. The clinical periodontal parameters investigated in all groups comprised the gingival index score, probing pocket depth, and clinical attachment level. The GCF and plasma levels of IL-33 and IL-1 α were quantitated using enzyme linked immunosorbent assay. The mean IL-33 concentrations in GCF and plasma were highest in Group GAP, followed by Group CP, with the lowest in Group H; the difference among them was statistically significant ($p < 0.05$). Concentration of IL-1 α followed the same trend as that of IL-33 in GCF, but was lower than detection levels in plasma. The GCF and plasma concentrations of IL-33 correlated with IL-1 α concentrations in GCF. Concentrations of IL-33 and IL-1 α in GCF varied significantly between healthy patients and those with disease, allowing healthy patients to be distinguished from those with GAP or CP. The results of this study suggest that IL-33 offers a potential inflammatory marker of periodontal disease, similar to IL-1 α .

Key words: Cytokine — Chronic periodontitis — Gingival crevicular fluid — Aggressive periodontitis — Alarmin

Introduction

Periodontal disease results from the inflammatory response to periodontopathic bacteria, where disease progression is due to a combination of high levels of proinflammatory cytokines and low levels of anti-inflammatory cytokines⁽²⁸⁾.

The immune response to infection is regulated by the balance between T helper (Th) 1- and Th2-type cytokines. The net effect of the former is to enhance cell mediated responses; while that of the latter is to suppress cell mediated responses and enhance humoral immunity. It has been suggested that stable periodontal lesions are mediated by Th1 cells, and progressive lesions by Th2 cells, although this remains controversial⁽²⁹⁾.

The classical Interleukin (IL)-1 family of cytokines especially has been known to play a key role in these disease processes, and hence have been identified as therapeutic targets, too. Excessive or dysregulated activity of these agents is associated with tissue destruction, which is a hallmark of periodontitis^(6,26).

Interleukin-33 (IL-1F11; previously known as NF-HEV) is the most recent addition to the IL-1 family. It has been shown to induce Th2 responses by signaling through the IL-1 receptor-related protein ST2 (IL-1R4), an orphan member of the IL-1 receptor family, as well as activate NF- κ B and MAP kinases⁽²⁷⁾. In addition, it is also involved in the regulation of innate immune response, particularly via mast cell activation⁽¹⁾.

Interleukin-33 is associated with endothelial cells in inflamed tissues in patients with rheumatoid arthritis and Crohn's disease, where it is a nuclear factor which regulates transcription⁽⁷⁾. It is a dual-function protein that may play important roles as both a cytokine and an intracellular nuclear factor^(7,24). It may function similarly to the prototypical alarmin IL-1 α as an endogenous danger signal to alert cells of the innate immune system to tissue damage during trauma or infection, as it can be released in the extracellular space after endothelial cell damage or mechanical injury⁽¹⁸⁾.

The facts that increased microvasculature is a prominent histological finding in periodontitis and that IL-33 plays a role in promoting Th2 responses, (such as those in destructive periodontitis), warrant a detailed investigation of the role of IL-33 in periodontitis⁽²⁶⁾. To date, little is known about the role of IL-33 in periodontal disease since the results of previous studies are varied and inconclusive. Therefore, the aim of the present study was to investigate the presence of IL-33 in gingival crevicular fluid (GCF) and plasma obtained from patients with periodontal disease and determine any correlation with IL-1 α , an established alarmin, and clinical parameters of periodontal inflammation.

Materials and Methods

1. Study population and clinical examination

All patients enrolled in this study attended the Outpatient Department of Periodontics at the Government Dental College and Research Institute in Bangalore between February and August 2017. All were selected at random from among individuals scheduled for routine oral examination. Written informed consent was obtained from each participant. Ethical clearance was obtained from the institution's ethical committee and review board (Reference No.: GDCRI/ACM/PG/Ph.D/1/2016-2017) and was conducted according to the guidelines of the Helsinki Declaration.

