Effect of Nonsurgical Periodontal Therapy on Interleukin-34 Levels in Periodontal Health and Disease

Abstract

Background: Interleukin-34 (IL-34) is a recently identified alternative ligand for colony-stimulating factor-1 receptor plays an important role in osteoclastogenesis. The aim of this study was to evaluate the IL-34 levels in gingival crevicular fluid (GCF) and plasma in subjects with chronic periodontitis and to evaluate the effect of nonsurgical periodontal therapy on the GCF and plasma IL-34 levels. Materials and Methods: Thirty individuals (age range: 30–56 years) were selected and divided into groups based on the gingival index, probing pocket depth, clinical attachment level, and radiologic parameters (bone loss): Group I (15 individuals with healthy periodontium), Group II (15 individuals with chronic generalized periodontitis) while Group II patients after 8 weeks of the treatment (scaling and root planing) constituted Group III. GCF samples and plasma samples were collected to estimate the levels of IL-34 using enzyme-linked immunosorbent assay kit. Results: The mean IL-34 concentration in GCF and plasma was highest for Group I and decreased after nonsurgical periodontal therapy in chronic generalized periodontitis group. The difference between them was statistically significant (P < 0.05). Conclusion: IL-34 can be considered as an “inflammatory marker” of periodontal disease and can be explored in the future as a potential therapeutic target in the treatment of periodontal disease.

Keywords: Gingival crevicular fluid, periodontitis, plasma

Introduction

Periodontitis is an inflammatory disease that results in the destruction of the supporting connective and bony tissues of the teeth. Human periodontal ligament (HPDL) cells are exposed to periopathogenic factors and inflammatory cytokines. HPDL cells possibly play a role in osteoclastogenesis through the expression of receptor activator of nuclear factor kappa B ligand (RANKL), on their cell surfaces. The differentiation of osteoclast is mainly dependent on RANKL, a tumor necrosis factor (TNF) family cytokine, as well as on macrophage colony-stimulating factor (M-CSF/CSF-1). It is widely accepted in a model of bone cells that formation of osteoclasts requires the interaction between receptor activator of nuclear factor kappa B (NF-kB/RANK), which is expressed on the surface of osteoclasts and RANKL, in the presence of M-CSF. The role of M-CSF has been demonstrated in osteopetrotic mutant mice, which suffer from congenital osteopetrosis due to deficiency of osteoclasts associated with an absence of M-CSF. Therefore, M-CSF is required for osteoclastogenesis, stimulating both adhesion and the proliferation of osteoclast precursors.

Recently, interleukin-34 (IL-34) was functionally identified as a cytokine by comprehensive proteomic analyses and was shown to act as an alternative ligand of CSF-1 receptor (CSF-1R, c-fms) although it shares no sequence homology with CSF-1. IL-34 mRNA is expressed in various tissues, including heart, brain, lung, liver, kidney, spleen, thymus, testes, ovary, small intestine, prostate, and colon, and it is most abundant in the spleen. IL-34 plays an important role in RANKL-induced osteoclastogenesis, as it can substitute for M-CSF and support osteoclast differentiation in the same way that M-CSF does.

IL-34 has been identified as a novel and moderate inflammatory cytokine that significantly induces macrophage activation and migration and plays an important role in inflammation, as it increases IL-6 and...
chemokine levels in human whole blood.\[11\textendash}13\] IL-34 is expressed in rheumatoid arthritis (RA) synovium where it relates to the synovitis severity and is elevated in serum and synovial fluid of RA patients.\[14\] Furthermore, IL-34 is produced by synovial and gingival fibroblasts in response to TNF-α and IL-1 β through NF-κB and c-JunN-terminal kinase pathways.\[14,15\] IL-34 expression is also upregulated in intestine from patients with inflammatory bowel disease (IBD)\[16\] and in inflamed salivary glands from patients with Sjögren’s syndrome.\[17\] IL-34 mRNA expression in periapical lesions was significantly higher than that of the normal periodontal ligament tissue in periapical lesions of chronic apical periodontitis.\[18\] CSF-1 and IL-34 have complementary roles in periodontal disease with IL-34 in steady-state and CSF-1 in inflammation.\[19\]

Until today, no studies have reported the levels of IL-34 in gingival crevicular fluid (GCF) and plasma in periodontal health, disease, and chronic periodontitis-affected individuals after the nonsurgical periodontal treatment. In the light of the above facts, the present study is designed to estimate the levels of IL-34 in GCF and plasma in periodontal health, disease and chronic generalized periodontitis-affected individuals after the nonsurgical periodontal treatment.