A total of 45 patients (28 men and 17 women; age, 20 to 60 years; mean age, 29.73 years) were divided into the following 3 groups: Group H, comprising healthy patients; Group CP, comprising those with chronic periodontitis; and Group GAP, comprising those with generalized aggressive periodontitis.

Each patient underwent full-mouth periodontal probing and charting, along with radiographic examination. For periodontal evaluation the Gingival Index⁽¹⁴⁾ (GI) score, probing pocket depth (PPD), and Clinical attachment level (CAL) were determined. All clinical measurements were performed by a

single examiner (SPB). Probing pocket depth and CAL were measured using a graduated William's periodontal probe (Hu-Friedy, Chicago, IL).

The CP patients showed signs of clinical inflammation, $GI > 1$, $PPD \geq 4$ mm, $CAL \geq 2$ mm, and moderate-to-severe alveolar bone loss at multiple sites in all 4 quadrants of the oral cavity, which was commensurate with the amount of plaque accumulation. The GAP patients showed signs of clinical inflammation, $GI > 1$, $PPD \geq 4$ mm, $CAL \geq 2$ mm, and radiographic evidence of bone loss affecting at least 3 permanent teeth other than first molars and incisors. The patients in the CP and GAP groups were selected based on primary features and clinical presentation in accordance with the definition of Lang *et al*¹³. Patients with a clinically healthy periodontium, $GI = 0$ (absence of clinical inflammation), $PPD \leq 3$ mm, $CAL = 0$, no evidence of bone loss on radiographs, and no bleeding on probing were included in Group H. The exclusion criteria were as follows: any chronic inflammatory disease such as rheumatoid arthritis; any autoimmune disease or other systemic disease that could alter the course of periodontal disease; a history of smoking or use of tobacco in any form; inflammatory bowel disease such as ulcerative colitis or Crohn's disease; a history of periodontal therapy in the preceding 6 months; pregnant/lactating women; patients with immunodeficiency such as those infected with the Human Immunodeficiency virus; use of medication like steroids, contraceptives, anti-inflammatory drugs, or antibiotics (in the last 6 months); and an unwillingness to participate in the study.

2. Site selection and GCF collection

In the periodontitis groups, only 1 site per patient was selected as a sampling site; whereas in the healthy group, multiple sites (3 to 5 sites per patient) with an absence of clinical inflammation were sampled to ensure the collection of an adequate volume of GCF. In patients with chronic or aggressive periodontitis, sites showing the greatest CAL and signs of inflammation along with radiographic evi-

dence of bone loss were selected for sampling.

On the subsequent day, the sample collection site was air-dried and well-isolated using sterile cotton rolls, and supragingival plaque was removed gently without touching the marginal gingiva, using a Universal Gracey curette #4R/4L (Hu-Friedy, Chicago, IL) to avoid contamination of the paper strips. The paper strips (Periopaper, Ora Flow Inc., Amityville, NY, USA) were carefully placed at the entrance of the gingival sulcus/crevice until light resistance was felt, care being taken to avoid mechanical injury, and left in place for 60 seconds. The volume of absorbed GCF in each strip was determined by electronic impedance using a Periotron 8000 (ProFlow Inc., Amityville, NY, USA). Samples suspected of being contaminated with blood or saliva were discarded. After collection of gingival fluid, the 2 periopaper strips per collection site that absorbed GCF from each patient were pooled and immediately transferred to microcentrifuge tubes (premarked with the biomarker name for identification) containing 400 μ l phosphate buffer saline and stored frozen at -70°C for subsequent analysis.

3. Blood collection and plasma extraction

On the same day as GCF collection, 2 ml blood was collected from the antecubital fossa by venipuncture using a 2-ml syringe with a 20-gauge needle and transferred immediately to EDTA containing vials. The plasma was separated from blood by centrifuging at 1,270 g for 5 min. The plasma was immediately transferred to a plastic vial and stored at -70°C until the time of assay.

4. ELISA

The GCF sample tubes were first allowed to reach room temperature, following which they were homogenized for 30 sec and centrifuged for 5 min at 1,500 g to elute. The elution was then used as sample for ELISA estimation of IL-33 and IL-1 α .

1) IL-33 assay

Interleukin-33 levels in GCF were measured using the Human IL-33 DuoSet ELISA kit

(R&D Systems, USA, Imported by Biotech-India, India) in accordance with the manufacturer's instructions. Absorbance in each well was read on an ELISA reader using 450 nm as the primary wavelength. Concentrations of IL-33 in the GCF samples were estimated using the standard curve.

2) IL-1 α assay

Interleukin-1 α levels in GCF obtained were measured using the Human IL-1 α Quantikine ELISA kit (R&D Systems, USA, Imported by Biotech-India, India) in accordance with the manufacturer's instructions. Absorbance in each well was read on an ELISA reader using 450 nm as the primary wavelength. Concentrations of IL-1 α in the GCF samples were estimated using the standard curve.

5. Statistical analyses

Power analysis was used to estimate sample size with a software program (G*power v.3.0.8 for Windows, Heinrich Heine University, Düsseldorf). The SPSS statistical software (SPSS version 18.5, Chicago, IL, USA) was utilized for all statistical analyses. The results were averaged (mean $\pm$ standard deviation) for continuous data. Normality assumptions were tested using the Shapiro-Wilks and Kolmogorov-Smirnov tests. The Kruskal Wallis and Mann-Whitney tests were used as non-parametric tests. Proportions were compared using the Chi square test. Spearman's rho correlation coefficients were calculated to determine whether there were any correlations among the clinical parameters and GCF or plasma concentrations of IL-33 and IL-1 α . A p-value of <0.05 was considered as statistically significant. Mean intra-examiner standard deviation of differences in repeated PPD and CAL measurements was obtained using single passes of measurements (correlation coefficients between duplicate measurements; $r = 0.95$).

Results

1. Clinical findings

Descriptive statistics of the study popula-

tion are shown in Table 1. All values were rounded off to the nearest whole number. Groups GAP and CP showed significantly higher PPD scores than group H ($p < 0.001$). The CAL scores were similar between groups CP and GAP ($p = 0.126$). Patients with periodontitis showed significantly higher GI scores ($p = 0.003$). No significant difference was observed in sex distribution between the groups ($p = 0.094$). Mean age in the CP group was significantly higher than that in the GAP or healthy groups ($p < 0.001$), whereas there was no statistically significant difference between the GAP and healthy group.

2. Biochemical findings

1) Analysis of IL-33 in GCF and plasma

Interleukin-33 was detected in all samples. Mean concentrations of IL-33 in GCF according to ELISA readings were 414.36 ± 49.461 pg/ml in group H; 466.93 ± 56.206 pg/ml in group CP; and 543.20 ± 95.819 pg/ml in group GAP. The mean IL-33 concentration in GCF was highest in group GAP. The mean GCF IL-33 concentration showed a significant increase, from the healthy to CP groups, and then from the CP to GAP groups (414.36 pg/ml < 466.93 pg/ml < 543.20 pg/ml). Mean concentrations of IL-33 in plasma according to ELISA readings were 13.40 ± 4.304 pg/ml in group H; 17.67 ± 8.371 pg/ml in group CP; and 216.04 ± 148.436 pg/ml in group GAP. The mean IL-33 concentration in plasma was highest in group GAP. The mean plasma IL-33 concentration showed a significant increase, from the healthy to CP groups, and then from the CP to GAP groups (13.40 pg/ml < 17.67 pg/ml < 216.04 pg/ml). To test the hypothesis of equality of means among the 3 groups, the Kruskal-Wallis test was carried out, the results of which indicated that the means differed significantly ($p < 0.001$) among groups in GCF and plasma concentrations of IL-33. Further multiple comparisons using the Mann Whitney *U* test were carried out to find which pair or pairs differed significantly. The results showed that the differences were statistically significant between all 3 groups for IL-33 in GCF