**Materials and Methods**

A total of 30 individuals (n = 30; 16 men and 14 women; age range: 30–56 years) were selected from outpatient pool, Periodontology Department, Government Dental College and Research Institute, Bengaluru, Karnataka, India. Ethical clearance for the study was obtained from the Institutional Ethical Committee and review board, Government Dental College and Research Institute, Bengaluru (approval no: GDCRI/ACM/PG/PhD/10/2013-2014) and was conducted in accordance with the Declaration of Helsinki 1975, as revised in 2013. The patients were explained regarding the study procedure and written informed consent was obtained from those who agreed to participate voluntarily in this study. Patient with chronic inflammatory diseases such as RA, IBD, and respiratory diseases such as chronic obstructive pulmonary disease, asthma and bronchitis, immunodeficiency state like those infected by human immunodeficiency virus, pregnancy, giant cell tumors of the bone, coronary heart disease, hypertension, aggressive periodontitis, diabetes mellitus, habits of smoking, betelnut/arecanut chewing, alcoholism and who had taken steroids, contraceptives, anti-inflammatory drugs, or antibiotics, and periodontal treatment in the preceding 6 months were excluded from the study.

Full mouth periodontal examination was done clinically for all individuals along with periapical radiographs using long cone technique. To differentiate chronic generalized periodontitis, participants from periodontally healthy group radiographic bone loss was recorded dichotomously (presence or absence). Furthermore, no demarcation was attempted within the chronic generalized periodontitis group based on the extent of alveolar bone loss. Based on the gingival index (GI),\[20\] pocket-probing depth (PPD), clinical attachment level (CAL)\[21\] and radiograph evidence of bone loss, body mass index in the range of 18.5–24.9 kg/m², and waist circumference <90 cm (men) or <80 cm (women) (WHO, 2004)\[22\] individuals were categorized into three groups. Group I: 30 samples (15 GCF and 15 plasma) from 15 periodontally healthy controls with clinically healthy periodontium, with a GI score of 0, a PPD of ≤3 mm and CAL of 0, with no evidence of bone loss on radiograph. Group II: 30 samples (15 GCF and 15 plasma) from 15 chronic generalized periodontitis participants who had signs of clinical inflammation, a GI score of ≥1, a PPD of ≥5 mm, and CAL ≥1 with radiographic evidence of bone loss.\[23\]

Patients with chronic generalized periodontitis (Group II) were treated with a nonsurgical periodontal therapy (i.e., scaling and root planning) and GCF samples were collected from the same sites 8 weeks after the treatment along with plasma samples to constitute Group III (the after-treatment group).

**Selection of site and gingival crevicular fluid collection**

Allocations of groups and sample site selections were performed by an examiner (ARP). A calibrated examiner (CNG) did the radiographic evaluation, clinical evaluation measuring the clinical parameters including PPD, CAL, and GI using a University of North Carolina-15 periodontal probe (Hu-friedy, Chicago, IL, USA) and collected the GCF samples on the subsequent day. This was undertaken to prevent the contamination of GCF with blood associated with the probing of inflamed sites. The sites showing the greatest CAL and signs of inflammation, along with radiographic confirmation of bone loss, were selected for sampling in Group II participants. In the periodontally healthy group, to ensure the collection of an adequate amount of GCF, multiple sites with absence of inflammation were sampled. Scaling and root planing was performed for Group II participants. The selected site was cleaned first, isolated, and air dried using sterile cotton rolls, and using a Universal curette (Hu-friedy, Chicago, IL, USA); the supragingival plaque was removed gently to avoid contamination of the paper strips (Periopaper, Ora Flow Inc., Amityville, NY, USA). The paper strips were placed gently at the entrance of the gingival sulcus/crevice until a light resistance was felt.\[24\] Care was taken to avoid mechanical injury and the absorbed GCF volume of each strip was determined by electronic impedance (Periotron 8000, ProFlow Inc., Amityville, NY, USA) where a digital readout was converted to microliters using software (MLCONVERT. EXE software version 2.52, OraFlow, Amityville, NY, USA). Samples that were suspected to be contaminated with blood and saliva were excluded from the study. After collection of the gingival fluid, the four periopaper strips/site that absorbed GCF from each subject were pooled and were immediately...
transferred in microcentrifuge tubes containing 200 μL of phosphate buffer saline and stored frozen at −70°C for subsequent analysis.