Table 1 Descriptive statistics of study population and gingival crevicular fluid and plasma IL-33 and IL-1 α concentrations

PARAMETERS	GROUP H (n = 15)	GROUP CP (n = 15)	GROUP GAP (n = 15)	F value/ Chi-square value	p-value
AGE (YEARS)	24.53 $\pm$ 4.658	37.20 $\pm$ 6.428	27.47 $\pm$ 7.945	15.685	<0.001*
SEX					
Women (n)	9	4	4		
Men (n)	6	11	11	4.727	0.094
GI	—	1.90 $\pm$ 0.186	2.36 $\pm$ 0.287	26.540	0.003*
PPD (mm)	1.87 $\pm$ 0.640	6.07 $\pm$ 1.387	6.93 $\pm$ 1.163	89.661	<0.001*
CAL (mm)	—	6.93 $\pm$ 1.624	7.80 $\pm$ 1.373	2.491	0.126*
GCF IL-33 concentration (pg/ml)	414.36 $\pm$ 49.461	466.93 $\pm$ 56.206	543.2 $\pm$ 95.819	22.031	<0.001*
Plasma IL-33 concentration (pg/ml)	13.40 $\pm$ 4.304	17.67 $\pm$ 8.371	216.04 $\pm$ 148.436	30.143	<0.001*
GCF IL-1 α concentration (pg/ml)	174.975 $\pm$ 101.593	309.464 $\pm$ 82.882	623.77 $\pm$ 251.686	33.269	<0.001*
Plasma IL-1 α concentration (pg/ml)	0.15 (n = 1)	0.60 (n = 2)	<0.15**	—	—

*Statistically significant at $p < 0.05$; F value-Kruskal Wallis test

**below detection limit;

— not applicable

All values except sex expressed as Mean $\pm$ SD

($p = 0.004$, $p < 0.001$, $p = 0.004$, respectively) and were statistically significant between groups H and GAP ($p < 0.001$) and groups CP and GAP ($p < 0.001$) for plasma IL-33 (Table 2).

2) Analysis of IL-1 α in GCF and plasma

Interleukin-1 α was detected in all GCF samples. Mean concentrations according to ELISA readings were 174.97 $\pm$ 101.593 pg/ml in group H; 309.96 $\pm$ 82.882 pg/ml in group CP; and 623.77 $\pm$ 251.686 pg/ml in group GAP. The mean IL-1 α concentration in GCF was highest in group GAP. The mean GCF IL-1 α concentration showed a significant increase, from the healthy to the CP groups, and then from the CP to the GAP groups (174.97 pg/ml < 309.96 pg/ml < 623.77 pg/ml). To test the hypothesis of equality of means among the 3 groups, the Kruskal-Wallis test was carried out, the results of which indicated that the means differed significantly ($p < 0.001$) among groups in GCF concentrations of IL-1 α . Further multiple comparisons

using the Mann Whitney U test were carried out to find which pair or pairs differed significantly. The results showed that the differences were statistically significant between all 3 groups for IL-1 α ($p = 0.001$, $p < 0.001$, $p < 0.001$, respectively) (Table 2). Interleukin-1 α was detected in only 3 of the 45 plasma samples. In the remaining 42 samples, it was below detection levels. In one of the samples in the H group, 0.15 pg/ml was detected; and in the CP group, 2 of the samples had a mean concentration of 0.60 pg/ml. It was not detected in any of the GAP group plasma samples. The remaining samples had <0.15 pg/ml of IL-1 α , so they were labelled as undetectable. The plasma concentration of IL-1 α was therefore not submitted for statistical analysis.

3) Correlations among IL-33, IL-1 α , and clinical parameters

The Spearman's rho correlation test was carried out to determine any possible correlations among IL-33, IL-1 α , and clinical param-

Table 2 Intergroup comparison of gingival crevicular fluid and plasma IL-33 and IL-1 α concentrations using Mann-Whitney Test.