**Blood collection and plasma extraction**

Using a 2 ml syringe, 20G needle 2 ml of blood was collected from the antecubital fossa by venepuncture and immediately transferred to ethylenediaminetetraacetic acid containing vials. The plasma was separated from blood by centrifuging at 3000 rpm for 5 min. The plasma was immediately transferred to a plastic vial and stored at −70°C until the time of assay.

**Interleukin-34 analysis**

The GCF and plasma samples were assayed for IL‑34 levels using a highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (human IL‑34 CatLog Number: DY5265 R and D systems, USA) according to the manufacturer instruction, and the samples were run in duplicate and mean was taken into consideration.

**Statistical analysis**

Statistical analysis was done using SPSS statistical software (SPSS version 18.0, Chicago, IL, USA) Power calculations were performed before initiation of the study. Based on the power of the study, grouping and sample size were determined at the 95% confidence interval (P < 0.05). The nonparametric Kruskal–Wallis test was performed to find the significant difference of IL‑34 concentration in GCF and plasma between the study groups. Pairwise t-test was used to determine statistical difference in the clinical parameters GI and CAL measured between groups, and Kruskal–Wallis test was used to find statistical difference in PPD between the groups. Pairwise comparison of IL‑34 concentration in GCF and plasma between Group I and Group II and Group I and Group III was performed using Mann–Whitney U test. Pairwise comparison of IL‑34 concentration in GCF and plasma between Group II and Group III was performed using Wilcoxon Signed-Rank test. P < 0.05 was considered statistically significant. The intragroup correlation of GCF and plasma concentrations of IL‑34 with clinical parameters was performed using Spearman’s rho correlation test. The mean intraexaminer standard deviation (SD) of differences in repeated PPD and CAL measurements was obtained using single passes of measurements (correlation coefficients between duplicate measurements; r = 0.95).

**Results**

Table 1 represents the descriptive statistics (mean ± SD) of the study population. All the samples, in each group tested positive for the presence of IL‑34. The mean IL‑34 concentration in GCF in Group I, Group II, and Group III were 414.49 ± 44.54 pg/ml, 1022.76 ± 168.21 pg/ml, and 604.17 ± 105.11, respectively. The mean IL‑34 concentration in plasma was observed to be the highest in Group II and least in Group I. The mean IL‑34 concentration in plasma was observed to be the highest in Group II and least in Group I. Kruskal–Wallis test was applied to compare the mean GCF and plasma concentration of IL‑34 and statistically significant (P < 0.05) difference existed between the groups [Table 1].

Pairwise comparison between Group I and Group II and Group I and Group III using Mann–Whitney U test showed statistically significant difference in IL‑34 concentration in GCF and plasma (P < 0.05) [Tables 2 and 3]. Pairwise comparison of IL‑34 concentration in GCF and plasma between Group II and Group III using Wilcoxon Signed-Rank test showed statistically significant difference in IL‑34 concentration in GCF and plasma (P < 0.05) [Tables 2 and 3]. Spearman’s rho Correlation demonstrated positive correlation between IL‑34 concentration in GCF and plasma in Group II and Group III except in Group I, there was negative correlation. There was statistically significant correlation between IL‑34 concentration in GCF and plasma in Group II. There was no statistical significant correlation between clinical parameters and IL‑34 concentration in GCF and plasma [Table 4].

**Discussion**

Newly discovered cytokine, IL‑34 regulates differentiation, proliferation, and survival of myeloid lineage acting through c-fms.[8] RANKL, a TNF family cytokine and M‑CSF/CSF-1, are important for differentiation of osteoclast. As an alternative ligand for M-CSF receptor, IL‑34 is now considered as a novel noncanonical pathway of osteoclast