Study groups	GCF IL-33			GCF IL-1 α			Plasma IL-33		
	H vs CP	H vs GAP	CP vs GAP	H vs CP	H vs GAP	CP vs GAP	H vs CP	H vs GAP	CP vs GAP
Mann-Whitney U value	44.500	12.000	44.000	34.500	0.000	4.000	80.500	0.000	0.000
'p' value	0.004*	<0.001*	<0.004*	0.001*	<0.001*	<0.001*	0.187*	<0.001*	<0.001*

*statistically significant at $p < 0.05$

eters. Correlations among GCF or plasma concentrations of IL-33 and IL-1 α and clinical parameters were statistically insignificant in all 3 groups. There was a weak-to-moderate, but statistically insignificant, negative relationship between PPD or CAL and GCF levels of IL-33. There was a weak, positive relationship between GCF and plasma concentrations of IL-33 in all 3 groups which did not reach statistical significance. The test showed a significant and very marked positive correlation between IL-33 and IL-1 α concentrations in GCF in the H group ($r = 0.997$, $p = 0.000$), CP group ($r = 0.999$, $p = 0.000$) and GAP group ($r = 0.884$, $p = 0.000$) There was a statistically insignificant, moderately positive relationship among IL-33 concentrations in plasma with that of both IL-33 and IL-1 α concentrations in GCF of all 3 groups (Table 3).

Discussion

The present cross-sectional study was undertaken to compare and evaluate GCF and plasma levels of IL-33 and IL-1 α among periodontally healthy individuals, those with chronic, and those with aggressive periodontitis. Numerous studies have investigated the relationship between IL-33 and periodontal disease. While most *in vitro* studies have found that IL-33 was a central mediator of periodontal disease, the results of human studies have been inconclusive at best²³.

As chronic inflammatory diseases such as rheumatoid arthritis have been reported to affect levels of IL-33¹⁷, they were included as

an exclusion criterion in the present study. To the best of our knowledge, this is the first study to report data on GCF and plasma concentrations of IL-33 and IL-1 α in periodontal disease.

Chronic periodontitis, which is prevalent mainly in adults, is a major cause of tooth loss, whereas aggressive periodontitis, which is characterized by severe and rapid progression, is found more in younger individuals. These two types of disease differ in many aspects, including age of onset, disease progression rate, major pathogenic mechanisms, familial aggregation, genetic susceptibility, and local factors. At present, clinical periodontal and radiographic findings along with patient history are commonly used in arriving at a diagnosis. At times, these periodontal parameters are insufficient to allow the establishment of an accurate diagnosis, however. Therefore, continued research is crucial in understanding the specific role of microbial factors as well as host factors if these two clinical phenotypes are to be better distinguished. One study has suggested an association between differences in the bacterial load in different types of periodontal disease and specific cytokine profiles²⁾. This is supported by the results of the current study, which revealed a statistically significant difference in GCF and plasma levels of IL-33 between CP and GAP. Probing pocket depth and the CAL neither indicate current disease activity nor predict future progression. It is a major challenge in clinical periodontics to find a reliable, highly specific, and sensitive molecular marker of periodontal tissue destruction⁴⁾.

Table 3 Correlations between gingival crevicular fluid IL-33, plasma IL-33, and GCF IL-1 α concentrations and clinical periodontal parameters.

STUDY GROUPS	GROUP H (r value)	GROUP CP (r value)	GROUP GAP (r value)
GCF IL-33 & GCF IL-1 α	0.997*	0.999*	0.884*
GCF IL-33 & GI	—	0.050	−0.075
GCF IL-33 & PPD	−0.258	−0.365	−0.114
GCF IL-33 & CAL	—	−0.307	−0.254
GCF IL-1 α & GI	—	0.061	0.066
GCF IL-1 α & PPD	−0.233	−0.364	−0.146
GCF IL-1 α & CAL	—	−0.301	−0.149
Plasma IL-33 & GI	—	0.406	−0.468
Plasma IL-33 & PPD	0.161	−0.218	−0.195
Plasma IL-33 & CAL	—	−0.171	−0.218
GCF IL-33 & Plasma IL-33	0.338	0.184	0.228
GCF IL-1 α & Plasma IL-33	0.343	0.188	0.346

Correlations between different parameters were analyzed by Spearman correlation test.