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**Table 1: Descriptive statistics of study population (mean±standard deviation)**

| Study group | Group I (n=15) | Group II (n=15) | Group III (n=15) | P |
|-------------|----------------|-----------------|------------------|---|
| Age (years) | 37.4±4.93      | 40.8±7.2        | 40.8±7.2         | 0.144 |
| Sex (male/female) | 8/7 | 8/7 | 8/7 | - |
| GI score | 0 | 2.29±0.34 | 1.29±0.25 | <0.001* |
| PPD (mm) | 1.67±0.49 | 6.00±1.13 | 3.73±0.79 | <0.001* |
| CAL (mm) | 0 | 4.67±1.11 | 2.07±0.59 | <0.001* |
| GCF IL‑34 (pg/ml) | 414.49±44.54 | 1022.76±168.21 | 604.17±105.11 | <0.001* |
| Plasma IL‑34 (pg/ml) | 82.71±8.88 | 614.44±171.83 | 235.65±50.87 | <0.001* |

*P<0.05. GI=Gingival index, PPD=Pocket-probing depth, CAL=Clinical attachment level, GCF=Gingival crevicular fluid, IL=Interleukin
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In the present study, the influence of age and gender of the participants on the IL-34 levels was diminished by including the equal number of males and females in each group and selecting the participants within the specified age group of 30–56 years. GCF was collected using the absorbent filter paper strips in the study. The advantages of the technique are that it is quick and easy to use, can be applied to individual sites and possibly, is the least traumatic when correctly used.

The results of the present study indicated that the concentration of IL-34 in GCF and plasma increased progressively from periodontally healthy controls (414.49 ± 44.54 pg/ml and 82.707 ± 8.88 pg/ml, respectively) to chronic generalized periodontitis participants (1022.76 ± 168.21 pg/ml and 614.44 ± 171.83, respectively). After treatment by nonsurgical periodontal therapy and instituting strict oral hygiene measures, the mean concentration of IL-34 in GCF and plasma in chronic periodontitis group reduced to an after-treatment level of 604.17 ± 105.11 pg/ml and 235.65 ± 50.87 pg/ml, respectively, which were statistically significant (P < 0.05).

In bone-degenerative diseases, such as RA and chronic periodontitis, pro-inflammatory cytokines promote inflammation, osteoclast formation, and bone resorption. IL-34 is expressed in synovial tissue and is elevated in serum and synovial fluid of RA patients. Fibroblasts in periodontitis are, similarly to in RA, active cells highly important in the inflammatory process through modulation of myeloid cells leading to osteoclast activation and bone destruction. The pro-inflammatory cytokines TNF-α and IL-1 β regulate IL-34 expression in synovial and gingival fibroblasts, by a mechanism involving NF-kB and MAPK. Ma et al. found IL-34 mRNA expression in periapical lesions was significantly higher than that of the normal periodontal ligament tissue and concluded that IL-34 may be closely related to inflammation of chronic apical periodontitis. Thus, the increase in GCF and plasma concentrations of IL-34 from periodontally healthy controls to chronic generalized periodontitis and decrease after nonsurgical periodontal treatment in the present study can be attributed to the proinflammatory properties of the protein. In our study, there was a statistically significant and positive correlation between IL-34 concentration in GCF and plasma in Group II. However, Martinez et al. found CSF-1 and IL-34 in saliva have complementary roles in periodontal disease with IL-34 in steady-state and CSF-1 in inflammation. The concentration of IL-34 in our study was higher in GCF than in plasma, which could be explained by

### Table 2: Comparison of interleukin-34 levels in gingival crevicular fluid between the groups

| Group          | Mann-Whitney | P     | Group          | Mann-Whitney | P     |
|----------------|--------------|-------|----------------|--------------|-------|
| Group I        | 120.000      | <0.001| Group II       | 126.500      | <0.001|
| Mann-Whitney   | P            |       | U-test         | Z*           | P     |
| Group II       | -            |       |                | -            | 3.408 | 0.001|

*Wilcoxon signed-rank test

### Table 3: Comparison of interleukin-34 levels in plasma between the groups

| Group          | Mann-Whitney | P     | Group          | Mann-Whitney | P     |
|----------------|--------------|-------|----------------|--------------|-------|
| Group I        | 120.000      | <0.001| Group II       | 126.500      | <0.001|
| Mann-Whitney   | P            |       | U-test         | Z*           | P     |
| Group II       | -            |       |                | -            | 3.408 | 0.001|