* statistically significant at $p = 0.000$;

— not applicable

Therefore, one of the goals of the present study was to determine possible correlations between clinical diagnostic parameters and IL-33 levels to evaluate their usefulness as a biomarker in identifying periodontal disease and monitoring treatment.

Expression of IL-33 increases in response to proteases, pathogen-associated molecular patterns, and proinflammatory stimuli. Tada *et al.* detected IL-33 in inflamed gingival epithelium of chronic periodontitis patients. They found that *Porphyromonas gingivalis* increased IL-33 expression in human gingival epithelial cells *in vitro*, and that protease-activated receptor-2 (PAR-2), as well as inhibitors of phospholipase C, p38, and NF- κ B, inhibited expression of IL-33 induced by *P. gingivalis*³⁰. In an *in vitro* study, it was reported that stimulation of human monocytes with *Escherichia coli* and *P. gingivalis* lipopolysaccharide upregulated IL-33 at both the mRNA and protein levels¹⁹. Beklen *et al.* reported that IL-33 levels significantly increased in TNF- α -stimulated human gingival fibroblasts as compared to in unstimulated fibroblasts³. In a ligature-induced periodontitis model,

Köseoglu *et al.* noticed that the experimental periodontitis group exhibited increased expression of IL-33 and RANKL compared with the healthy group. They concluded that IL-33 could be associated with the pathogenesis of periodontal disease¹¹. In a murine model of periodontal disease, Malcolm *et al.* demonstrated that expression of IL-33 and its receptor ST2 was elevated in gingival tissues of CP patients, and concluded that IL-33 plays a role in exacerbating bone loss in a RANKL-dependent manner in the context of bacterial infection, suggesting that this pathway may be amenable to manipulation as a novel therapeutic target in periodontitis¹⁵. The findings of the present study showed a gradual increase in IL-33 levels in GCF and plasma, from healthy patients to CP, and from CP to GAP. This suggests that IL-33 may indeed play a role in the pathogenic pathways of periodontal disease. Further studies are required to quantify virulence factors such as gingipains and leukotoxin A in periodontitis patients, along with GCF and gingival tissue expression of IL-33 to establish if there is actually a concentration-dependent relationship among

them.

In the present study, IL-33 was detected in all of the GCF and plasma samples. This was in contrast with in a previous study by Papatasiou *et al.*, who found that IL-33 was below detection level in all the GCF samples collected. They attributed many reasons to this finding, which included greater dilution of GCF during the assay; the inability of IL-33 to cross the junctional epithelium into the gingival crevice; degradation of IL-33 by proteases before reaching the GCF; and levels of IL-33 being much lower than the detection levels of the assay used²²⁾.

Buduneli *et al.* found that differences among GCF, saliva, and plasma levels of IL-33 were insufficient to allow differentiation of chronic periodontitis from healthy periodontium. Their results were attributed to low sensitivity of the ELISA kits, a significantly lower volume of GCF in periodontally healthy patients, and ethnic differences between study populations. They suggest that analyzing other members of the IL-1 family along with IL-33 may provide a better understanding of their possible role in periodontal disease⁵⁾. Kurşunlu *et al.* reported that total amounts of IL-33 were similar among GAgP, CP, gingivitis, and periodontally healthy individuals. They concluded that IL-33 does not contribute to the pathogenesis of periodontal disease¹²⁾.