*Wilcoxon signed-rank test

### Table 4: Spearman’s rho correlation coefficient between gingival crevicular fluid, plasma interleukin-34 versus clinical parameter

| Group          | Plasma IL-34 pg/ml | GI PPD (mm) | CAL (mm) |
|----------------|--------------------|-------------|----------|
| GCF IL-34 pg/ml|                   |             |          |
| Correlation P  | −0.371             | 0.557       |          |
| Plasma IL-34 pg/ml |                |             |          |
| Correlation P  | 0.174              | 0.031       |          |
| GCF IL-34 pg/ml|                   |             |          |
| Correlation P  | 0.614              | 0.052       | 0.027    | 0.110|
| Plasma IL-34 pg/ml |                |             |          |
| Correlation P  | 0.015              | 0.854       | 0.925    | 0.698|
| GCF IL-34 pg/ml|                   |             |          |
| Correlation P  | 0.293              | 0.037       | 0.228    | −0.110|
| Plasma IL-34 pg/ml |                |             |          |
| Correlation P  | 0.289              | 0.894       | 0.414    | 0.696|

*P<0.05. IL=Interleukin, GCF=Gingival crevicular fluid, GI=Gingival index, PPD=Pocket-probing depth, CAL=Clinical attachment level

formation as it can substitute M-CSF for osteoclast differentiation and play important role in RANKL-induced osteoclastogenesis.

Although numerous studies have underlined the role of M-CSF in periodontal tissue destruction,[25] there are no studies which have investigated the IL-34 concentration in GCF and correlated them to that of plasma in periodontal health, disease, and after treatment of periodontal disease. The present study is thus the first study that investigated the levels of IL-34 in GCF and plasma in periodontal health and disease and also assessed the role of periodontal therapy on IL-34 levels.

In bone-degenerative diseases, such as RA and chronic periodontitis, pro-inflammatory cytokines promote inflammation, osteoclast formation, and bone resorption. IL-34 is expressed in synovial tissue and is elevated in serum and synovial fluid of RA patients. Fibroblasts in periodontitis are, similarly to in RA, active cells highly important in the inflammatory process through modulation of myeloid cells leading to osteoclast activation and bone destruction. The pro-inflammatory cytokines TNF-α and IL-1 β regulate IL-34 expression in synovial and gingival fibroblasts, by a mechanism involving NF-kB and MAPK. Ma et al. found IL-34 mRNA expression in periapical lesions was significantly higher than that of the normal periodontal ligament tissue and concluded that IL-34 may be closely related to inflammation of chronic apical periodontitis. Thus, the increase in GCF and plasma concentrations of IL-34 from periodontally healthy controls to chronic generalized periodontitis and decrease after nonsurgical periodontal treatment in the present study can be attributed to the proinflammatory properties of the protein. In our study, there was a statistically significant and positive correlation between IL-34 concentration in GCF and plasma in Group II. However, Martinez et al. found CSF-1 and IL-34 in saliva have complementary roles in periodontal disease with IL-34 in steady-state and CSF-1 in inflammation. The concentration of IL-34 in our study was higher in GCF than in plasma, which could be explained by
local production of IL-34 in diseased periodontal tissues, suggesting that IL-34 levels might serve as a marker for local disease activity. The probability of local production of IL-34 by the recruiting inflammatory cells cannot be overlooked.

To the best of our knowledge, this is the first study evaluating IL-34 levels in GCF and plasma in periodontally healthy, chronic generalized periodontitis, and effect of nonsurgical periodontal therapy on IL-34 levels in the Indian population. Further, multicenter, longitudinal, and prospective studies can be carried out along with other proinflammatory cytokines like IL-1β, TNF-α, IL-6, and regulators of osteoclastogenesis such as RANK/RANKL/Osteoprotegerin to confirm the findings of the study and better understand the role of IL-34 as an inflammatory marker in periodontal health and disease.

**Conclusion**

Within the limitations of the present study, it can be postulated that with an increase in the amount of periodontal destruction there is substantial increase in the concentration of IL-34 both in GCF and plasma. In addition, treatment of periodontal disease leads to a proportional reduction in GCF and plasma levels of IL-34. Thus, IL-34 can be considered as potential inflammatory marker of periodontal disease. Furthermore, the finding that the concentrations of IL-34 in GCF are significantly greater than those in plasma samples suggesting a possible local IL-34 synthesis and/or storage within the periodontium. However, further multicenter, longitudinal, and prospective studies with larger sample size and long-term follow-up are needed to validate IL-34 as an inflammatory marker in periodontal disease as well as its possible therapeutic applications in periodontal health and disease.

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**Conflicts of interest**

There are no conflicts of interest.

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