In the present study, levels of IL-1 α showed an increase in GCF obtained from patients with periodontitis as compared to that obtained from healthy controls, a finding similar to that obtained in previous studies¹⁶⁾. The results of previous studies on GCF levels of IL-33 in periodontitis have been inconclusive, however. Therefore, the present study compared them with GCF concentrations of an already established alarmin, namely, IL-1 α . Interleukin-1 α alerts the cells of the innate immune system to tissue damage during trauma or infection, as it can be released in the extracellular space after endothelial cell damage or mechanical injury¹⁸⁾. The present study also included an additional study group of patients with GAP.

Gümü *et al.* reported statistically significant higher saliva IL-33 levels in CP patients than in aggressive periodontitis patients or those who were periodontitis-free, suggesting a relationship between periodontopathogens and salivary cytokine levels⁹⁾. Sağlam *et al.* compared GCF levels of IL-33, saliva, and plasma obtained from periodontitis patients. They found that IL-33 concentrations were lower in the CP group than in the healthy or gingivitis groups, whereas salivary and plasma levels were similar²⁵⁾. In another study, it was reported that salivary IL-33 concentrations were significantly higher in patients with obstructive sleep apnea syndrome than in the healthy control group, but no relationship was noted with periodontal parameters²⁰⁾. The present study showed that IL-33 concentrations were greater in GCF or plasma obtained from patients with chronic or aggressive periodontitis than from healthy controls. Whole saliva represents a pooled sample, with contributions from all periodontal sites, whereas GCF is site-specific. Proteases are known to be elevated in the saliva of periodontitis patients³³⁾ and may further reduce the levels of protein biomarkers in GCF. In the present study, GCF and plasma samples were selected for assessment taking into consideration the results of the previous studies. Concentrations of IL-33 and IL-1 α in GCF showed no correlation with the clinical parameters of inflammation. This finding may be explained by the episodic nature of periodontitis. This disease may have been in different phases (active or quiescent) in the patients in the periodontitis groups. Also, the present study recorded mainly soft tissue parameters, which might not be true indicators of underlying active bone resorption. Further studies are needed both before and after intervention (such as Phase I therapy) to determine whether there is a correlation between IL-33 levels and clinical indicators of disease such as PPD or CAL. Moreover, further study is also needed to establish whether IL-33 levels are associated with alveolar bone loss.

The negative, although statistically insignificant, correlation between PPD or CAL

and IL-33 concentrations seen here may be attributable to the fact that in the initial stages of inflammation IL-33 participates in the anti-inflammatory response by an augmentation of the Th2 response. In the absence of treatment, however, extremely high levels of IL-33 and other pro-inflammatory cytokines are released, and these have been shown to activate mast cells and favour osteoclastogenesis^{8,21,31,32}.

In the present study, plasma levels of IL-1 α were below detection levels. Previous studies have detected IL-1 α in plasma from healthy and chronic periodontitis patients¹⁰. This discrepancy between the current and earlier results may be partly attributable to the different assay kits used, as sensitivities will vary. To our knowledge, no previous studies have analyzed IL-1 α in plasma obtained from patients with aggressive periodontitis.

Conclusion

Interleukin-33 was detected in GCF and plasma obtained from healthy, chronic, and aggressive periodontitis patients. The levels of IL-33 were highest in GCF and plasma obtained from patients with aggressive periodontitis and lowest in those with healthy periodontium, with those observed in patients with chronic periodontitis in between. Interleukin-1 α was detected in GCF obtained from healthy, chronic, and aggressive periodontitis patients, but not in plasma. Concentrations of IL-1 α in GCF showed a significant correlation with those of IL-33. This suggests that IL-33 offers a useful biomarker of periodontal disease, as it can differentiate between healthy and diseased periodontal sites, similarly to the previously established alarmin IL-1 α . Future studies with larger sample sizes, quantification of virulence factors, and intervention clinical trials will enhance the knowledge of the role of IL-33 in periodontal health and disease. Understanding the association between IL-33 levels and severity of chronic or aggressive periodontitis might open a new pathway for periodontal therapy in the future.

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